Reliability of models that predict the fate of organic trace pollutants in municipal activated sludge plants

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Stellingen

1 Het is een verwarrende bezigheid om nieuwe, ongestructureerde biodegradatiemodellen te ontwikkelen, zolang op basis van experimentele gegevens geen onderscheid met bestaande modellen gemaakt kan worden.

Kovárová-Kovar, K., Egli, T. (1998), Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics, Microbiol. Mol. Biol. Rev., Vol. 62, No. 3, 646-666.
Dit proefschrift.

2 In tegenstelling tot wat Birch (1991) en Van Wijk *et al.* (1996) beweren, betekent het feit dat de concentratie van een milieuvreemde biodegradeerbare organische stof in het effluent van een biologische reactor onafhankelijk is van de influentconcentratie, niet zonder meer dat de biodegradatie van die stof met het Monod model kan worden beschreven.

Birch, R.R. (1991), Prediction of the fate of detergent chemicals during sewage treatment, J. Chem. Biotechn. Biol. 50, 411-422.
Van Wijk et al. (1996), The performance of models in predicting biodegradability in wastewater treatment plants, Proceedings of the 4th World Surfactants Congress, Barcelona, 3-7 VI.
Dit proefschrift.

3 Volgens Chen en Horan (1998) zijn ultra- en nanofiltratiemembranen te duur voor de zuivering van papierafvalwater. Echter, zij hebben niet goed gekeken naar de ontwikkelingen van de afgelopen 15 jaar en houden daardoor geen rekening met de zeer snel dalende prijs en toenemende kwaliteit van membranen, en met het positieve effect van hogere temperaturen op de flux van membranen indien het gezuiverde water wordt hergebruikt.

Chen, W., Horan, N.J. (1998), The treatment of a high-strength pulp and paper mill effluent for wastewater re-use: tertiairy treatment options for pulp and paper mill wastewater to achieve effluent recycle, Env. Tech., Vol. 19, 173-182.

4 Confer en Logan (1997) zijn een van de weinigen die beseffen dat 'biosorptie' van organische verontreinigingen door biofilms de resultante is van een groot aantal deelprocessen (ad- en desorptie, diffusie, ionenwisseling, bacteriële opslag, hydrolyse, etc.) en dat kennis over deze processen en de interactie tussen deze processen onontbeerlijk is voor een goed ontwerp van biofilmprocessen.

Confer, D.R., Logan, B.E. (1997), Molecular weight distribution of hydrolysis products during biodegradation of model macromolecules in suspended and biofilm cultures, Wat. Res., Vol. 31, No. 9., 2127-2145.

5 De enorme hoeveelheid tijd, geld en energie die tot nu toe is gespendeerd aan de praktische toepassing van de actiefslibmodellen No. 1 en No. 2, waarmee CZV-, stikstof en fosfaatverwijdering in actiefslibinstallaties wordt beschreven, heeft vooralsnog niet geresulteerd in het gewenste effect, d.w.z. kleinere en goedkopere installaties en een verbeterde effluentkwaliteit.

6 Er is een duidelijke grens aan de eigen bijdrage die universiteiten aan projecten kunnen leveren. Die grens wordt vaak overschreden, met als gevolg dat het onderzoek van matige kwaliteit is of de onderzoekers en laboranten een groot aantal overuren moeten draaien.

7 Er is een directe relatie tussen het weer en de spontaniteit van mensen. De enige uitzondering op deze regel lijken de Engelsen en Ieren te zijn.

8 Dat veel bewoners van bejaardentehuizen als gevolg van de bezuinigingen geen koekje meer bij de koffie krijgen, terwijl menige yup op zijn 30-ste al meer dan een ton op de bank heeft staan, laat zien dat het met onze beschaving niet best is gesteld.

Stellingen behorende bij het proefschrift "Reliability of models that predict the fate of organic trace compounds in municipal activated sludge plants" van Hardy Temmink. Wageningen, 25 juni 2001

Voor mijn ouders

Abstract

Temmink, H (2001). Reliability of models that predict the fate of organic trace pollutants in municipal activated sludge plants, PhD-thesis, Wageningen University, Wageningen: The Netherlands, 184 pages.

The production, use and disposal of many compounds inevitably leads to their presence in the environment as organic trace pollutants. Although their concentration may be low, these trace compounds can present an environmental hazard associated with their toxicity for human beings, their potential to accumulate in biota and ecosystems and in some cases their function as a catalyst in the destruction of the ozone in the stratosphere.

In many cases these organic trace compounds enter the sewage system and will finally appear in municipal activated sludge plants. Several processes determine their distribution in these plants. Hydrophobic compounds can partition to the sludge and in this manner may create a potential hazard associated with sludge disposal. Volatile organic compounds are amenable to air stripping and surface desorption and therefore are frequently found in the off-gas of activated sludge plants. Whereas sorption and volatilisation merely rearrange the distribution of compounds among the different environmental compartments (sludge, air and water), only a destructive process such as biodegradation can actually remove them from the environment.

During the last two decades several activated sludge fate models have been developed. These models are used (i) to predict the environmental exposure to specific organic trace compounds expected to appear in municipal wastewater's, (ii) to optimise the design and control of treatment plants with respect to the removal of these pollutants and (iii) to establish limits on treatment plant influent loads based upon allowable effluent loads. The reliability of these models only has been demonstrated to a limited extent and therefore their applicability remains uncertain. A validation study may help to gain some confidence in these models, but also can reveal some of their shortcomings.

Based on an extensive literature review such a validation study was set-up. The fate of three test trace compounds in a pilot-scale municipal activated sludge plant was investigated: the C_{12}-homologue of linear alkylbenzene sulfonate (LAS-C_{12}), nitrilotriacetic acid (NTA) and toluene. The sludge retention time (SRT) of the plant and influent concentration of the trace compounds were varied. In addition, for each of the test compounds their biodegradation kinetics by activated sludge were assessed with a method which was specifically developed for this purpose. The results showed that existing models do not yield accurate predictions, in particular because the biodegradation kinetics they employ are incorrect. A generic assessment of the fate of organic trace compounds in activated sludge plants therefore should not rely on model calculations alone. Still, existing models can be used for a first screening of new priority compounds or to design field monitoring studies for existing priority compounds. More accurate models can only be developed if more fundamental research is directed towards biodegradation of mixtures of organic substrates by mixed microbial cultures such as activated sludge.

Keywords : activated sludge, organic trace pollutants, fate modelling, wastewater

Contents

Chapter 1

General introduction

Exposure assessment of organic trace compounds

The production, use and disposal of many compounds inevitably leads to their presence in the environment as organic priority pollutants (Figure 1). This did not receive much attention until the 1970s when more sophisticated analytical techniques became available and their presence could be demonstrated in municipal and industrial wastewater, surface water, groundwater, off-gases and soil. Although their concentrations may be low, these trace compounds (and synonyms thereof such as xenobiotic compounds, organic micropollutants, priority pollutants, etc.) can present an environmental hazard associated with their toxicity for human beings, their potential to accumulate in biota and ecosystems and in some cases their function as a catalyst in the destruction of the ozone in the stratosphere.

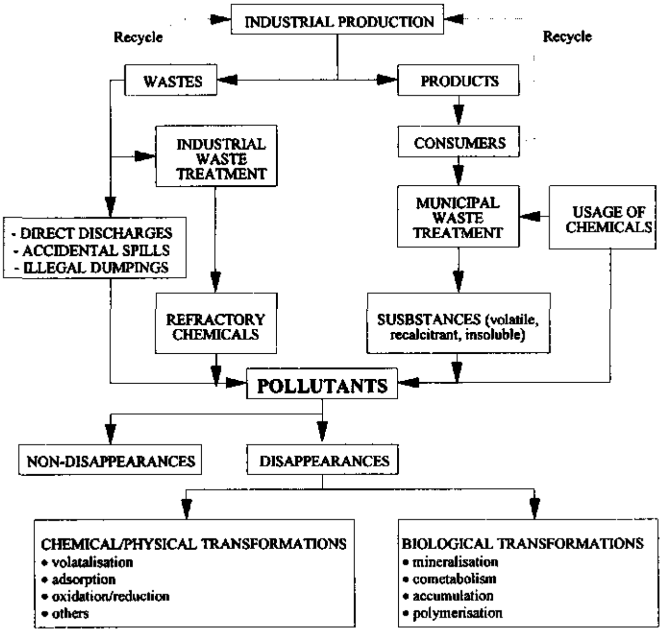

Fig. 1 Origination, routes and fates of priority pollutants (after Richards and Shieh, 1986).

To avoid these risks, Commission Directive 93/67/EEC and Commission Regulation (EC) No. 1488/94 require that an environmental risk assessment be carried out on notified new substances and on existing priority substances. For this purpose a hazard assessment scheme has to be implemented according to the "7th Amendment" of Directive 67/548/EEC (EC, 1992). Such a scheme (Figure 2) includes an exposure assessment leading to a predicted environmental concentration (PEC) and an effect assessment to estimate a no-effect concentration (PNEC). These PECs and PNECs are to be determined for all three environmental compartments: the aquatic environment, the terrestrial environment and the atmosphere. Based upon the PEC/PNEC ratio a decision will be made whether a compound is admissible or not, or to collect more detailed (field) information.

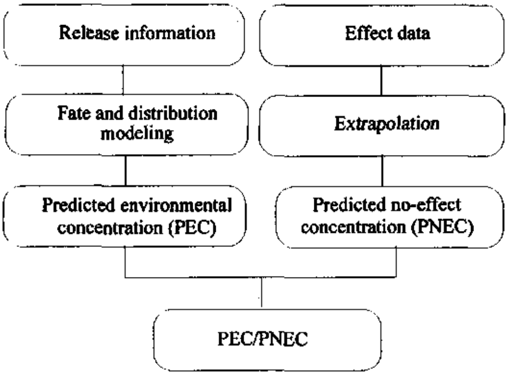

Fig. 2 Hazard assessment scheme (after ECETOC, 1992).

In accordance with the procedure in the other member states of the EC, the Netherlands Substances Acts (WMS) prescribes that each new chemical be registered at the Department of Housing, Physical Planning and Environment (VROM) and judged on its admissibility by the Office of Environmentally Hazardous Compounds (BMS). In support of the admission procedure, the National Institute of Public Health and Environmental Protection (RIVM) has developed a risk assessment software package USES 1.0 (Uniform System for the Evaluation of Substances) to predict the exposure concentrations in the environment (RIVM, 1994). Typical for USES is a tiered approach dictated by the availability and accuracy of exposure data (Figure 3).

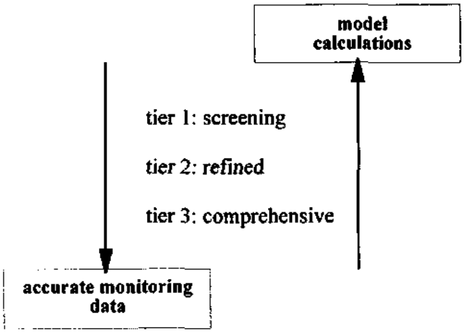

Fig. 3 Tiers in exposure assessment.

Table 1 - Mandatory base-set of compound properties.

physical-chemical properties
molecular weight
water solubility
vapour pressure
octanol-water partitioning coefficient
biodegradation
result of standardised biodegradability tests*

* according to the guidelines prescribed by the OECD (OECD, 1993)

At the highest tier relevant information comes directly from measurements in the environment whereas at the lowest tier exposure assessment will have to rely on model calculations using a minimal set of production and consumption data, combined with the mandatory chemical and biological compound properties listed in Table 1. Its physical-chemical properties provide essential information about the distribution of a compound between the different environmental compartments. For example, water solubility can be used in combination with vapour pressure to predict the tendency of trace pollutants to escape from water to the atmosphere. Information about the biodegradability of trace pollutants usually is not available and has to be assessed in (standardised) biodegradation tests. In accordance with Figure 3 these tests are organised at different tiers with ready biodegradability tests carried out under standardised conditions (tier 1), inherent biodegradability tests carried out under favourable conditions (tier 2) and simulation tests mimicking realistic environmental conditions (tier 3).

Fate of organic trace compounds in municipal activated sludge plants

In many cases trace compounds enter the sewage system and will finally appear in municipal activated sludge plants (Figure 4). Examples of such compounds can be found in Table 2. In contrast to industrial wastewater treatment plants, which usually are designed to remove specific pollutants, municipal plants have to eliminate the bulk of organic compounds, expressed by the sum parameter biochemical oxygen demand (BOD) or chemical oxygen demand (COD). More recently municipal plants also have to comply with stringent effluent demands for nitrogen and phosphorus.

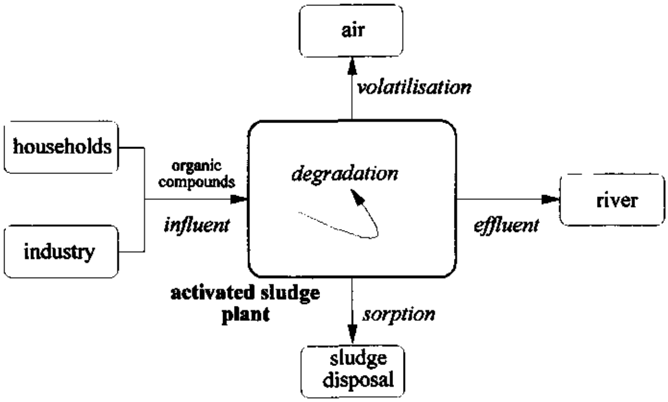

Fig. 4 Distribution of organic compounds in municipal activated sludge plants.

Several processes determine the fate of organic trace compounds in activated sludge plants. In particular if they are strongly hydrophobic (with a high octanol-water partition coefficient, K_{ow}) they can partition to the sludge flocs and in this manner create a potential hazard associated with sludge disposal (Petrasek *et al.*, 1983; Hannah *et al.*, 1986; Klöpfer, 1996; Rogers, 1996; Beck *et al.*, 1996). Typical examples are pesticides, phenols, phthalates and polycyclic aromatic hydrocarbons. Volatile organic compounds (VOCs) (with a high Henry's law constant, H) are frequently found in the off-gas of municipal activated sludge plants because they can be removed by air stripping and surface volatilisation (Lurker *et al.*, 1982; Namkung and Rittmann, 1987; Thompson *et al.*, 1993).

Table 2 - Examples of organic trace compounds and their concentrations in the influent and effluent of municipal wastewater treatment plants (nd = not detected).

organic trace compound	influent $\mu g \cdot l^{-1}$	effluent $\mu g \cdot l^{-1}$	removal* %	reference
Volatile organic compounds				
dichloromethane	175	21	88	1
1,2-dichloroethane	130	45	65	1
toluene	85.47	6.2	93	2
benzene	23.3	nd	high	2
ethylbenzene	17.7	0.5	97	2
tetrachloroethylene	16.13	2.1	87	2
1,1,1,-trichloroethane	12.77	2.9	77	2
trichloroethylene	9.7	0.5	95	2
chlorobenzene	0.47	nd	high	2
Surfactants				
LAS	5000	40	99	3
DTDMAC	1000	40	96	3
C_{12}TMAC	82	2.4	97	3
Phthalates				
bis(2-ethylhexyl)phthalate	26.3	7.17	73	4
diethylphthalate	9.33	nd	high	4
dibuthylphthalate	3.8	nd	high	4
dimethylphthalate	0.93	nd	high	4
Polynuclear aromatic hydrocarbons				
fluoranthene	0.77	0.076	90	4
naphtalene	0.16	nd	high	4
anthracene	0.056	0.009	84	4
Chlorophenols				
2,4-dichlorophenol	0.27	nd	high	4
2,4,6-trichlorophenol	0.1	nd	high	4
pentachlorophenol	0.16	0.27	69	4

reference 1: van Luin and Starkenburg (1985), 2: Namkung and Rittmann (1987),
3: Cowan *et al.* (1993), 4: van der Spoel *et al.* (1990)

* overall removal from wastewater

Whereas sorption and volatilisation merely rearrange the distribution of compounds among the environmental compartments in a wastewater treatment plant (sludge, air and water), only a destructive process such as biodegradation can actually remove them from the environment. Municipal activated sludge plants have a large biodegradation potential, both qualitatively and quantitatively (Grady, 1985), mainly because the sludge consists of a diverse population of micro-organisms growing on a complex mixture of carbon sources supplied with the (domestic) wastewater. At the same time, biodegradation is a very complicated process involving numerous factors such as the molecular structure of trace compounds, biomass composition, environmental and operating conditions, the presence of other substances, etc. Besides, biodegradation has to compete with the abiotic mass-transfer processes sorption an volatilisation. For example, non-chlorinated readily biodegradable VOCs are effectively eliminated from the wastewater by biodegradation whereas chlorinated and less biodegradable VOCs are primarily removed by volatilisation (Kincannon *et al.*, 1983; Melcer *et al.*, 1992; Bell *et al.*, 1993).

Activated sludge fate models for organic trace compounds

To assess the behaviour of organic trace substances in wastewater treatment plants, the Technical Guidance Document (TGUD) in support of risk assessment (EC, 1994) advises to use the following order of preference:

1. **Monitoring data** - In principal, the results of long-term monitoring studies in which the influent and effluent concentrations of pollutants are determined could be used for exposure assessment. For instance, the data in Table 2 can be used to estimate the overall removal efficiency of the trace compound in a wastewater treatment as well as their distribution to the effluent. However, relevant information about air stripping, sorption and biodegradation cannot be derived. Furthermore, the reported removal efficiencies depend on plant operational characteristics (e.g., hydraulic- and sludge retention time and aeration intensity), which makes extrapolation of these results to other plants questionable. Finally, the sludge in the plant the monitoring data were reported for may have been acclimated to high-volume, site-specific compounds. This may result in an overestimation of the removal capacity in other treatment plants.

2. **Simulation test data** - Many tests are described in the literature to simulate, in the laboratory, the removal of organic compounds by activated sludge. In these tests different analytical methodologies are used including parent compound analysis, respirometry (e.g. Grady *et al.*, 1989; Brown *et al.*, 1990; Tabak *et al*, 1990 and Ellis *et al.*, 1996) and ^{14}C-radiolabelled techniques (Berg and Nyholm, 1995; Nyholm *et al.*, 1996; Federle and Itrich, 1997). However, only the so called Coupled Unit Test (OECD, 1993) is a generally accepted method. In this test elimination of a compound is determined by changes in dissolved organic carbon (DOC) or chemical oxygen demand (COD). The main disadvantages of this method are that (i) it is an open test and therefore is not suitable for volatile compounds and (ii) DOC and COD measurements cannot be used for the extremely low concentrations of trace compounds that generally prevail in activated sludge plants.

3. **Modelling** - For new substances no relevant monitoring or simulation test data are available and the first step of an exposure assessment has to rely on model calculations. For existing substances it may seem that monitoring data always give more reliable results than model calculations. However, monitoring data often have a considerable uncertainty associated with them (temporal and spatial variations, high detection limits, etc.). Consequently, both approaches should complement each other and modelling is mentioned by the TGUD as a third possibility for exposure assessment. Although several models have been developed for this purpose (e.g., Blackburn *et al.*, 1984; Weber *et al.*, 1987; Namkung and Rittmann, 1987; Govind *et al.*, 1991; Birch, 1991; Cowan *et al.*, 1993), the TGUD advises to use a revised version of a model called SimpleTreat (Struijs, 1996). This model is constructed such that the required output (distribution and concentrations in the effluent, waste sludge and air) can be calculated from the release rate of a compound into the sewer system and the mandatory base-set of compound properties of Table 1. Figure 5 shows the set-up of an activated sludge plant in SimpleTreat.

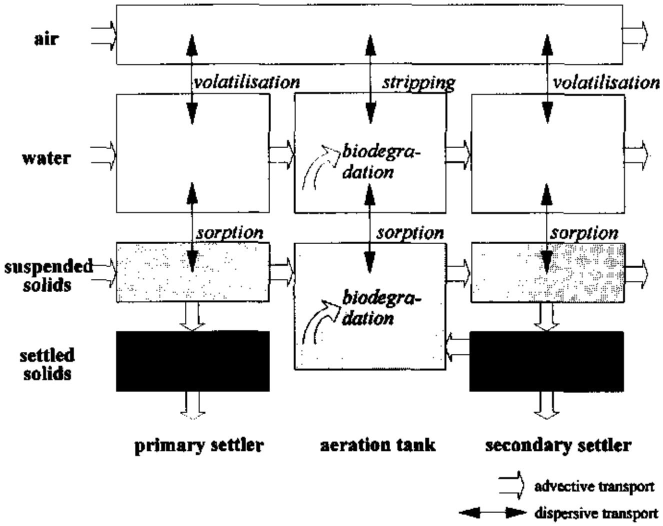

Fig. 5 Activated sludge plant in SimpleTreat, version 3.0 (Struijs, 1996).

Research objectives and outline of this thesis

The reliability of activated sludge fate models such as SimpleTreat only has been demonstrated to a limited extent and therefore their applicability remains uncertain. A validation study may help to gain some confidence in these models, but at the same time can also reveal their shortcomings. Although it seems logic to focus such a study on one particular model, SimpleTreat for example, this would ignore that other models employ completely different mathematical equations. In particular for biodegradation numerous mathematical approaches are used, including those based on Monod, secondary utilisation and pragmatic first-order kinetics (ECETOC, 1991). Because activated sludge fate models are extremely sensitive to the rate of biodegradation, it can be expected that these differences in the biodegradation kinetics also will generate highly different model predictions.

The aim of this thesis is to analyse the shortcomings of activated sludge fate models and to develop scientific knowledge, including the development of a biodegradation test and validation experiments, which may help further improvement of the various models and their implementation in practice. Figure 6 presents a general overview of the set-up of this study.

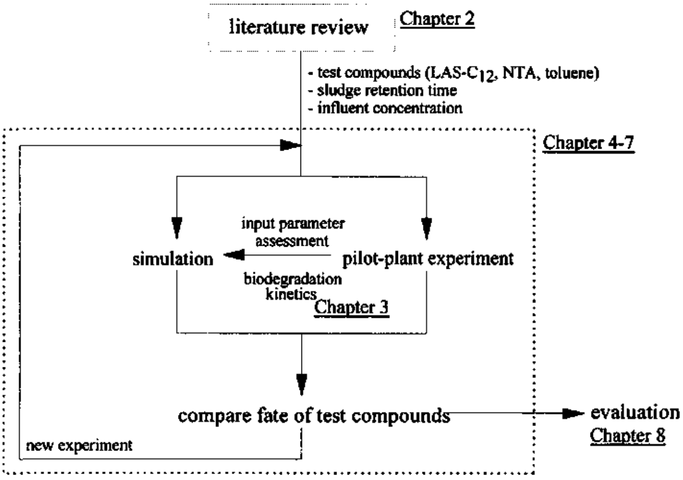

Fig. 6 General set-up of this study.

First, a literature review was carried out (chapter 2) to provide an overview of the mathematical equations that are used for biodegradation, as well as for sorption and volatilisation which are the other two important processes determining the behaviour of organic trace compounds in activated sludge plants. Also, methods to assess the parameters associated with these processes are addressed in chapter 2 because the accuracy of these parameters is essential for the quality of the model predictions. Finally, some background information is given about activated sludge fate models that are proposed in the literature and efforts undertaken to validate these models.

The information produced by the literature review was used to assist in setting up further research. One important result was that there is a lack of accurate biodegradation tests. Most (standardised) biodegradation test are not designed to yield kinetic information and extrapolation of their results to activated sludge plant conditions is difficult and dubious. To overcome these difficulties a new biodegradation test was developed, which mimics as closely as possible the conditions in the plant of interest (chapter 3).

Apart from an activated sludge system (aerated bioreactor and a settler to retain the biomass in the system), most municipal wastewater treatment plants include several pre-treatment steps (primary settler, grit removal, screens, etc.) and facilities to treat excess sludge (thickeners, digesters, etc.).

Although it is recognised that these process units all may have an effect on the removal of trace pollutants, the (aerated) bioreactor generally is considered the most important treatment unit in this regard. For that reason it was decided to focus this study entirely on the bioreactor.

Although in a way it is a model itself, it was decided to use a pilot-scale activated sludge plant for the experiments rather than to collect monitoring data in a full-scale plant because (i) the conditions in a pilot-scale plant can be more conveniently manipulated , (ii) test compounds can be discharged without having to worry about detrimental effects on plant performance with respect to conventional wastewater parameters like COD, N and P and (iii) a pilot-scale plant can be covered which facilitates off-gas sampling. As they may have an important effect on the actual fate of the test compounds, the operational parameters of the pilot-plant (sludge retention time, hydraulic retention time, COD loading rate, release rate of the test compounds, aeration intensity) were varied in the experiments.

Activated sludge fate models are extremely sensitive to the physical-chemical properties and the biodegradability of trace compounds. Because these properties vary strongly among trace pollutants it is necessary to investigate a large number of test compounds. Initially, a validation matrix was constructed consisting of 17 test compounds possessing different volatility, hydrophobicity and biodegradability. Unfortunately, a lack of reliable and accurate analytical techniques forced us to reduce this number to three compounds only: the C_{12}-homologue of linear alkylbenzene sulfonate (LAS-C_{12}) which is a highly sorptive compound, toluene which is a volatile compound and nitrilotriacetic acid (NTA) to represent the class of non-sorptive and non-volatile compounds.

Monitoring sessions to assess the behaviour of LAS-C_{12}, NTA and toluene in a pilot-scale activated sludge plant are described in chapters 4, 5 and 6, respectively. In addition, the biodegradation kinetics for these compounds were determined using the new biodegradation test mentioned earlier. The results were used to check the model equations employed by existing activated sludge fate models, to obtain an impression of the reliability of these models and to identify model imperfections that need improvement and further research.

During the monitoring sessions the test compounds were discharged at a constant rate, i.e., their fate was determined under "steady-state" conditions. In reality the influent concentration of trace compounds fluctuate in time and this may have a strong impact on the average removal efficiency that can be achieved. For that reason the dynamic behaviour of the test compounds was investigated in a number of step discharge experiments. The results of this part of the investigations are described in chapter 7.

Finally, all the results obtained in this study are assembled and evaluated in chapter 8. In this chapter also some directions are given for future research activities.

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Chapter 2

Literature review

Introduction

To reduce the risks associated with the use, production and disposal of organic compounds, EC regulations require that an environmental risk assessment be carried out on notified new substances as well as on priority existing substances. An important element of this risk assessment is an estimation of the environmental exposure, leading to a predicted concentration (PEC) for the aquatic and terrestrial environments and the atmosphere. In industrialised countries consumer products, and in many cases also industrial waste products, enter the environment after passing through municipal wastewater treatment plants. Hence, a prediction of the fate of individual (trace) organic compounds in these plants with a mathematical model would be a useful element of an initial exposure assessment of notified new chemicals for which no environmental monitoring data are available. For existing (priority) chemicals such a model could be used to help interpreting field monitoring data which often are incomplete or have considerable uncertainty (temporal and spatial variations, high detection limits, etc.) associated with them. Finally, local water authorities could use an activated sludge fate model to estimate the effect of industrial or polluted groundwater discharges to their wastewater treatment plants.

Domestic wastewater usually is treated by activated sludge systems (Figure 1). Here, a certain portion of a trace compound may be effectively eliminated from the environment by biodegradation whereas the remainder is released into the environment with the treated wastewater, the (dewatered) waste sludge or the off-gas. The distribution between these pathways is largely determined by the physical-chemical and biodegradation properties of the target compound and by the operational characteristics of the plant.

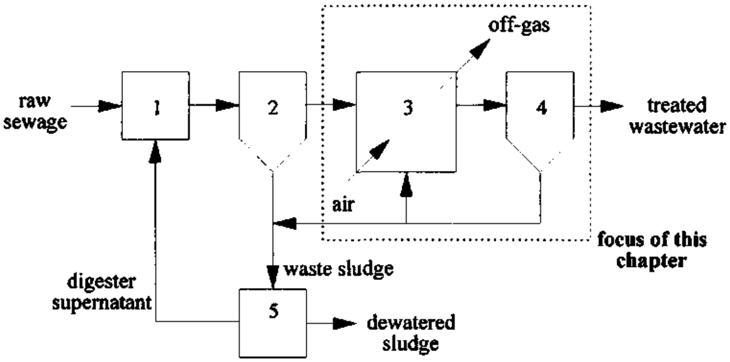

Fig. 1 Municipal activated sludge plant (1 mechanical treatment, 2 primary settler, 3 bioreactor, 4 secondary settler, 5 sludge digestion and dewatering).

The main objective of this literature review is to provide information on (i) the relevant processes determining the fate of organic trace compounds in activated sludge plants, (ii) mathematical equations that can be used to predict the rates at which these processes proceed, (iii) methods to assess the model parameters, (iv) existing activated sludge fate models and (v) studies that have been undertaken to validate these models.

It is emphasised that this review is not in any way complete because several restrictions were applied. First, although the sewer system, mechanical (pre-)treatment steps (e.g., grit removal and primary settling) and sludge treatment all may have an effect on the behaviour of organic trace compounds in wastewater treatment plants, it is assumed that the most relevant processes only take place in the bioreactor (Figure 1). A second assumption is that this bioreactor is completely mixed and aerated. It is recognised however that other hydraulic regimes are employed as well. Also, contemporary municipal wastewater treatment plants consist of aerated and non-aerated compartments to accommodate biological nitrogen and phosphorus removal .

Figure 2 shows a hierarchical diagram of the processes that can take place in an aerated bioreactor and which have an effect on the behaviour of trace compounds. Of these processes, biodegradation and the mass-transfer processes volatilisation and sorption will be included in this review. Abiotic conversion processes such as photolysis and chemical oxidation/reduction are not considered because for most compounds they are of minor importance as compared to biochemical oxidation by micro-organisms (CEC, 1993).

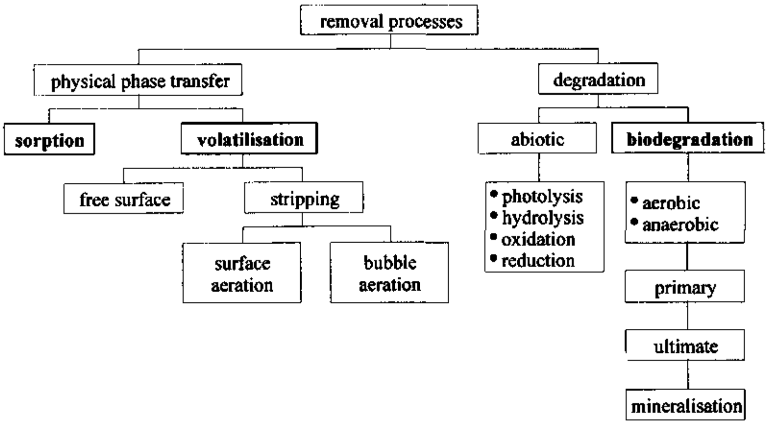

Fig. 2 Hierarchical diagram for removal processes in a bioreactor (after CEC, 1993).

Sorption

The literature provides ample evidence that (non or slowly biodegradable) organic trace compounds, in particular if they are strongly hydrophobic, can be taken up by activated sludge from the wastewater and in this manner accumulate in the sludge at concentrations which are several orders of magnitude greater than influent concentrations (Hannah and Rossman, 1982; Petrasek *et al.*, 1983; Hannah *et al.*, 1986; Klöpfer, 1996; Rogers, 1996; Beck *et al.*, 1996). Typical examples are pesticides, phenols, phthalates and polycyclic aromatic hydrocarbons.

Sorption is a complicated process determined by several compound specific properties such as water solubility, acidity, molecular size and polarity, but also by sludge and environmental characteristics such as the organic carbon content of the sludge and the pH (Haigh, 1996). A detailed description of sorption mechanisms is beyond the scope of this review but an excellent overview is given by Weber *et al.* (1991).

Sorption equilibrium

Tsezos and Bell (1989) have shown that the majority of organic trace compound uptake by biomass is located in the cell interior, although an appreciable amount can be found in the cell wall and cell membrane. Based on this observation, Wang *et al.* (1993) suggested that sorption is a two step process with (i) adsorption of the compound from the bulk liquid onto the surface of the sludge and (ii) partitioning of the compound between the aqueous phase and the organic matter in the sludge. To describe these phenomena they developed a combined adsorption-partition equilibrium model. The first step of adsorption onto the sludge surface is represented by a Langmuir equation and the second step of partitioning in the organic matter by a linear equation:

$$q = q_a + q_p = q_{a\max} k \frac{S}{1+kS} + K_p S \qquad (1)$$

with q, q_a, $q_{a\max}$ and q_p the total uptake by the sludge, uptake by adsorption, maximum uptake by adsorption and partitioning uptake, respectively ($\mu g \cdot g^{-1}$), k the ratio of the adsorption rate constant to the desorption rate constant (-), K_p the sludge-water partition coefficient and S the liquid-phase concentration of the trace compound ($\mu g \cdot l^{-1}$).

Wang *et al.* (1993) also showed that for strongly hydrophobic compounds partitioning uptake will dominate whereas for compounds of low hydrophobicity adsorption uptake will dominate. Usually, for the generally low concentrations of trace compounds in activated sludge systems it is assumed that only (linear) partitioning is important (Bell and Tsezos, 1987, Urano and Saito, 1984; Dobbs *et al.*, 1989; Jacobsen *et al.*, 1993; Kördel *et al.*, 1997). Equation (1) then can be reduced to:

$$q = K_p S \qquad (2)$$

Sorption kinetics

The rate of sorption of trace compounds to activated sludge is determined by several processes including diffusion of the compound through the liquid film surrounding the sludge flocs, diffusion through the pores in these flocs, adsorption onto the sludge-water interface and finally uptake by the biomass. In activated sludge fate modelling sorption usually is considered to be an instantaneous equilibration process. Considering typical hydraulic retention times (HRTs) in activated sludge bioreactors of 8 hours or longer, several (batch-type) sorption studies would support this assumption. For example, Matter-Müller (1979) determined the kinetics of sorption of several trace compounds to activated sludge and observed that an equilibrium already was achieved within 7 to 20 minutes. Dobbs *et al.* (1989) measured the kinetics of chlorobenzene and 1,1-dichloroethylene sorption and observed an equilibration time of about 30 minutes. Jacobsen *et al.* (1993) reported the equilibration of a number of chlorinated phenols to be within 2 hours. The time required to reach a sorption equilibrium for a number of surfactants was investigated by Kerr and McAvoy (1993) and was within 30 minutes.

Sometimes, for extremely hydrophobic compounds and at short HRTs, sorption and desorption may be sufficiently slow as to invalidate the use of equilibrium models and their rates also have to be taken into account. The most simple kinetic model is a one-box model in which sorption and desorption are assumed to be reversible processes and their overall rate is a first-order function of the concentration difference between the sludge and the liquid phase (Matter-Müller, 1979; Liljestrand and Lee, 1991):

$$r_{sor} = k_{sor}(S - \frac{q}{K_p}) \qquad (3)$$

where r_{sor} is the (volumetric) rate of sorption ($\mu g \cdot l^{-1} \cdot h^{-1}$) and k_{sor} is the sorption rate constant (h^{-1}).

Unfortunately this simple one-box model cannot explain sorption and desorption behaviour with an initial rapid rate followed by a much slower rate observed by Dobbs *et al.* (1989) for activated sludge. This initiated the development of numerous two-box models in which the sorbent (the sludge) is divided into two separate boxes. One box is an easily accessible exterior part and the other box a slowly exchanging interior part. However, it is virtually impossible to estimate unique values for the three parameters that are required (exchange rate constants between the bulk and between the first and second box and the fraction of the total sorption capacity taken by the first box). Moreover, extrapolation to different conditions is difficult because these parameters cannot be related to the physical properties of the sorbent (the sludge) and sorbate (the organic compound).

To avoid similar problems, associated with sorption to suspended river sediments, a different approach was followed by Wu and Gschwend (1986). They proposed a radial diffusion model with only one fitting parameter, the effective diffusity:

$$\frac{\delta S(r)}{\delta t} = D_{eff}[\frac{\delta^2 S(r)}{\delta r^2} + \frac{2}{r}\frac{\delta S(r)}{\delta r}] \qquad (4)$$

with S(r) the total local volumetric compound concentration ($\mu g \cdot l^{-1}$), D_{eff} the effective diffusity coefficient ($m^2 \cdot h^{-1}$), t the time (h) and r the coordinate in the direction of transport (m).

In this model sorption is assumed to be limited by intrapartical diffusion. The effective diffusity D_{eff} is related to the sorbent properties and to the hydrophobicity of the compound. Stronger hydrophobicity will slow down the sorption process. With this model sorption and desorption behaviour of several compounds to suspended river sediments could be described. An application of this model for sorption by activated sludge has not been investigated.

Sorption parameters

Partition coefficient - The linear sludge-water partition coefficient K_p of trace compounds can be determined directly in batch sorption experiments with activated sludge that are spiked with different amounts of the target compound.

As an alternative, the (obligatory) standard test to determine sorption and desorption of organic chemicals to soil could be used (OECD, 1993). From the results of this test the sorption coefficient K_{oc} can be calculated, which is the amount of trace compound taken up per unit weight of organic matter in the soil. Kördel *et al.* (1997) compared the K_{oc} for soil with the K_{oc} for activated sludges from different origins. They concluded that they are comparable because in both cases organic matter is the most important constituent determining sorption. Only at extremely low values of K_{oc} the sorption capacity to soil is somewhat higher because soil contains more inorganic constituents (clay, ferric oxides, etc.) to which polar compounds may adsorb.

Another promising test method could be the HPLC-screening method (OECD, 1993) (Kördel *et al.*, 1993; Kördel *et al.*, 1997). In this method the retention time of an organic compound on a HPLC column is related to its sludge-water partition coefficient. The main advantage of this method is that no quantitative analytical method is required.

An even less elaborate, but probably also less accurate method, is to estimate the sludge-water partition coefficient K_p from known trace compound and sludge properties. Blackburn *et al.* (1984) used an empirical relationship with the octanol-water partition coefficient K_{ow}, the lipid weight of the biological solids and the density of the lipids:

$$K_p = \frac{K_{ow} f_l}{\rho_l} \qquad (5)$$

with f_l the lipid weight fraction of biological solids ($g \cdot g^{-1}$) and ρ_l the density of the lipids ($g \cdot l^{-1}$).

Other workers proposed to use an empirical relationship with the K_{ow} and/or the fraction of organic carbon f_{oc} of the sludge. Table 1 shows some examples of these relationships and Figure 3 shows the K_p these relationships would predict for a number of selected trace pollutants.

Table 1 -Empirical relationships between the linear sludge-water partition coefficient K_p, the octanol-water partition coefficient K_{ow} and the organic carbon content of sludge f_{oc} (K_p in $l \cdot g^{-1}$ suspended solids).

Relation	reference
$\log(K_p) = 0.67 \cdot \log(K_{ow}) - 2.369$	Bell and Tsezos (1987)
$\log(K_p) = 0.58 \cdot \log K_{ow}) + \log(f_{oc}) - 1.86$	Dobbs *et al.* (1989)
$\log(K_p) = 0.82 \cdot \log(K_{ow}) + \log(f_{oc}) - 2.98$	Schwarzenbach and Westall (1985)

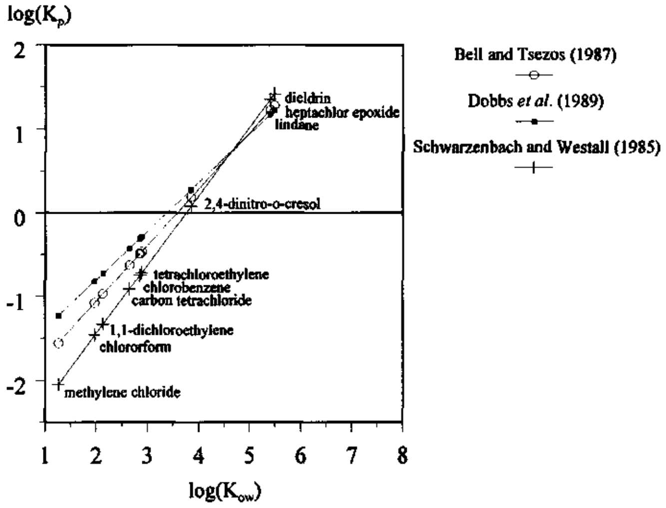

Fig. 3 The sludge-water partition coefficient K_p as a function of the octanol-water partition coefficient K_{ow} (a f_{oc} of 0.4 was assumed).

According to Schwarzenbach and Westall (1985) a correction should be applied for ionic compounds because only the non-ionised fraction is available for sorption. Given a certain pH and the dissociation constant pK_a, this fraction equals:

$$\frac{1}{1+10^{(pH-pK_a)}} \tag{6}$$

Rate constants - Hardly any attention has been paid to the estimation of sorption rate parameters. Although the rate parameters in one-box and two-box models may be estimated from experimental data, the validity of the one-box model of equation (3) is questionable and it is impossible to find unique parameters for the two-box model. The effective diffusity D_{eff} in the radial diffusion model of Wu and Gschwend (1986) of equation (4) can be estimated from batch sorption experiments, but this value cannot be extrapolated to other compounds and systems. Therefore, they correlated D_{eff} to sorbent and sorbate properties:

$$D_{eff} = \frac{D_m \varepsilon}{K_p (1-\varepsilon)\rho_s} f(\tau, c) \tag{7}$$

where D_m is the pore fluid diffusity coefficient ($m^2 \cdot h^{-1}$), ε is the porosity of the sludge floc (-) and ρ_s is the density of the sludge ($g \cdot l^{-1}$). D_{eff} is corrected for turtuosity (τ) and constrictivity (c) effects by $f(\tau,c)$. It is unclear whether this relationship can also be applied to activated sludge.

Volatilisation

Volatile organic compounds are frequently detected in the influent of municipal activated sludge plants (Lurker *et al.*, 1982; Petrasek *et al.*, 1983; Dunovant *et al.*, 1986; Namkung and Rittmann, 1987; Melcer *et al.*, 1992; Bell *et al.*, 1993; Thompson, 1993). They can be removed from the wastewater by air stripping and surface volatilisation, and once transferred to the atmosphere they may constitute an environmental hazard. Air stripping and surface volatilisation also may be competing with the other relevant processes, in particular with biodegradation. For example, several workers found that readily biodegradable non-chlorinated compounds are mainly eliminated by biodegradation whereas for less biodegradable chlorinated compounds volatilisation constitutes the most important removal mechanism (Kincannon *et al.*, 1983; Dunovant *et al.*, 1986; Melcer *et al.*, 1992; Bell *et al.*, 1993).

Air Stripping

Although in aerated bioreactors air stripping and surface volatilisation occur simultaneously, air stripping usually is considered to be the dominant mechanism. In activated sludge systems air stripping can be caused by (sub-surface) bubble aeration or by surface aeration. In the following only bubble aeration will be described.

Using the two-film model of gas-transfer (Skelland, 1974) as a starting-point, Matter-Müller *et al.* (1981) developed a rigorous mechanistic model describing the transfer of a volatile compound to an air bubble as it rises to the surface. In their model it is assumed that the rate of mass-transfer is controlled by the liquid film resistance:

$$\frac{1}{K_{ol}} = \frac{1}{k_l} + \frac{1}{k_g H} \cong \frac{1}{k_l} \tag{8}$$

where K_{ol} is the overall mass-transfer coefficient ($m \cdot h^{-1}$), k_l is the liquid phase mass-transfer coefficient ($m \cdot h^{-1}$), k_g is the gas phase mass-transfer coefficient ($m \cdot h^{-1}$) and H is Henry's law constant, defined here as the concentration in the gas-phase over the concentration in the liquid phase at equilibrium (-).

If it is assumed that (i) the bulk liquid is completely mixed, (ii) a series of air bubbles rises through the liquid as a plug-flow and (iii) pressure and volume changes in these bubbles can be neglected, the liquid concentration of the compound in equilibrium with the gas concentration in the rising air bubble S* (in $\mu g \cdot l^{-1}$) over the bulk liquid concentration S (also in $\mu g \cdot l^{-1}$) equals:

$$\frac{S^*}{S} = 1 - e^{(-\alpha \frac{t}{\tau_g})} \quad \text{with} \quad \alpha = \frac{K_{ol} a V_l}{H Q_g} \tag{9}$$

with t the time (h), τ_g the total contact time between the air bubbles and the liquid (h), a the interfacial area per unit of volume ($m^2 \cdot m^{-3}$), V_l the liquid volume (l) and Q_g the gas flow rate ($l \cdot h^{-1}$). Figure 4 shows examples of batch stripping of three volatile compounds. This clearly demonstrates the importance of the α factor in equation (9). For high values of α (low H or long τ_g) the bubbles will become saturated with the compound whereas for low values of α (high H or short τ_g) they will not.

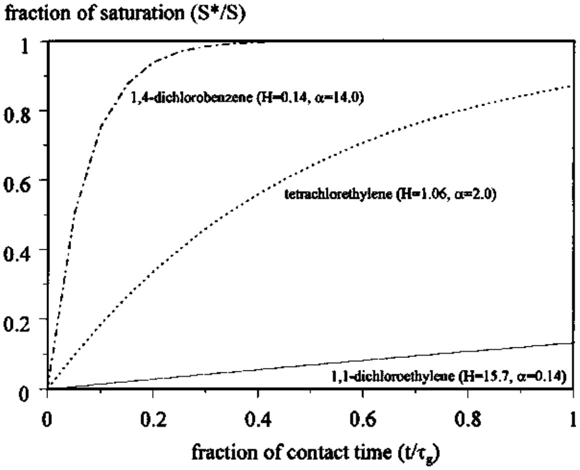

Fig. 4 Liquid concentration in equilibrium with gas phase (S*) over bulk liquid concentration (S) as function of the contact time of a rising air bubble (t/τ_g) (V_l=500 l, Q_g=4000 $l \cdot h^{-1}$, H and $K_{ol}a$ were taken from Matter-Müller *et al.*, 1981).

The volumetric rate of stripping r_{str} (in $\mu g \cdot l^{-1} \cdot h^{-1}$) can be derived from equation (9) by substituting the concentration in the gas phase S_g (in $\mu g \cdot l^{-1}$) for S^*H when the air bubble leaves the liquid phase at $t=\tau_g$:

$$r_{str} = \frac{Q_g S_g(t=\tau_g)}{V_l} = \frac{Q_g H}{V_l}(1-e^{-\alpha})S \qquad (10)$$

For high values of α (α » 1) this reduces to:

$$r_{str} = \frac{Q_g H}{V_l} S \qquad (11)$$

and for low values of α (α « 1) to:

$$r_{str} = K_{ol} a S \qquad (12)$$

K_{ol} and a often are often lumped as a combined characteristic of the trace compound and the aeration equipment.

Surface volatilisation

Apart from air stripping trace compounds can also be exchanged at the interface between the bulk liquid and the atmosphere at the surface of aeration tanks. This is called surface volatilisation or surface desorption. Although this process is often ignored, Kyosai and Rittmann (1991) have shown it to be an important process if the air bubbles quickly saturate with a compound. As was explained previously this would be the case for compounds with a low Henry's law constant and/or long air bubble contact times τ_g (Figure 4). For example, Kyosai and Rittmann (1991) reported that 17 to 60% of the total transfer rates can come from surface volatilisation in the order tetrachloroethylene (H=0.895) < carbontetrachloride (H=1.152) < trichloroethylene (H=0.419) < chloroform (H=0.159). Similar to stripping, the rate of surface volatilisation, r_{suv} (in $\mu g \cdot l^{-1} \cdot h^{-1}$) can be derived from the two-film theory:

$$r_{suv} = \frac{K_{ol} a_s}{V_l} (S - S^*) \tag{13}$$

Where a_s is the surface area of the aeration tank (m^2) and K_{ol} is the overall mass-transfer coefficient ($m^2 \cdot h^{-1}$). The ratio between a_s and V_l is equivalent to the reciprocal height of the aeration column 1/h. Furthermore, if the air above the aeration tank is continuously renewed it can be assumed that S^* equals zero. The rate of surface volatilisation then reduces to:

$$r_{suv} = \frac{K_{ol}}{h} S \tag{14}$$

Volatilisation parameters

Only the non-ionic fraction of a compound is amenable to volatilisation and a correction similar to equation (6) has to be applied. Two essentially different methods can be used to obtain the volatilisation parameters:

1. Relate the parameters to a known mass transfer rate for oxygen - The $K_{ol}a$ of a trace compound can be correlated to the $K_{ol}a$ of oxygen, which usually is a known parameter for aeration systems (e.g., Hsieh *et al.*, 1993):

$$\psi = \frac{K_{ol} a}{K_{ol} a_{O2}} = \left(\frac{D}{D_{O_2}}\right)^n = \left(\frac{v_c}{v_{cO_2}}\right)^\delta \tag{15}$$

with ψ a relational parameter, D the diffusion coefficient ($m^2 \cdot h^{-1}$) and v_c the critical molar volume

($l\cdot mol^{-1}$). The power constant n is dependent on the mixing conditions of the liquid, with a value ranging between 1 for laminar conditions and 0.5 for turbulent conditions. In systems with bubble aeration usually a value of 1.0 is applied (Matter-Müller *et al.*, 1981; Bielefeldt and Stensel, 1999). For δ several values have been reported which all are in a range of $^1/_3<\delta<^2/_3$ (Matter-Müller *et al.*, 1981; Kyosai and Rittmann, 1991).

When establishing the rate of air stripping (equation 10), it was assumed that mass-transfer is controlled by the liquid film resistance. However, this is valid only for compounds with a Henry's law constant greater than 1.2 (Hsieh *et al.*, 1993). For other compounds the gas film resistance has to be taken into account as well according to:

$$\psi_m = \psi \frac{H\frac{k_g}{k_l}}{1+H\frac{k_g}{k_l}} \tag{16}$$

where ψ_m is the modified ψ (equation 15) taking gas film resistance into account (-). Although k_g and k_l are dependent on the air flow rate, according to Hsieh *et al.* (1993) the ratio k_g/k_l in equation (16) is independent of the air flow rate with an average value of 2.9.

The $K_{ol}a_{O2}$ of an aeration system in an activated sludge plant can be measured (ASCE, 1984) or can be estimated from a mass-balance for dissolved oxygen (Roberts *et al.*, 1984):

$$K_{ol}a_{O_2} = \frac{R_O}{\theta_h \Delta O_2} \tag{17}$$

with R_O the oxygen demand of the wastewater ($mg\ O_2\cdot l^{-1}$), θ_h the hydraulic retention time (h) and ΔO_2 the difference between the actual and saturation concentration of oxygen ($mg\ O_2\cdot l^{-1}$).

The overall mass-transfer rate K_{ol} in surface volatilisation is calculated with equation (8) from the individual transfer rates in the liquid and gas phase k_l and k_g. MacKay *et al.* (1985) reported values of $k_l=0.1\ m\cdot h^{-1}$ and $k_g=10\ m\cdot h^{-1}$. Alternatively, similar to stripping, the transfer rate in the liquid phase can be related to a known surface transfer rate for oxygen.

2. Use an empirical relationship - Blackburn *et al.* (1984) used a more empirical approach. Based on observations with several trace compounds at various air flow rates, they found that the first-order stripping rate constant k_{str} was a function of Henry's law constant and of the air flow rate according to:

$$k_{str} = \phi \cdot \frac{Q_g}{V_l} \cdot H^{\kappa} \tag{18}$$

where the air flow rate Q_g is in $l\cdot h^{-1}$, the liquid volume V_l is in l and H is dimensionless. For ϕ and κ

they found values of 1.8 and 1.05, respectively. Hsieh *et al.* (1993) found a similar relationship with values for ϕ and κ of 2.9 and 1.04, respectively. Remark that strictly speaking air stripping in this approach is the sum of air stripping and surface volatilisation. Also, an application of equation (18) for systems other than the one the stripping parameters were assessed for is doubtful because ϕ and κ depend on the aeration equipment that is used.

Effect of other contaminants on volatilisation of trace compounds

The presence of contaminants can have an effect on air stripping and surface desorption. For example, oil, salts and surfactants change the rate of stripping by ± 50%, depending on the nature of the contaminant (Truong and Blackburn, 1984). Particularly interesting is the effect of surfactants as these have two opposing effects: they decrease the mass-transfer coefficient by producing a "skin" around the air bubbles but at the same time they enhance mass-transfer by decreasing the size of the air bubbles which is accompanied by a larger interfacial surface area. Lurker *et al.* (1984) found that the stripping rate of hexachlorobicycloheptadiene (Hex-BCH) increased with an increasing concentration of the detergent linear alkylbenzene sulfonate. Matter-Müller *et al.* (1981) however did not find a significant effect of this same detergent on the stripping rate of tetrachloroethylene. Differences in the experimental set-up may have caused these different observations.

Biodegradation

The mass transfer processes of sorption and volatilisation merely distribute compounds among the different environmental compartments whereas biodegradation can effectively reduce the rate at which these compounds are released into the environment. Municipal activated sludge plants exhibit a large biodegradation potential, both qualitatively and quantitatively (Grady, 1985). Because activated sludge fate models are extremely sensitive to the rate of biodegradation, they also require an accurate input of these rates. A theoretical approach using quantitative structure biodegradability relationships (QSBRs) to estimate biodegradation rates from the molecular structure (Desai *et al.*, 1989; Tabak *et al.*, 1990; Tabak *et al.*, 1992; Tabak and Govind, 1993; Okey and Stensel, 1996) is promising but far from a practical implementation (Struijs, 1995). Hence, we still have to rely on a more experimental approach, using the results of standardised biodegradation tests. These tests, and extrapolation of their results to activated sludge plant conditions will also be described in the following.

Basic concepts of biodegradation

Organic compounds can be classified as biodegradable, persistent or recalcitrant (Bull, 1980). In contrast to recalcitrant compounds which are resistant to any degree of biodegradation, persistent compounds may be inherently biodegradable but fail to undergo biodegradation given a specified set of conditions. If a compound is classified as biodegradable, different levels can be distinguished (ECETOC, 1991): (i) primary biodegradation refers to the alteration of the structure of the parent compound such that its basic physical-chemical properties are lost; (ii) in ultimate biodegradation the compound is broken down entirely to simple inorganic molecules such as CO_2, H_2O and Cl^- and to biomass and finally, (iii) the biomass that is formed may in turn degrade leading to complete mineralisation. Although from an environmental point of view ultimate biodegradation may be the most important process, it is the rate of primary biodegradation that is required in exposure assessment.

Biodegradation requires the presence of capable bacteria and the proper conditions for their growth. Growth depends on energy and electrons to synthesise new biomass and maintain existing biomass (Figure 5). Both are generated by the oxidation of an electron-donor and the reduction of an electron-acceptor. For instance, in activated sludge plants the wastewater biochemical oxygen demand (BOD) can be considered as a lumped electron-donor and the oxygen supplied by aeration as the main electron-acceptor. Because both are essential for growth they are called primary substrates. Oxidation of the electron-donor yields electrons which are transferred to the electron-acceptor to generate the internal energy carrier ATP. This energy is used for cell synthesis and maintenance.

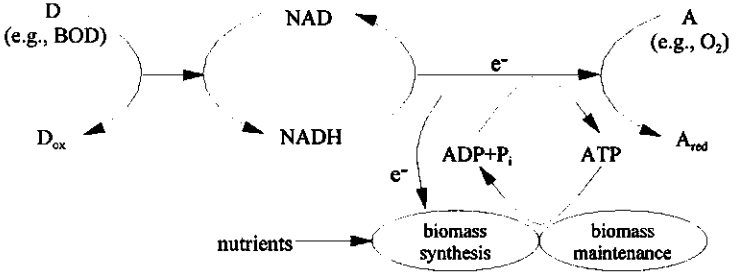

Fig. 5 Electron and energy flows in a bacterial cell (D=organic electron-donor, A= inorganic electron-acceptor) (after Rittmann, 1992).

Energy generation and bacterial growth can be reduced or even completely stopped by decoupling or primary substrate inhibition. In decoupling the generation of energy via ATP is blocked. From Figure 5 it can be deduced that this would result in a decreased biomass yield or an increased maintenance requirement. In primary substrate inhibition the inhibitor reduces the oxidation of the electron-donor or reduction of the electron-acceptor because a chemical analogue of the primary substrate binds to the reaction site of the responsible enzyme (competitive inhibition) or a site different from the reaction site making the enzyme a poor catalyst (non-competitive inhibition).

In contrast to BOD, which is abundantly present in domestic wastewater, the concentration of trace organic compounds may be too low to provide sufficient energy and electrons to support growth and maintenance. In this case their biotransformation is dependent on the presence of other (primary) substrates. This situation is referred to as secondary utilisation (Kobayashi and Rittmann, 1982), fortuitous biodegradation (Knackmuss, 1981) or gratuitous biodegradation (Slater and Bull, 1982). Remark that compounds which are degraded by secondary utilisation could be primary substrates as long as they are present in sufficient quantities. On the other hand, so called co-metabolic compounds can provide no energy at all: they can be degraded but there is an obligatory need for primary organic substrates.

All the steps in biodegradation are controlled by enzymes. Metabolic control operates to regulate enzyme formation and activity with the main purpose to conserve carbon sources and energy when the bacteria would not really benefit from having the enzyme present. These enzymes, called inducable enzymes are not synthesised unless their substrates are present. Induction seems to be an "on" and "off" phenomenon and a certain threshold substrate concentration is required before biodegradation is initiated (Boethling and Alexander, 1979; Alexander, 1985). In contrast to inducable enzymes, constitutive enzymes are always produced. Consequently, if the "natural" substrate for these enzymes is present in sufficient amounts to produce all of the necessary electrons and energy, there no longer is a need to induce the enzymes which would be required to metabolise the organic trace compound of interest, even when it is available in a sufficiently high concentration. This phenomenon is referred to as catabolic repression.

Apart from control of enzyme synthesis also enzymes activity may be controlled. For instance, if bacteria all of a sudden find themselves in an environment rich in substrate they may reduce the activity of inducable enzymes, which results in a decreased biodegradation rate. Together with the phenomenon of catabolic repression this leads to the conclusion that for a proper operation with respect to removal of organic trace compounds, carbon sources have to be continuously supplied while at the same time substrate-limited conditions should be maintained. Activated sludge system which are operated at long sludge retention times (SRTs) usually provide such conditions (Grady, 1985).

All of the above concentrated on single species in the presence of an organic trace compound and possible other organic substances. However, in activated sludge systems a mixed-culture is grown and therefore also interspecial relations have to be considered because the ultimate biodegradation of a compound may require the combined effort of a consortium of micro-organisms having different enzymes. Van Ginkel (1996) gives an example for the biodegradation of linear alkylbenzene sulfonates (LAS). The alkyl chain of LAS is biodegraded by combined ω/β-oxidation to yield sulphophenyl alkanoates (SPCs). These SPCs are subsequently degraded by other bacteria through desulphonation and ring opening. Another important phenomenon in mixed-cultures is the possibility to exchange genetic information (plasmids) between the different species. These plasmids contain the information to produce enzymes for partial or even complete degradative pathways. An example of the latter category is the TOL plasmid for toluene and xylene degradation (Kellogg *et al.*, 1981).

The characteristics of bacterial populations are largely determined by the conditions imposed upon them. Therefore, also the operating characteristics of activated sludge plants and in particular the SRT have to be considered. It was already mentioned earlier that plants operated at long SRTs offer the best opportunity for induction of those enzymes that are necessary for degradation. The SRT dictates the selection of micro-organisms as well as their physiological state. In selection some members of the population will be favoured over others. At long SRTs and corresponding low substrate concentrations "oligotrophic" organisms will dominate which have high affinity enzymes but relatively slow maximum growth rates (Dijkhuizen and Hartle, 1983; Chiu *et al.*, 1972, Battersby, 1990). On the other hand, at short SRTs "eutrophic" organisms will dominate which have low affinity enzymes and fast growth rates. This selection mechanism not only depends on the SRT but also is determined by the hydraulic characteristics. For example, Cech *et al.* (1984) and Ellis *et al.* (1996b) have shown that the growth rate and affinity of mixed-cultures grown in completely mixed reactors is considerably lower than in plug-flow reactors.

In mixed-cultures not only selection but also the physiological condition of single species is important. Several studies (Chudoba *et al.*, 1992; Ellis *et al.*, 1996b) indicate that at low substrate to biomass ratio's insufficient energy is produced for cell multiplication and the cells will store the substrate as polymers. At high substrate to biomass ratio's when cell multiplication is possible, the population is allowed to develop in an optimal way.

Finally, some attention should be directed towards adaptation or acclimation of activated sludge to trace compounds. Very often it is observed that the onset of biodegradation is preceded by a certain period of acclimation. There are several possibilities to explain this phenomenon: (1) induction of the proper enzymes, (2) mutation and genetic exchange, (3) growth of a population towards sufficient numbers to allow detection of biodegradation (Spain *et al*, 1980; Thouand *et al.*, 1996) and (4) preferential usage of other organic substances before the compound can be degraded (de-repression) (Kuiper and Hanstveit, 1984; Nyholm *et al.*, 1984).

Biodegradation Kinetics

To predict the degradation of trace compounds their rate of biodegradation should be quantified by a mathematical equation. A multitude of such equations exists. In the following a classification is used with Monod kinetics, secondary utilisation kinetics and pragmatic first- and second-order kinetics. Table 2 provides a summary of the most important kinetic models.

Monod kinetics - Initially, the Monod model was developed to describe pure cultures growing on a single, growth-limiting substrate. In many studies it was shown that the Monod model also can provide a good approximation for the degradation by mixed-cultures of single pollutants such as ammonia or of a lumped group of substrates such as BOD. An excellent example of this application of the Monod model is the Activated Sludge Model No. 1 (ASM1) developed by Henze *et al.* (1987). When applied to activated sludge processes, two important characteristics of the Monod model are that (i) a certain minimum SRT is required to obtain growth and (ii) the effluent concentration is dependent on the SRT but not on the influent concentration. These characteristics were verified in several studies. Using porous pots reactors, Birch (1991) investigated the effect of the SRT on the effluent concentration of a number of compounds. In particular for nitrilotriacetic acid (NTA) and nonylphenol ethoxylate the dependency of the effluent concentration on the SRT was fully in agreement with the Monod model.

Table 2- Overview of biodegradation kinetics used in activated sludge fate models.

model	competent biomass	special case	rate of biodegradation ($\mu g\cdot l^{-1}\cdot h^{-1}$)
Monod	grows on organic trace compound		$\frac{\mu_{max}}{Y}\frac{S}{K_S+S}B$
		$S \ll K_S$	k_2SB with $k_2=\frac{\mu_{max}}{YK_S}$
		$S \gg K_S$	k_1B with $k_1=\frac{\mu_{max}}{Y}$
secondary utilisation	grows on primary substrate or is equivalent to total biomass	competition and co-metabolism	$v_{max}\frac{S}{K_m+S}B$
		$S \ll K_m$	k_2BS with $k_2=\frac{v_{max}}{K_m}$
		$S \gg K_m$	k_1B with $k_1=v_{max}$
		competitive inhibition	$v_{max}\frac{S}{K_m+(1+\frac{S_1}{K_{i1}})}B$
pragmatic	equivalent to total active biomass	first-order	k_1S
		second-order	k_2SB
		first-order dissolved and sorbed	$k_1(1+K_pX)S$

B	concentration of specific (competent) biomass ($\mu g\cdot l^{-1}$)
k_1	first-order biodegradation rate constant (h^{-1})
k_2	second-order biodegradation rate constant ($l\cdot g^{-1}VSS\cdot h^{-1}$)
K_{i1}	inhibition constant for repression by primary substrate ($\mu g\cdot l^{-1}$)
K_m	half-saturation constant in Michaelis-Menten kinetics ($\mu g\cdot l^{-1}$)
K_p	sludge-water partition coefficient ($l\cdot g^{-1}$ VSS)
K_S	half-saturation constant in Monod kinetics ($\mu g\cdot l^{-1}$)
S	concentration of organic trace compound ($\mu g\cdot l^{-1}$)
S_1	concentration of primary substrate ($\mu g\cdot l^{-1}$)
v_{max}	maximum specific rate (h^{-1})
X	concentration of total biomass (g $VSS\cdot l^{-1}$)
Y	biomass yield (g $VSS\cdot g^{-1}$)
μ_{max}	maximum specific growth rate (h^{-1})

Cech and Chudoba (1988) measured the maximum volumetric removal rate of morpholine, sulphonic acid, NTA and ethylenediaminetetraacetic acid (EDTA) in semi-continuously operated activated sludge systems at different SRTs and with peptone as the main carbon source. Also their results confirmed the applicability of the Monod model. Similarly, the Monod model could describe the results obtained by Chudoba *et al.* (1989) who measured the maximum volumetric removal rate of a mixture of 2,4-dichlorophenol and methanol in a continuously operated system. Simkins and Alexander (1984) applied the Monod model to describe the batch disappearance of benzoate at different initial concentrations and population densities. They concluded that in all cases the Monod model or one of its simplifications accurately fitted the measurements. In the Monod model net bacterial growth is given by:

$$r_B = (\mu - k_d)B \qquad (19)$$

where r_B is the net bacterial growth rate ($\mu g \cdot l^{-1} \cdot h^{-1}$), k_d is the biomass decay rate constant (h^{-1}), B is the concentration of biomass that is responsible for degradation of the target compound ($\mu g \cdot l^{-1}$) and μ is the specific growth rate (h^{-1}).

The rate of compound removal is coupled to growth according to:

$$r_{bio} = \frac{1}{Y}\mu B \qquad (20)$$

where r_{bio} is the biodegradation rate ($\mu g \cdot l^{-1} \cdot h^{-1}$) and Y is the biomass yield ($\mu g \cdot \mu g^{-1}$).

The specific growth rate μ is described by a saturation function with respect to the trace compound concentration S (in $\mu g \cdot l^{-1}$):

$$\mu = \mu_{max} \frac{S}{K_S + S} \qquad (21)$$

where μ_{max} is the maximum specific growth rate (h^{-1}) and K_s is a half-saturation constant ($\mu g \cdot l^{-1}$).

The biodegradation rate r_{bio} now can be rewritten as:

$$r_{bio} = \frac{\mu_{max}}{Y} \frac{S}{K_S + S} B \qquad (22)$$

Equation (22) can be simplified in several ways (Simkins and Alexander, 1984). In particular a second-order rate at low concentrations ($S \ll K_s$) and a first-order rate at high concentrations ($S \gg K_s$) can be useful (Table 2). If the Monod model is applied to an activated sludge plant with a completely mixed aeration tank, biomass recycle and removal of excess biomass, steady-state values for B and S can easily be obtained from their respective mass balances (Metcalf and Eddy, 1991):

$$S = \frac{K_S(1 + k_d\theta_c)}{\theta_c(\mu_{max} - k_d) - 1} \tag{23}$$

$$B = \frac{\theta_c Y(S_i - S)}{\theta_h(1 + k_d\theta_c)} \tag{24}$$

where θ_c is the SRT as controlled by the flow rate of excess biomass (h), θ_h is the hydraulic retention time (h) and S_i is the influent concentration of the trace compound ($\mu g \cdot l^{-1}$).

The effect of the SRT on the biomass concentration B and effluent concentration S is illustrated in Figure 6, which elucidates three important characteristics of the Monod model: (i) the effluent concentration S is independent of the influent concentration and can be predicted from the operational parameters θ_c and θ_h and the biodegradation parameters μ_{max}, K_s and k_d, (ii) there is a minimum SRT θ_{cmin} below which the competent micro-organisms are wasted from the system and biodegradation will cease:

$$\theta_{c\,min} = \frac{1}{\mu_{max}\frac{S_i}{K_S + S_i} - k_d} \tag{25}$$

and (iii) it is impossible to achieve effluent concentrations below a concentration S_{min}:

$$S_{min} = \frac{k_d K_S}{\mu_{max} - k_d} \tag{26}$$

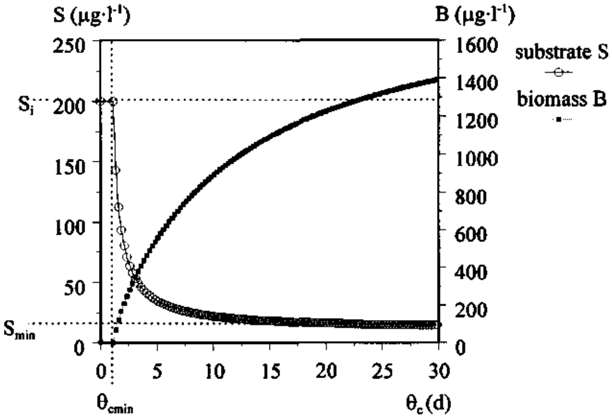

Fig. 6 Effluent trace compound concentration (S) and concentration of competent biomass (B) as a function of the SRT (θ_c), predicted by the Monod model for a completely mixed bioreactor with biomass recycle.

Although the Monod model often is employed by activated sludge fate models, it is subject to several restrictions:

- When secondary utilisation or co-metabolism dominates, growth on the compound of interest is negligible or even absent and the Monod model has to be replaced by another model.
- Sometimes a compound is degraded in several steps by a consortium of micro-organisms. In this case lumped Monod parameters can be used, but only if primary biodegradation is the rate-limiting step.
- The kinetic parameters may be affected by the operational characteristics, in particular by the SRT which not only determines the selected micro-organisms but also their physiological state.
- The Monod model allows a prediction of steady-state effluent concentrations regardless of the occurrence of competing processes such as volatilisation or sorption. However, these mass-transfer processes will take away part of influent load which otherwise would have been available for growth. As a result, the minimum SRT θ_{cmin} required for growth may exceed the θ_{cmin} predicted by equation (25) (Watkin and Eckenfelder, 1985).

Secondary utilisation kinetics - Nyholm and Ingerslev (1996) suggested to use an influent trace compound concentration of 100 $\mu g \cdot l^{-1}$ as the lower limit for the applicability of the Monod model. At lower concentrations organic compounds do not provide sufficient energy to support growth and maintenance and degradation become dependent on the presence of another (primary) substrate. This situation is referred to as secondary utilisation (Rittmann, 1992).

Suppose that a trace compound (S) is biodegraded by secondary utilisation because another suitable (primary) substrate (S_1) is available at sufficiently high concentrations. The three different possibilities of Table 3 now can be distinguished. In substrate competition the responsible biomass can grow on either substrate. However, assuming that S_1 is present at much higher concentrations than the trace compound S, growth can be considered entirely on S_1. Co-metabolism is a sub-set of secondary utilisation in which S, irrespective of its concentration, can provide no energy at all but can still be biodegraded in the obligatory presence of S_1. Finally, in competitive inhibition the competent biomass has a preferential growth on S_1 and therefore high concentrations of S_1 will inhibit the biodegradation of S.

Table 3 - Possibilities in secondary utilisation (S=trace compound and S_1=primary substrate)

situation	micro-organism grows on	preference for
Substrate competition	S_1 and S	neither
Co-metabolism	S_1	S_1
Competitive inhibition	S_1 and S	S_1

The first two cases of substrate competition and co-metabolism can be expected to follow some form of saturation kinetics for which the Michaelis-Menten equation can be used (Schmidt *et al.*, 1985):

$$r_{bio} = v_{max} \frac{S}{K_m + S} B \tag{27}$$

where v_{max} is the maximum specific reaction rate (h^{-1}) and K_m is a half-saturation constant ($\mu g \cdot l^{-1}$).
At extremely low and extremely high substrate concentrations this equation can be simplified to yield second- and first-order kinetics, respectively (Table 2). In the third case of competitive inhibition an inhibition term for the presence of primary substrate S_1 is required (Broholm, 1992; Jacobsen *et al.*, 1996):

$$r_{bio} = v_{max} \frac{S}{K_m + (1 + \frac{S_1}{K_{i1}})S} B \tag{28}$$

where K_{i1} is an inhibition constant for inhibition by the primary substrate ($\mu g \cdot l^{-1}$).
The main difference with Monod kinetics (equation 22) is that in secondary utilisation kinetics the biomass concentration B is determined by the supply of a primary substrates S_1 rather than by the organic trace compound itself (S), although the Monod model still can be used to describe growth of B on the primary substrate. Jacobsen and Arvin (1996) give an example for biodegradation of pentachlorophenol. Remark that for this approach the concentration of primary substrate should be known. Because this usually is not the case, it often is assumed that the primary substrate is equivalent to the abundant BOD supplied with the wastewater and that the biomass concentration B can be approximated by the total biomass concentration in the reactor (Namkung and Rittmann, 1987).
The most important difference with the Monod model is that according to secondary utilisation the effluent concentration of a trace compound is determined by its influent concentration. Prediction of this effluent concentration is difficult because similar to the Monod parameters all the parameters may be influenced by the environmental conditions, the presence of inhibitors and the operational characteristics of the activated sludge plant.

Pragmatic first- and second-order kinetics - To avoid separate determination of the competent biomass concentration B and the maximum rate v_{max}, many workers have adopted simple pragmatic first-order kinetics to describe the biodegradation rate r_{bio} (e.g., Larson and Payne, 1981; Games *et al.*, 1982; Larson, 1983; Shimp and Larson, 1996; Federle and Itrich, 1997):

$$r_{bio} = k_1 S \tag{29}$$

where k_1 is the first-order rate constant (h^{-1}).

Struijs *et al.* (1991) use first-order kinetics in their SimpleTreat model to predict effluent trace compound concentrations from activated sludge plants. However, a disadvantages of equation (29) is that the rate of biodegradation is independent of the biomass concentration. To compensate for this, Mikkelsen *et al.* (1996) and others proposed a second-order rate including the total biomass concentration:

$$r_{bio} = k_2SX \tag{30}$$

where k_2 is a second-order rate constant ($l \cdot g^{-1} \cdot h^{-1}$) and X represents the total (non-specific) biomass concentration ($g \cdot l^{-1}$).

Remark that the kinetics in equation (30) are equivalent to secondary utilisation kinetics at low substrate concentrations (Table 2) if it were assumed that the competent biomass B utilises the bulk BOD as a carbon- and energy source.

Cowan *et al.* (1993) observed that elimination of surfactants cannot be explained by biodegradation of the dissolved surfactants alone but biodegradation of the sorbed fraction also had to be taken into account. Therefore, they included a separate first-order biodegradation rate for the sorbed fraction and assumed this rate to be equal to the biodegradation rate of dissolved surfactant:

$$r_{bio} = k_1(1 + K_pX)S \tag{31}$$

Remark that this phenomenon is not necessarily limited to pragmatic kinetics but also can be incorporated into the Monod model or secondary utilisation models by replacing the dissolved concentration S with the total concentration $C=(1+K_pX)S$. Also remark that both equation (29) and equation (31) always predicts a certain rate of biodegradation, even in the absence of biomass.

The Monod model and the secondary utilisation models include the effect of the SRT and the BOD loading rate on the rate of biodegradation via the concentration of competent biomass. This is not the case for pragmatic kinetics models. One of the consequences is that pragmatic biodegradation parameters are directly related to a specific activated sludge plant, given the influent trace compound concentration, BOD loading rate, SRT, etc.

Biodegradation tests

The predictions generated by activated sludge fate models not only are determined by the selected biodegradation kinetics, but also by the biodegradation parameters. In many cases these parameters are assessed in standardised laboratory tests.

Extrapolation from test results to activated sludge plant conditions

When the results of laboratory tests are extrapolated to activated sludge plants, two levels can be distinguished (Figure 7). On the first level sludge is sampled from the plant of interest and the laboratory test results are used to predict the rate of biodegradation in this particular plant. Useful results are most likely if the test conditions closely mimic those in the activated sludge plant of mineralised.

Table 4 - OECD biodegradability tests (OECD, 1993).

test type	name	variable
ready		
301A	DOC die-away	DOC
301B	CO_2-evolution	CO_2
301C	MITI (I)	O_2
301D	closed bottle	O_2
301E	modified screening	DOC
301F	manometric respirometry	O_2
inherent		
302B	Zahn-Wellens/EMPA	DOC
302C	MITI (II)	O_2
simulation		
303A	coupled units	DOC

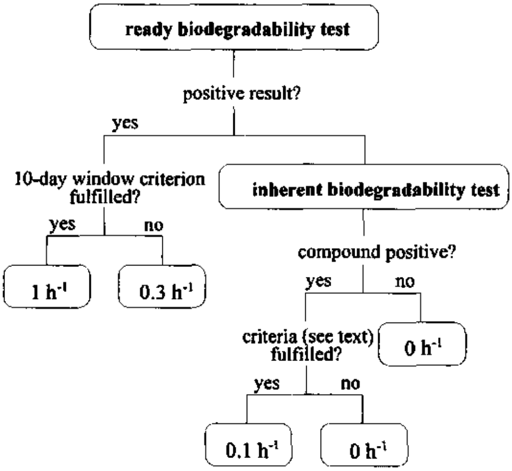

Fig. 8 Extrapolation from biodegradability tests to first-order rate constant in activated sludge plants (EC, 1994).

Ready tests are designed as a fail/pass test to determine the potential of a compound to undergo biodegradation rather than to generate kinetic information. However, if sufficient data points are available, for instance O_2-consumption data from an automated electrolytic respirometer, in combination with a non-linear regression technique some kinetic information could be derived (Blok, 1994; Hales, 1996 and Painter, 1996).

A more simple approach is followed in the Technical Guidance Document (TGUD) on risk assessment (EC, 1994). Depending on the 10-day window criterion, the TGUD advises to extrapolate the results of ready tests to a (pragmatic) first-order biodegradation rate in an activated sludge plant of 1 or 0.3 h^{-1} (Figure 8). These values are restricted to the water dissolved fraction of compounds and in some cases higher biodegradation rate constants are justified if they can be confirmed by experimental data. These rate constants apply to activated sludge plants with a total biomass concentration of 3 g suspended solids (SS)$\cdot l^{-1}$. Mikkelsen *et al.* (1996) extended this to accommodate variable sludge concentrations by using the second-order rate equation (33) with a rate constant k_2 of 0.3 $l\cdot g^{-1}$ SS$\cdot h^{-1}$ or 0.1 $l\cdot g^{-1}$ SS$\cdot h^{-1}$.

Blok and Struijs (1996) detected a general trend in Monod parameters which were estimated from O_2-consumption data of 52 different compounds. They concluded that most compounds which pass a ready test have a maximum specific growth rate μ_{max} greater than 1.5 d^{-1} and a half-saturation constant K_s lower than 1 mg ThOD$\cdot l^{-1}$. This observation suggests that these values can be used to predict "worst-case" scenario's for ready biodegradable compounds.

Although due to their stringent character ready tests give useful results for ranking and labelling of organic compounds, the kinetic parameters which are obtained must be treated with extreme caution because (i) as was mentioned earlier, the high substrate to biomass ratio will yield intrinsic rather than extant parameters, (ii) the absence of substrates other than the compound of interest may prevent the occurrence of secondary utilisation and (iii) the rate of mineralisation is measured instead of the rate of primary biodegradation required for exposure assessment.

Inherent biodegradability tests

Failure in ready tests may be due to recalcitrance of the organic compound or to the severe conditions imposed during testing. Inherent tests coded 302B and 302C in the OECD guidelines (OECD, 1993) are designed to satisfy all the criteria to obtain biodegradation and making it possible to assume that any failure to observe biodegradation is due to recalcitrance.

Inherent biodegradability tests 302B and 302C are similar to ready biodegradability tests but apply higher biomass concentrations. Furthermore, the biomass is allowed to be pre-exposed to the target compound. Tests 302B is a so-called Zahn-Wellens test with a high sludge concentration of 1-3 g $SS \cdot l^{-1}$ and with the target compound added as the only (external) carbon source at a concentration of 300 mg $DOC \cdot l^{-1}$. Starvation of the sludge is an additional source of carbon. Biodegradation is followed by DOC, COD or compound specific analysis. Test 302C (MITI II) uses an activated sludge concentration of 100 mg $SS \cdot l^{-1}$ and the compound is added at a concentration of 30 $mg \cdot l^{-1}$. Biodegradation is followed by oxygen consumption. In both tests a compound is considered to be inherently biodegradable if more than 70% biodegradation is achieved within a time window of 28 days, regardless of the duration of the test. The latter means that a long adaptation time before the onset of biodegradation is allowed.

The TGUD (EC, 1994) advises to extrapolate the results of inherent biodegradability tests to a first-order biodegradation rate constant of 0.1 h^{-1} if the lag-phase is no longer than 3 days and, in case of the Zahn-Wellens test the pass level has been reached within 7 days and for the MITI (II) within 14 days (Figure 8).

Although frequent analysis of the trace compound may give some kinetic information, in general the test conditions may be too favourable as compared to activated sludge conditions and may lead to an overestimation of the biodegradation rate.

Activated sludge simulation tests

In contrast to ready and inherent tests, simulation tests attempt to mimic activated sludge plants and for this reason could generate more reliable kinetic data. The OECD provides in one activated sludge simulation test, the coupled units test encoded 303A. Unfortunately, this particular test does not generate kinetic data which explains why many alternative simulation tests have been proposed in the literature. These alternatives can be classified by their mode of operation (Figure 9) with short-term batch tests, long-term continuous tests, semi-continuous tests or a combination of these.

Batch activated sludge (BAS) tests - In BAS tests sludge is collected from a laboratory or full-scale plant and is spiked with the test compound. Usually, the total biomass concentration is not changed from the original concentration. An advantage of laboratory parent plants as compared to full-scale plants is that they allow a controlled acclimation of the sludge to the target compound. Biodegradation in the BAS tests can be followed over time by O_2-consumption, CO_2-evolution,

Table 5 - Overview of activated sludge fate models developed over the last 15-20 years.

		processes included						
reference	dynamic model	biodegradation	stripping	surface volatilisation	sorption	biodegradation kinetics	volatilisation rate constant	sludge-water partition coefficient
Blackburn *et al.* (1984)	yes	yes	yes	no	yes	pragmatic first-order	empirical	empirical
Watkin and Eckenfelder (1984)	no	yes	yes	no	no	Monod	from $K_{ol}aO_2$	not relevant
Weber *et al.* (1987)	yes	yes	yes	no	yes	pragmatic first-order	empirical	measured
Barton (1987)	yes	yes	yes	yes	yes	pragmatic second-order	from $K_{ol}aO_2$	empirical
Namkung and Rittman (1987)	yes	yes	yes	no	yes	pragmatic second-order	empirical	empirical
Blackburn *et al.* (1987)	no	yes	yes	no	yes	pragmatic first-order	empirical	empirical
Siegrist *et al.* (1989)	yes	yes	no	no	no	Monod	not relevant	not relevant
Govind *et al.* (1991)(1)	no	yes	yes	yes	yes	pragmatic second-order	from $K_{ol}aO_2$	empirical
Birch (1991)	no	yes	no	no	no	Monod	not relevant	not relevant
Struijs *et al.* (1991)(2)	no	yes	yes	yes	yes	pragmatic first-order	from $K_{ol}aO_2$	empirical
Cowan *et al.* (1993)(3)	no	yes	no	no	yes	pragmatic first-order	not relevant	measured
Melcer *et al.* (1993)(4)	yes	yes	yes	yes	yes	pragmatic second-order	from $K_{ol}aO_2$	empirical
O'Brien and Teather (1995)	yes	yes	yes	no	no	pragmatic second-order	unknown	not relevant
Struijs (1996)(5)	no	yes	yes	yes	yes	pragmatic first-order/Monod	from $K_{ol}aO_2$	empirical
Jabobsen and Arvin (1996)	yes	yes	no	no	yes	secondary utilisation	not relevant	empirical
Jacobsen *et al.* (1996)	yes	yes	yes	yes	yes	pragmatic second-order	unknown	unknown
van Wijk *et al.* (1996)	yes	yes	no	no	yes	Monod	not relevant	measured
Siegrist (1996)	no	yes	yes	no	yes	pragmatic second-order	empirical	measured

(1) General Fate Model

(2) SimpleTreat version 1.0

(3) WWTreat

(4) Toxchem

(5) SimpleTreat version 3.0

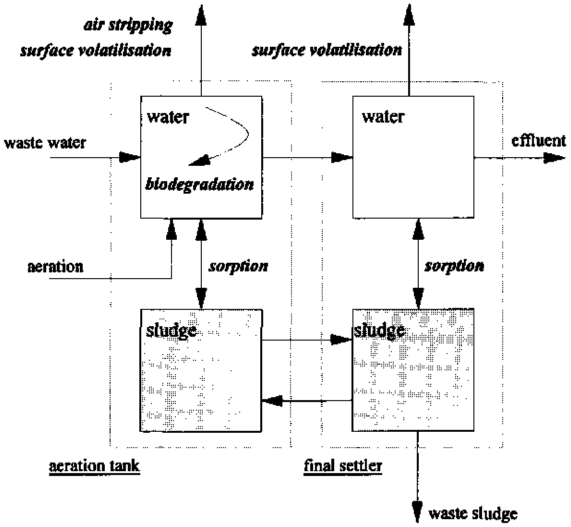

Fig. 10 Simple lay-out of a model activated sludge plant.

Although contemporary activated sludge plants include non-aerated compartments to accommodate nutrient removal, the models in Table 5 without exception are restricted to aerobic conditions. Recently, Boeije *et al.* (1998) proposed an extension of the SimpleTreat model developed by Struijs (1996) to include non-aerated compartments. Obviously, this should be accompanied by additional experiments to assess the biodegradation kinetics under non-aerated conditions.

It is a common simplification to assume that the occurrence of the relevant processes (biodegradation, surface volatilisation, air stripping and sorption) is limited to the bioreactor. In addition, the TOXCHEM model (Melcer *et al.*, 1993) and the SimpleTreat models (Struijs *et al.*, 1991 and Struijs, 1996) also consider the possibility of surface volatilisation and sorption in the secondary settler.

All the models are build from mass balances around the different compartments. For instance, a mass balance around the water compartment of a completely mixed bioreactor would be:

$$V\frac{dS}{dt} = Q(S_i - S) - V(r_{sor} + r_{str} + r_{bio}) \tag{32}$$

where S and S_i are the dissolved concentrations in the influent and effluent of the bioreactor respectively ($\mu g \cdot l^{-1}$), V is the volume of the reactor (l), Q is the influent flow rate ($l \cdot h^{-1}$) and r_{sor}, r_{str} and r_{bio} are the volumetric rates of sorption, stripping and biodegradation ($\mu g \cdot l^{-1} \cdot h^{-1}$), respectively.

Mathematical expressions for these rates can be found in previous paragraphs. In some models the effect of discontinuous discharges can be simulated by numerically solving the differential (mass balance) equations. Most of the models however only consider steady-state conditions, i.e., they assume a constant supply of the target compound with the wastewater. The accumulation term

(V·dS/dt) is then set to zero and the concentration S can be computed directly from the mass balance or can be found by iteration.

The large variety in biodegradation kinetics described earlier can also be found among the models of Table 5. Monod and pragmatic first- and second-order are the most popular kinetic models. Although many models claim to use secondary utilisation kinetics, they often ignore that in true secondary utilisation the competent biomass utilises another, growth-limiting primary substrate. For that reason in Table 5 most of these models were allotted pragmatic first- or second-order kinetics instead. Only Jacobsen and Arvin (1996) incorporated true secondary utilisation kinetics.

The mathematical equations that are used to describe air stripping for bubble aeration systems and surface volatilisation are similar in all the models. However, the rate constants are calculated according to (i) a relationship with a known oxygen mass transfer coefficient $K_{ol}a_{O2}$ (equation 15) or, (ii) an empirical relationship with Henry's law constant and the air flow rate (equation 18). The latter alternative is highly aeration equipment specific and may result in erroneous model predictions when the same parameters are applied to different plants.

Apart from the SimpleTreat models, which take the kinetics of sorption into account, all the other models assume that sorption of trace compounds to activated sludge is an instantaneous process that can be described by linear sludge-water partitioning. The sludge-water partition coefficient K_p is assessed in sorption experiments or is calculated from an empirical relationship with known compound (K_{ow}) and sludge (f_{oc}) properties.

Monitoring studies and model validation

Before being put into practice, every mathematical model needs to be validated against a set of experimental data. Obviously, this set of data is extremely important because it determines the quality of the validation exercise and the confidence in the generated model output. Table 6 presents an overview of experimental data which could be used to validate activated sludge fate models.

The plants the monitoring data were collected from varied in size from laboratory-scale to full-scale. A disadvantage associated with plants on laboratory or pilot-scale is that they in itself are models of full-scale plants. On the other hand, they offer better possibilities for experimentation. For example, influent concentrations of the target compounds, air flow rate, SRT and HRT can all be manipulated without having to worry about the primary function of the plant, i.e., removal of bulk COD, nitrogen and phosphorus. Most of the monitoring studies were carried out in plants treating domestic wastewater. Only a few plants were fed with industrial or synthetic wastewater.

Jacobsen (1993) distinguishes three different levels of monitoring studies, depending on the types of measurements included (Figure 11). On the first level only influent and effluent concentrations are available. Although this allows for an evaluation of the treatment efficiency, no distinction can be made between removal by biodegradation, sorption and volatilisation. In addition to influent and effluent concentrations, monitoring studies on the second level include in-plant measurements such as off-gas and waste sludge concentrations. From these studies mass balances can be established and they provide an indication of the dominant removal mechanism.

Table 6 - Monitoring studies and validation efforts.

reference	monitoring level(8)	scale	type of wastewater	# SRTs	chemical addition	feed pattern	compounds
Blackburn *et al.* (1984)	3	lab	industrial	2	spiked	constant	phenol, toluene , aniline, pentachlorophenol
Weber *et al.* (1987)	3	lab	synthetic	1	spiked	constant	7 volatile compounds
Barton (1987)	1	full	industrial	1	unknown	unknown	phenol, chloroform, tetrachloroguaiacol, hexachlorobenzene
Namkung and Rittman (1987)	3	full	domestic	2	wastewater	natural	31 volatile compounds
Siegrist *et al.* (1989)	3	full	domestic	1	wastewater	natural	NTA
Govind *et al.* (1991)(1)	2	pilot	domestic	1	spiked	constant	28 RCRA(6) and 19 CERCLA(7) compounds
Birch (1991)	1	lab	domestic	several	spiked	constant	4-nitrophenol, LAS, alcohol ethoxylate, NTA, nonylphenol ethoxylate
Struijs *et al.* (1991)(2)	3	full	domestic	1	wastewater	averaged	several volatile compounds (data from Namkung and Rittmann, 1987)
Cowan *et al.* (1993)(3)	*1*	*full*	domestic	several	wastewater	averaged	NTA and *the surfactant LAS,* C_{12}*TMAC and DTDMAC*
Melcer *et al.* (1993)(4)	2	full	domestic	1	wastewater	averaged	5 volatile compounds
	2	full	industrial	1	wastewater	averaged	5 volatile compounds
	2	full	domestic	1	wastewater	natural	5 volatile compounds
data Parker *et al.* (1992)	3	pilot	domestic	1	spiked	pulse	3 chlorinated phenols
data Monteith *et al.* (1995)	2	pilot	domestic	2	spiked	pulse	5 pesticides
O'Brien and Teather (1995)	3	pilot	industrial	1	wastewater	natural	1,2-dichloroethane and chloroform
Jabobsen and Arvin (1996)	unknown	lab	domestic	*several*	spiked	constant	*pentachlorophenol*
Jacobsen *et al.* (1996)	3	full	domestic	1	wastewater	natural	5 volatile compounds (data from Namkung and Rittmann, 1987)
	2	pilot	domestic	1	spiked	constant	pesticides, phenols, phthalates and PAHs (data from Petrasek *et al.*, 1983)
	1	pilot	domestic	1	spiked	constant	pentachlorophenol (data from Melcer and Bedford, 1988)
Wijk *et al.* (1996)	1	full	domestic	several	wastewater	natural	LAS

(1) GFM (General Fate Model)

(2) TOXCHEM model

(3) WWTREAT

(4) SimpleTreat version 1.0

(5) SimpleTreat version 3.0

(6) Resource Conservation and Recovery Act

(7) Comprehensive Environmental Response, Compensation and Liability Act

(8) See the text and Figure 11 for an explanation

Finally, on the third level measurements are carried out under specific conditions and can be used to quantify the relevant processes. For example, if the occurrence of biodegradation and volatilisation in a batch experiment somehow can be excluded, measurements at varying initial bulk concentrations could be used to estimate a sludge-water partition coefficient or to assess the rate of sorption.

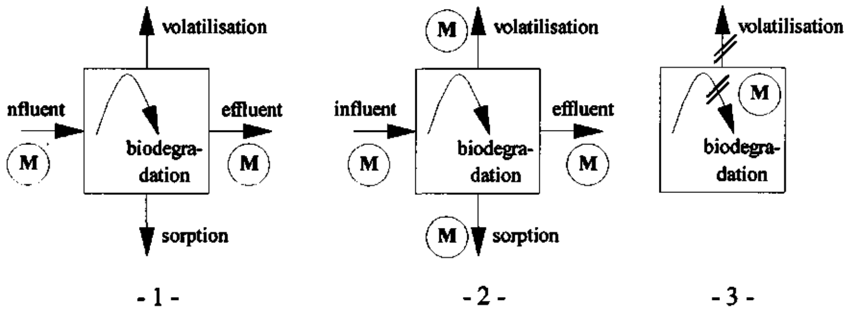

Fig. 11 Levels of monitoring studies (M=monitoring data are available).

Activated sludge fate models are extremely sensitive to a trace compound's physical-chemical and biodegradation properties. Hence, the quality of a validation exercise is largely determined by the number of test compounds that is used. However, at the same time this variation is restricted by available time, money and analytical techniques. For example, assume that the properties biodegradability, sorption capacity (or hydrophobicity represented by the compound's K_{ow}) and volatility (or the compound's Henry-coefficient) are the factors determining the fate of a compound in an activated sludge plant. Furthermore, if we assume that these properties can take one out of two values, low or high, these simplifications already result in a validation matrix of 8 test compounds (Table 7). Remark that combinations 4 and 8 in Table 7 are merely hypothetical because consultation of several lists of priority trace compounds did not result in compounds combining an extremely high Henry's law constant with an extremely high octanol-water partition coefficient.

The quality of the data-sets in Table 6 now can be reviewed with respect to their variety in compound properties. Most of the monitoring studies include one compound only, or a few at most and therefore have a limited validation value. When, similar to Blackburn *et al.* (1984), one compound is selected for each of the combinations in Table 7, this makes a qualitative validation possible. However, extrapolation to other compounds, for instance those which exhibit intermediate biodegradability, is doubtful. For the combination of volatilisation and biodegradation excellent data-sets, at least with respect to compound properties, have been produced by Weber *et al.* (1987), Namkung and Rittmann (1987), Govind *et al.* (1991) and Melcer *et al.* (1993). The combination of biodegradation and sorption may be validated using data provided by Govind *et al.* (1991), Parker and Melcer (1992) and Monteith *et al.* (1995).

Table 7- Reduced validation matrix for compound properties.

combination	compound properties biodegradability	sorption capacity (K_{ow})	volatility (H)	example compound
1	low	low	low	ethylenediaminetetraacetic acid (EDTA)
2	low	low	high	chloroethane
3	low	high	low	fluoranthene
4	low	high	high	-
5	high	low	low	nitrilotriacetic acid (NTA)
6	high	low	high	toluene
7	high	high	low	linear alkylbenzene sulfonate (LAS)
8	high	high	high	-

As was mentioned earlier, the (average) sludge retention time (SRT) can have a large effect on the biodegradation parameters. Consequently, the quality of a validation procedure would improve significantly if it includes a wide range of SRTs. Nevertheless, most of the monitoring studies in Table 6 were restricted to one or two SRTs only. Only data sets provided by Birch (1991), Cowan *et al.* (1993), Jacobsen and Arvin and van Wijk *et al.* (1996) consisted of more than two SRTs. Birch (1991) measured effluent concentrations from porous-pot reactors operated at several SRTs. He concluded that the results can best be described by a Monod model. Cowan *et al.* (1993) validated their WWtreat model against field-monitoring data for nitrilotriacetic acid (NTA) and three surfactants in full-scale activated sludge plants operated at different SRTs. Even though the model predictions were in good agreement with the observations, the validation procedure was incomplete because some of the plant's operational parameters were missing. Jacobsen and Arvin (1996) followed pentachlorophenol (PCP) removal in three reactors operated at different SRTs. They measured the concentration of the competent biomass and the concentration of PCP. They concluded that the biomass concentration was overestimated due to losses with the effluent and that PCP degradation rates were 3-4 times higher than predicted by their model. Van Wijk *et al.* (1996) calibrated a Monod model for linear alkylbenzene sulfonate (LAS) using data from a monitoring study in one particular full-scale activated sludge plant. They used the calibrated model to predict LAS effluent data of other activated sludge plants. In general, the model predictions were in good agreement with the field data.

Most activated sludge fate models assume steady-state conditions and should be validated against monitoring data which are obtained in a pilot-scale activated sludge plant that treat a constant load of the target compound. Alternatively, data from full-scale plants can be used, but this requires composite sampling and statistical techniques to obtain average concentrations representing the (quasi) steady-state of the plant. Dynamic models have to be validated against data from plants which are exposed to pulse loads or by following the natural variation in influent and effluent concentrations. For example, Melcer *et al.* (1991) investigated the effect of 12-hour pulse perturbations on the behaviour of several volatile organic compounds. They observed that the

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Chapter 3

A new method to study the biodegradation kinetics of organic trace pollutants by activated sludge

Abstract - A reliable prediction of the behaviour of organic trace compounds in activated sludge plants requires an accurate input of the biodegradation kinetics. Often these kinetics are extrapolated from the results of standardised biodegradation tests. However, these tests generally are not designed to yield kinetic information and do not reflect the conditions in activated sludge plants. To overcome these problems a new test method was developed which is referred to as a "by-pass" test. The test methodology is explained and examples are given for three compounds: the C_{12}-homologue of linear alkylbenzene sulfonate, nitrilotriacetic acid and toluene. More experience with the test is required, particularly with respect to selection of the proper test settings, which are compound related. The test seems to be a suitable tool in a research environment, for example to investigate the effect of plant operational parameters on the biodegradation kinetics.

Introduction

Mathematical models which predict the fate of organic trace pollutants in municipal activated sludge plants could be used to support an environmental risk assessment of these compounds. Examples of such models are given by Namkung and Rittmann (1987), Cowan *et al.* (1993) and Struijs *et al.* (1996). Because these models are extremely sensitive to the rate of biodegradation and they require an accurate input of the biodegradation kinetics. The Technical Guidance Document (TGUD) in support of risk assessment (EC, 1994) advises to use the results of standardised biodegradability tests for this purpose, for instance the tests prescribed by the Organisation for Economic Co-operation and Development (OECD, 1993). However, these tests were not designed to yield kinetic information but rather to classify compounds for their amenability to biodegradation.

It is evident that biodegradation tests most likely will provide useful kinetic data if the test conditions closely mimic the conditions in the activated sludge plant of interest. In most available biodegradation tests this is not the case and many differences can be pointed out between test and plant conditions (Figure 1, first extrapolation level).

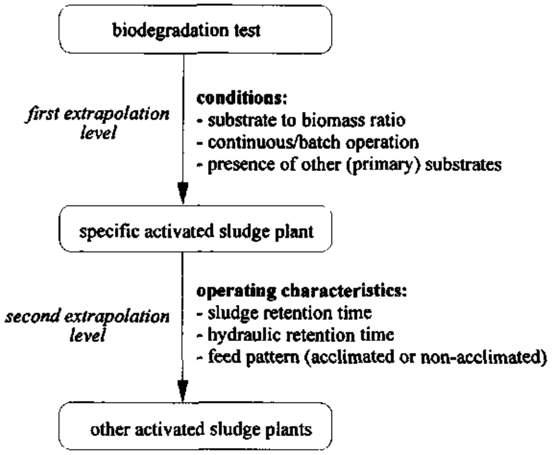

Fig. 1 Extrapolation of biodegradation test results to activated sludge plants conditions.

One of those differences is that biodegradation tests are often carried out in batch mode with a steep substrate gradient in time whereas activated sludge plants usually employ well-mixed bioreactors. This sudden change in growth conditions may have an enormous impact on the metabolic control actions by the biomass and herewith on the biodegradation kinetics (Kovárová-Kovar and Egli, 1998). Besides, the changing substrate concentration in batch tests prevents the biomass to reach a true steady-state with respect to growth (Philbrook and Grady, 1985).

Biodegradation of trace pollutants by activated sludge often is determined by a non-specific analytical technique such as COD removal, CO_2 evolution or respirometry. To allow a detectable response, an initial trace compound to biomass ratio has to be employed which is much higher than in activated sludge plants. This not only can change the members of the microbial population during the test period but also their physiological state (Chudoba *et al.*, 1992; Grady *et al.*, 1996; Kovárová-Kovar and Egli, 1998). As a result, the observed biodegradation kinetics will deviate from the original kinetics at the time of sampling. Another reason to avoid high substrate to biomass ratio's is that this may inhibit biodegradation.

Finally, in activated sludge plants biodegradation of trace pollutants can be repressed by the presence of other, more easily biodegradable compounds. This does not occur in standardised biodegradation tests where mostly the target compound is the only source of carbon and energy. On the other hand, there is also some experimental evidence for the opposite, that is, the presence of supplemental carbon sources may stimulate growth of competent biomass and induction of the proper enzymes (Kovárová-Kovar and Egli, 1998).

In chapters 4 to 7 the reliability of activated sludge fate models will be investigated using three different trace pollutants: the C_{12}-homologue of linear alkylbenzene sulfonate (LAS-C_{12}), nitrilotriacetic acid (NTA) and toluene. Based on the results of the literature review of chapter 2 it was suspected that the sludge retention time (SRT) and wastewater pollutant concentration may be important factors determining the biodegradation kinetics of these compounds (Figure 1, second extrapolation level) and therefore their effect should be examined in more detail. This is only possible with a biodegradation test which gives reliable results on the first level of extrapolation, that is, with a test closely mimicking the conditions in the plant of interest. Because available (standardised) biodegradation tests do not fulfil this requirement, a new test was developed which will be referred to as a "by-pass" test. In this chapter the methodology of this test will be explained and some examples will be given.

Test methodology

In the by-pass test activated sludge is continuously recirculated between the aeration tank of a (parent) activated sludge plant and a small, completely mixed and aerated test vessel (Figure 2). This set-up guarantees a continuous supply of fresh sludge, carbon sources and other nutrients at concentrations similar to those in the parent plant. The parent plant can be a full-scale plant or a pilot-scale plant, for instance when the effects of exposure history and plant operational parameters on the biodegradation kinetics are to be investigated. The sludge in the plant can be acclimated to background concentrations of trace compounds in the wastewater or, for research purposes, to enhanced concentrations of these compounds.

Just before entering the test vessel the sludge is mixed with a small, continuous flow of a stock solution of the target pollutant. After at least three times the hydraulic retention time (HRT) a pseudo steady-state can be assumed and samples are taken from the influent and effluent of the test vessel. In the case of volatile compounds the vessel is covered and to account for loss by volatilisation also an off-gas sample is taken after three times the HRT.

The rate of primary biodegradation (first step in the biodegradation pathway of the parent compound as opposed to ultimate biodegradation which refers to the complete breakdown into inorganic molecules such as CO_2 and H_2O and into biomass) can be derived from a mass balance for the target compound around the test vessel:

$$q_{bio} = \frac{(Q_c + Q_s)(C_i - C) - Q_g C_g}{XV} \tag{1}$$

with C the (total) trace compound concentration ($\mu g \cdot l^{-1}$), Q the flow rate ($l \cdot h^{-1}$), q_{bio} the rate of biodegradation ($\mu g \cdot g^{-1}$ volatile suspended solids [VSS]$\cdot h^{-1}$), V the volume of the test vessel (l) and X the concentration of volatile suspended solids (g VSS$\cdot l^{-1}$). The subscripts c, g, i and s refer to the stock solution, off-gas, influent of the test vessel and sludge from the parent plant, respectively (Figure 2).

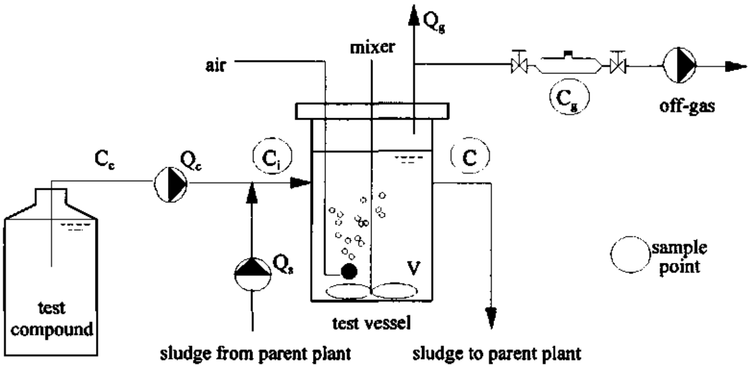

Fig. 2 Experimental set-up for the by-pass test (for an explanation of the symbols refer to the text).

The procedure above is repeated several times at increasing flow rates Q_c, as is schematically shown in the simulated example of Figure 3. This yields a set of values for C with corresponding values for q_{bio}. From this set the primary biodegradation kinetics can be estimated (Figure 4). In the simulated example of Figures 3 and 4 Michaelis-Menten like kinetics were assumed:

$$q_{bio} = k_0 \frac{C}{K_C + C} \tag{2}$$

where k_0 is a zero-order biodegradation rate constant ($\mu g \cdot g^{-1}$ VSS$\cdot h^{-1}$) and K_c is a half-velocity constant ($\mu g \cdot l^{-1}$).

For sorptive compounds it is assumed that a sorption equilibrium will be achieved within the HRT of the test vessel and C_i and C in equations (1) and (2) refer to total trace compound concentrations, i.e., to the sum of dissolved and sorbed concentrations. Consequently, also the biodegradation parameters k_0 and K_C relate to this total concentration.

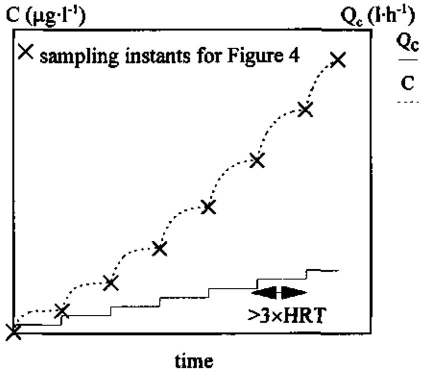

Fig. 3 Flow rate Q_c of a stock solution with the trace compound and the effluent concentration of this compound C in a simulated by-pass test.

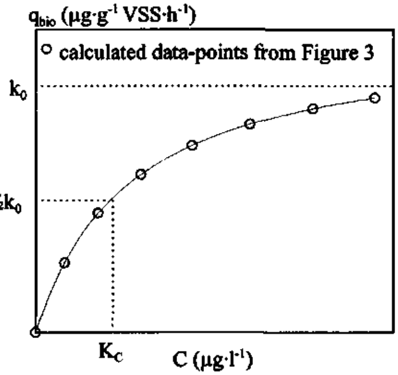

Fig. 4 Biodegradation rate q_{bio},calculated from the data in Figure 3 as a function of the trace compound concentration C.

Because this test is based on compound specific analysis it can only give useful results if sufficiently accurate measurements are available and the trace compound can be detected at concentrations far below the half-saturation constant K_C. Furthermore, a proper selection of the test settings, being the compound addition rate (Q_cC_c) and the HRT of the test vessel, is essential because several requirements have to be met. First, to calculate an accurate biodegradation rate (q_{bio}) the difference between the influent and effluent concentrations (C_i-C) should be sufficiently large. This imposes a minimum upon the HRT of the test vessel. At the same time, to avoid additional growth and acclimation of the biomass in the parent plant the test period should be short, which imposes a maximum upon the HRT. For small (parent) pilot-plants and slowly biodegradable compounds these two requirements for the HRT can be in conflict. Finally, the set of effluent concentrations C should allow an accurate estimation of K_C and k_0. This determines the set of compound addition rates (Q_cC_c). Because the optimum test settings strongly depend on the biodegradation kinetics some preliminary knowledge about these kinetics would be extremely useful. However, this knowledge usually is not available and one or more preliminary tests, each resulting in improved test settings, cannot be avoided.

Material and methods

Test compounds - The three test pollutants NTA, LAS-C_{12} and toluene were selected because they all are positive in ready or inherent biodegradability tests carried out according to guidelines of the Organisation for Economic Co-operation and Development (OECD, 1993), but have different characteristics with respect to sorption and volatilisation. NTA is a non-sorptive, non-volatile compound and several studies have shown that NTA can be readily biodegraded by activated sludge (e.g., Heide, 1984; Alder *et al.*, 1990, chapter 5 of this thesis). LAS products are widely applied anionic surfactants. Although they are highly soluble in water, they strongly sorb to biomass (Urano *et al.*, 1984; Waters and Feijtel, 1995). Commercially available LAS products are mixtures of different homologues ranging from 10-13 carbon units with a typical mean alkyl chain length of 12. All the homologues are known to be readily biodegraded by activated sludge (Larson *et al.*, 1993). Finally, toluene was selected to represent the class of volatile and readily biodegradable (Tabak *et al.*, 1990) trace compounds.

Activated sludge plant operation and sampling - A pilot-scale activated sludge plant was employed which consisted of a completely mixed and covered aeration tank of 490 l and a final settler of 280 l. The plant was operated with pre-settled domestic wastewater at SRTs of 10 and 27.3 days. To maintain the desired SRT sludge was wasted continuously from the aeration tank. The pH in the aeration tank was controlled in a range of 7.2-7.4 by addition of 0.3 M NaOH and 0.3 M HCl. A constant air flow rate was maintained by a mass flow controller. As a result, the oxygen concentration was variable but never below 3 mg $O_2 \cdot l^{-1}$. The wastewater contained average background concentrations of LAS-C_{12} and NTA of about 0.3 and 2 $mg \cdot l^{-1}$, respectively (also see Table 3). Toluene was added externally at 0.7 $mg \cdot l^{-1}$ because it was suspected that the wastewater itself did not contain sufficiently high toluene concentrations to permit development of a toluene degrading population. The pilot-plant was operated for at least four times the SRT before the by-pass tests were carried out. Starting 12 days before these tests, daily composite samples were collected from the influent, and (final) effluent of the plant to determine NTA and LAS-C_{12} concentrations as well as the water quality parameters COD, soluble COD and suspended solids (SS). In addition, every day a grab sample was taken from the effluent of the aeration tank to determine the concentration of toluene. Details of the sampling procedures for LAS, NTA and toluene can be found in chapters 4, 5 and 6, respectively.

By-pass test - An adjustable peristaltic pump was operated to circulate sludge between the aeration tank of the pilot-plant and a 6.1 l polyvinylchloride (PVC) vessel (Figure 2). This vessel was mechanically stirred at 20 rpm and aerated at a flow rate of 43.2 $l \cdot h^{-1}$ with dry, compressed air provided through a fine-pore diffuser stone. Using a second adjustable peristaltic pump, just before entering the test vessel, the sludge was mixed with a stock solution of the target compound. All the stock solutions were prepared in a 25 l polyethylene (PE) container by dilution in suprapur water of NTA (Baker, analytical grade), a commercial 154.2 g $LAS \cdot l^{-1}$ mixture (Isorchem 113/S-Na) containing 31.4 % (w/w) of LAS-C_{12} or toluene (Merck, analytical grade). The settings for the by-pass tests are given in Table 1 and were based on the results of one or two preliminary tests.

At each flow rate of the test compounds, after three times the HRT, grab samples were taken from the influent and effluent of the test vessel and from the stock solution. At the same time instant samples were taken from the effluent mixed-liquor to determine suspended and volatile suspended solids. In the test with toluene, the test vessel was covered with a PVC lid. The off-gas was continuously sampled with a vacuum-pump at 28 $l \cdot h^{-1}$ and guided through 50 or 100 ml glass sample bulbs provided with a polytetrafluorethylene (TFE) faced silicon septum and two valves located at the inlet and outlet of the sample bulbs (Figure 2). The actual sample was taken by subsequently closing the outlet and inlet valve. Approximately 20-30% of all the samples were taken in duplicate. To verify the occurrence of a steady-state, occasional samples were taken after 6 times the HRT. The pH, temperature and dissolved oxygen concentration in the test vessel were not controlled but were frequently monitored and did not significantly change during the tests.

Table 1 - Settings for the by-pass tests

	trace compound		
	LAS-C_{12}	NTA	toluene
sludge flow rate Q_s ($l \cdot h^{-1}$)	14.2	9.6	13.9
compound flow rate Q_c ($l \cdot h^{-1}$)	0-1.5	0-1.2	0-0.8
hydraulic retention time (h)*	0.4	0.6	0.4
stock solution C_c ($mg \cdot l^{-1}$)	40.5	19	26 and 200**
number of data-points (-)	6	7	7
total test duration (h)	8	14	10

* based on sludge flow rate Q_s

** higher stock concentration at higher flow rates of the compound.

Analytical procedures - COD, soluble COD, SS and VSS were all determined according to Dutch standard practice (NEN). Analytical methodologies for LAS-C_{12}, NTA and toluene can be found in chapters 4, 5 and 6, respectively.

Results

By-pass tests - Concentrations in occasional samples taken after 6 times the HRT of the test vessel were not significantly different from concentrations in samples taken after 3 times the HRT. This verifies the assumption made earlier that 3 times the HRT was long enough to achieve a steady-state and samples taken after this period could be used to calculate the rate of biodegradation from a steady-state mass balance around the test vessel (equation 1).

Mass balances showed that in the test vessel, without exception, a minimum of 30% of the load of the test pollutants (Q_cC_c) was removed by biodegradation. This means that the differences between the influent and effluent concentrations of the test vessel always were sufficiently large to calculate an accurate biodegradation rate.

The amount of test pollutant that was fed to the test vessel during the tests was extremely low: 1-3% of the amount of test pollutant fed directly to the pilot-plant during the same period with the wastewater (LAS-C_{12} and NTA) or by an external addition (toluene). Additional acclimation and growth of the biomass in the plant during the tests therefore can safely be neglected.

Figure 5 shows the results of the by-pass tests with LAS-C_{12}, NTA and toluene. For all three trace compounds a similar deviation from the Michaelis-Menten like equation (2) can be observed with a somewhat lower biodegradation rate at low concentrations and a maximum rate which is approached more rapidly at higher concentrations. This phenomenon has frequently been reported in the literature and several models, both empirical and mechanistic, have been proposed to describe this type of behaviour. With the data of Figure 5 it is impossible to distinguish between these models and a simple empirical first and zero-order model is used instead:

$$q_{bio} = k_1 C \quad \text{for} \quad C < C_{1/0} \tag{3a}$$

$$q_{bio} = k_0 \quad \text{for} \quad C \geq C_{1/0} \tag{3b}$$

where k_1 is a first-order rate constant ($l \cdot g^{-1}\ VSS \cdot h^{-1}$), k_0 is a zero-order rate constant ($\mu g \cdot g^{-1}\ VSS \cdot h^{-1}$) and $C_{1/0}$ is the concentration where the kinetics change from first- to zero-order ($\mu g \cdot l^{-1}$).

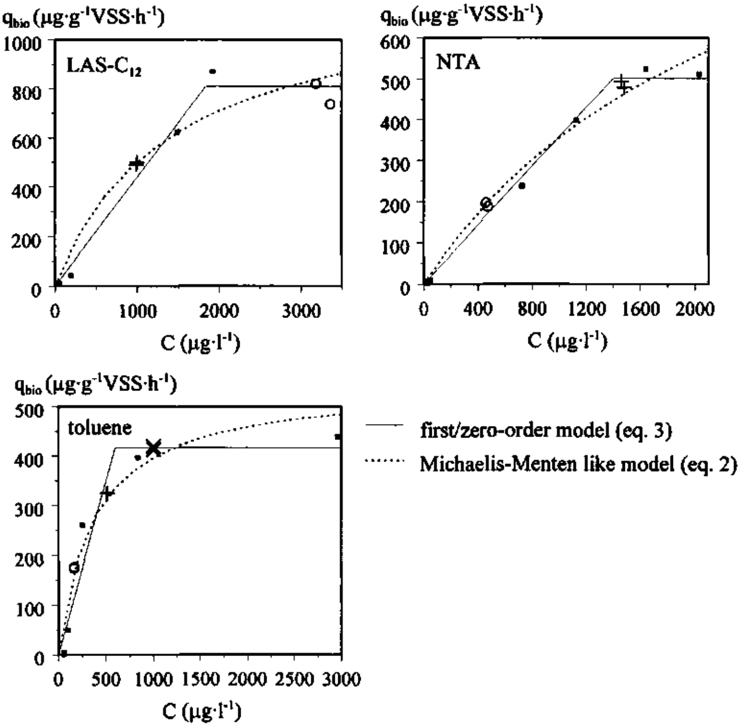

Fig. 5 Biodegradation kinetics of LAS-C_{12}, NTA and toluene as determined in by-pass tests (▪ denotes single measurements and +, o and × duplicate measurements).

Estimated model parameters of the combined first and zero-order model of equation (3) and of the Michaelis-Menten like model of equation (2) are presented in Table 2.

Table 2 - First/zero-order and Michaelis-Menten like biodegradation parameters estimated from the by-pass tests.

	trace compound		
parameter	LAS-C_{12}	NTA	toluene
Michaelis-Menten like model (eq. 2)			
k_0 ($\mu g \cdot g^{-1} VSS \cdot h^{-1}$)	1217	1261	545
K_c ($\mu g \cdot l^{-1}$)	1434	2557	376
First/zero-order model (eq. 3)			
k_0 ($\mu g \cdot g^{-1} VSS \cdot h^{-1}$)	810 ± 68*	501 ± 20	420 ± 17
k_1 ($l \cdot g^{-1} VSS \cdot h^{-1}$) (correlation coefficient)	0.46 (0.974)	0.36 (0.995)	0.70 (0.965)
$C_{1/0}$ ($\mu g \cdot l^{-1}$) = k_0/k_1	1760	1392	600

* ± one standard deviation

Pilot-plant monitoring data - Average monitoring data for the three test pollutants are given in Table 3. For all three pollutants extremely low effluent concentrations were achieved. For NTA the average effluent concentration even was below the method detection limit of 30 $\mu g \cdot l^{-1}$. Remark that the effluent concentration of LAS-C_{12} reflects the total concentration of LAS-C_{12}, i.e., dissolved LAS-C_{12} as well as LAS-C_{12} sorbed to the effluent suspended solids.

Table 3 - Pilot-plant performance with respect to removal of the trace compounds.

	concentration ($\mu g \cdot l^{-1}$)		
trace compound	influent	effluent	removal (%)
LAS-C_{12}	2039 ± 72	10 ± 1	99.5
NTA	304 ± 68	< 30*	> 90
toluene	730 ± 67	0.20 ± 0.05	99.9

* below method detection limit of 30 $\mu g \cdot l^{-1}$.

Discussion

Verification of the biodegradation kinetics

The biodegradation kinetics that were found in the by-pass tests can be verified in two ways: (i) by comparing the biodegradation parameters of Table 2 to literature values for these parameters or (ii) by comparing effluent concentrations calculated from the biodegradation parameters in Table 2 to the observed effluent concentrations of Table 3.

A comparison to literature data is a difficult affair. For example, for NTA half-saturation constants K_C have been reported in the literature from as low as 80 $\mu g \cdot l^{-1}$ to as high as 5 $mg \cdot l^{-1}$ (Bernhardt, 1984; Chudoba *et al.*, 1989 and Alder *et al.*, 1990). This large degree of variation probably can be explained by the many factors that are involved and which can vary between different systems and tests, such as (i) the specific microbial population responsible for degradation of NTA, (ii) the exposure history of this population to NTA, (iii) transport of NTA to the micro-organisms, (iv) the affinity of the responsible enzymes, (v) the formation of NTA complexes with different metals having different biodegradability (Madsen and Alexander, 1985) and (vi) the presence or absence of other (easily biodegradable) carbon sources which may stimulate NTA degradation (Egli and Bally, 1996). Consequently, the fact that the K_C and $C_{1/0}$ that were found in the by-pass test fit well into the range of literature data certainly is promising, but this does not provide detailed information concerning their accuracy. A similar reasoning probably also applies to the other biodegradation parameters and trace compounds. A further comparison to literature data therefore is omitted.

Effluent concentrations from the pilot-plant were calculated with a simple model described in the appendix. In this model sorption of LAS-C_{12} to activated sludge was assumed to be an instantaneous process and was accounted for by a linear sludge-water distribution coefficient of 3.2 $l \cdot g^{-1}$ VSS (chapter 4). Volatilisation had a negligible contribution to overall toluene removal (chapter 6) and was neglected in the calculations. The first-order kinetics of equation (3a) were used to calculate the biodegradation kinetics. Calculated and observed concentrations of LAS-C_{12} , NTA and toluene agree in that they are all extremely low (Figure 6). This indicates that the first-order biodegradation rate constant determined in the by-pass tests at least provide a good approximation of the true kinetics in the pilot-plant. A more precise verification of the biodegradation kinetics is not possible as this would require a more accurate assessment of the kinetics at the extremely low concentrations which were also observed in the pilot-plant.

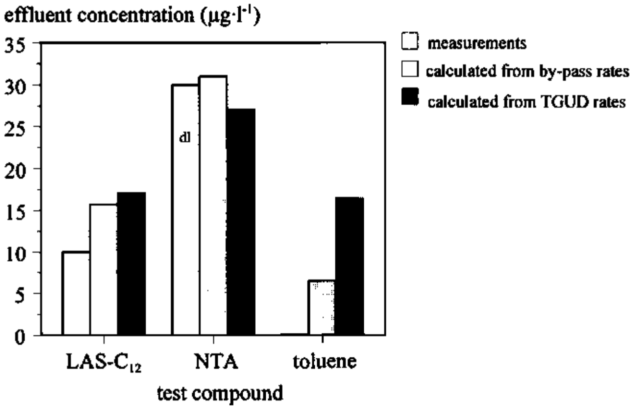

Fig. 6 Observed and calculated effluent concentrations (dl = detection limit for NTA, TGUD = Technical Guidance Document in support of risk assessment)

Figure 6 also shows effluent concentrations which were calculated using first-order rate constants advised by the Technical Guidance Document (TGUD) in support of risk assessment (EC, 1994). According to the TGUD readily biodegradable compounds such as LAS-C_{12}, toluene and NTA should be assigned a pragmatic (first-order) rate constant of 1 h^{-1}, which applies to a sludge concentration of 3 g $SS\cdot l^{-1}$ (Mikkelsen *et al.*, 1996). Translated to the sludge in the pilot-plant, with an average VSS/SS ratio of 0.8, this corresponds to a first-order rate constant k_1 in equation (3a) of 0.42 $l\cdot g^{-1}VSS\cdot h^{-1}$. Figure 6 shows that for all three test compounds this rate constant yields a similar prediction of the effluent concentration as the rate constants determined in the by-pass tests (Table 2).

Extrapolation of the biodegradation kinetics to other plants

The biodegradation kinetics determined in the by-pass test are directly related to the sludge in the pilot-plant, given the characteristics of the wastewater (BOD and pollutant concentration) this sludge is exposed to and the plant operational parameters (SRT, HRT, air flow rate, etc.). For a more generic application other wastewater characteristics and operational parameters have to be considered as well (Figure 1, second extrapolation level). The method that should be applied for this second level of extrapolation largely depends on whether the target compound is degraded as a primary or as a secondary substrate. In the first case a growth Monod model can be used, but in the last case a non-growth secondary utilisation model would be more appropriate.

In secondary utilisation the target compound itself cannot support growth and maintenance because its concentration is too low to provide a sufficient amount of energy or, as in co-metabolism, irrespective of its concentration cannot be used as a carbon and electron source (Rittmann, 1992). Although the compound can still be degraded, this depends on the presence of another (primary) substrate. Secondary utilisation has been adopted in many fate models as the responsible mechanism for biodegradation of trace compounds by activated sludge (e.g. Blackburn *et al.*, 1984; Namkung and Rittmann, 1987; Struijs *et al.*, 1991; Jacobsen *et al.*, 1996). The volumetric rate of biodegradation r_{bio} (in $\mu g\cdot l^{-1}\cdot h^{-1}$) follows from:

$$r_{bio} = q_{bio} X \tag{4}$$

The specific biodegradation rate q_{bio} can be evaluated from equation (2), (3a) or (3b) and is expected to be more or less plant independent. The (non-specific) sludge concentration X varies from plant to plant, depending on operational parameters such as the BOD loading and the SRT, but can easily be assessed as the total concentration of volatile suspended solids.

Nyholm and Ingerslev (1996) suggested that specific growth on a trace pollutant can be obtained if this compound is supplied at concentrations greater than 100 $\mu g\cdot l^{-1}$. Because all the test compounds were supplied to the pilot-plant at significantly higher concentrations, this would imply that their biodegradation should be described by (growth) Monod kinetics rather than by (non-growth) secondary utilisation kinetics. When the Monod model is used to extrapolate the by-pass results to other plants, a maximum specific growth rate is required. This maximum growth rate is related to the zero-order rate constant k_0 in equation (2) or (3b) according to:

$$k_0 = \frac{\mu_{max}}{Y}\frac{B}{X} \qquad (5)$$

where μ_{max} is the maximum specific growth rate of the competent biomass, i.e. the biomass responsible for degradation of the pollutant (h^{-1}), Y is the biomass-yield (g VSS·g^{-1}) and B is the concentration of competent biomass (g VSS·l^{-1}).

Y can be assessed in a respirometry test (Ellis *et al.*, 1996) or a default value of 0.5 g·g^{-1} can be assumed (Blok, 1994). According to Ellis *et al.* (1996) the fraction of competent biomass (B/X) in equation (5) can be approximated by the fraction of feed COD supplied by the target compound. Care should be taken however because this probably only is valid when practically the entire influent load of the target compound becomes available for biodegradation and this compound cannot (partly) escape by other removal pathways (off-gas or waste sludge). Table 4 gives values for μ_{max} which were calculated according to equation (5) using the k_0 from the combined first/zero-order model (Table 2). Table 4 also compares this μ_{max} to literature data. The μ_{max} of LAS-C_{12} and NTA is somewhat higher than reported values, the μ_{max} for toluene falls within the range of literature data. However, for reasons mentioned earlier, such a comparison may not be very useful.

Table 4 - Maximum specific growth rates μ_{max} calculated from the by-pass results and compared to literature values.

compound	fraction of feed COD to pilot-plant* = B/X in eq. (5)	μ_{max} (h^{-1}) calculated from results by-pass test	literature data
LAS-C_{12}	0.03	0.06	$0.036^a - 0.042^a$
NTA	0.0004	0.32	$0.036^b - 0.229^b$
toluene	0.0006	0.13	$0.121^c - 0.379^d$

* average COD of the wastewater was 500 mg·l^{-1}

[a] Hrsak, 1982; [b] Egli *et al.*, 1988; [c] Kincannon *et al.*, 1982; [d] Tabak *et al.*, 1990

Practical application of the by-pass test

The by-pass test does not seem to lend itself for a routine application, in particular because multiple "trial-and-error" tests are required to obtain the proper test settings and because more experience is required with the test method. Although a precise verification of the biodegradation kinetics that were found was not possible, it appears that the test is suitable as a research tool. In particular, it could be used in an investigation of the effect of plant operational parameters and wastewater characteristics on the biodegradation kinetics of trace pollutants. Partly because there is a lack of reliable biodegradation tests, these effects are still poorly understood and need more explanation before more reliable activated sludge fate models can be developed.

Conclusions

A new biodegradation test was developed to investigate the kinetics of primary biodegradation of trace organic compounds by activated sludge. The purpose of this so-called by-pass test was to circumvent difficulties which arise when results from standardised biodegradation tests are extrapolated to activated sludge plant conditions. The methodology was tested for three different trace compounds: NTA, LAS-C_{12} and toluene. Although it was not possible to precisely verify the biodegradation kinetics that were found, it is concluded that the test method can lend itself for an application in a research environment.

Appendix - Model predictions

A.1 Nomenclature and parameters

symbol	meaning	units	value LAS-C_{12}	 NTA	 toluene
C_i	influent concentration pilot-plant (total)	$\mu g \cdot l^{-1}$	2039	304	730
k_1	first-order biodegradation rate constant	$l \cdot g^{-1} VSS \cdot h^{-1}$	0.46	0.36	0.70
K_p	linear sludge-water distribution coefficient	$l \cdot g^{-1} VSS$	3.21*	not relevant	not relevant
Q_i	influent flowrate	$l \cdot h^{-1}$	67.1	67.1	39.5
Q_w	waste sludge flow rate	$l \cdot h^{-1}$	2.04	2.04	0.75
V_a	volume aeration tank	l	490	490	490
X_a	volatile suspended solids aeration tank	$gVSS \cdot l^{-1}$	3.35	3.35	5.59
X_e	volatile suspended solids final effluent	$gVSS \cdot l^{-1}$	0.008	0.008	0.022

* taken from chapter 4

A.2 Model

Consider an activated sludge plant consisting of a completely-mixed aeration tank and a secondary settler. Sludge is wasted from the aeration tank. If it is assumed that (1) biodegradation can only take place in the aeration tank, (2) volatilisation can be neglected and (3) first-order kinetics (eq. 3a) prevail, the following mass balance can be written around the plant:

$$Q_i C_i - Q_w C_a - (Q_i - Q_w) C_e - V_a k_1 X_a C_a = 0 \qquad (A.1)$$

For non-sorptive compounds such as NTA and toluene the concentrations in the aeration tank C_a and final effluent C_e are equal. For sorptive compounds such as LAS-C_{12} the final effluent and aeration tank concentrations are related according to:

$$\frac{C_e}{C_a} = \frac{1 + K_p X_e}{1 + K_p X_a} \qquad (A.2)$$

Equations (A.1) and (A.2) can be combined to give the final effluent concentration C_e:

$$C_e = \frac{Q_i C_i}{\frac{1 + K_p X_a}{1 + K_p X_e}(Q_w + V_a k_1 X_a) + Q_i - Q_w} \tag{A.3}$$

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Chapter 4.1

Fate of Linear Alkylbenzene Sulfonates (LAS) in activated sludge plants

- monitoring data -

Abstract - As part of a model validation study monitoring data were collected in a pilot-scale municipal activated sludge plant to establish the fate of the C_{12}-homologue of linear alkyl benzene sulfonate (LAS-C_{12}). The pilot-plant was operated at influent LAS-C_{12} concentrations between 2000 and 15000 $\mu g \cdot l^{-1}$ and at sludge retention times of 10 and 27.3 days. In general, of the influent load of LAS-C_{12} less than 0.5% was discharged with the effluent and less than 0.4% was wasted with the excess sludge, irrespective of the influent concentration of LAS-C_{12} and of the operational characteristics of the plant. Effluent and waste sludge concentrations were between 5 and 10 $\mu g \cdot l^{-1}$ and between 37 and 69 $\mu g \cdot g^{-1}$ VSS, respectively. In sludge samples which were taken from the aeration tank of the plant only a small fraction, 2 to 8%, was present as dissolved LAS-C_{12}. The remaining 92 to 98% was found to be sorbed to the sludge. In spite of this high degree of sorption, more than 99% of LAS-C_{12} could be removed by biodegradation, showing that not only the soluble fraction but also the sorbed fraction is readily available for biodegradation.

Introduction

Linear alkylbenzene sulfonate (LAS) was one of the test compounds in a validation study of models that predict the fate of organic trace compounds in municipal activated sludge plants. LAS products (Figure 1) are widely applied anionic surfactants and account for approximately 25-30% of the world total of synthetic surfactants (Larson *et al.*, 1993). They are mixtures of different phenyl isomers and homologues with an alkyl chain ranging from 10 to 13 carbon units. Considering a typical mean alkyl chain length of commercial LAS products of 12, we decided to focus specifically on the C_{12}-homologue (LAS-C_{12}), although some information will also be provided about the other homologues.

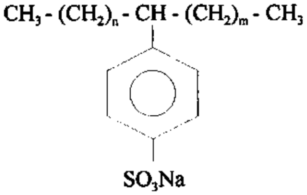

Fig. 1 Chemical structure of LAS.

Sorption and biodegradation are the main processes dominating the fate of LAS in activated sludge plants, i.e., they determine whether LAS is discharged with the effluent, is wasted with the excess sludge or is eliminated from the environment by biodegradation. Although LAS is highly soluble, it strongly sorbs to wastewater solids (Brunner *et al.*, 1988), activated sludge biomass (Urano and Saito, 1984) and sediments (Westall *et al.*, 1999). Due to its ionic character, the degree of sorption is not determined by hydrophobic interactions alone, but also by chemical and electrostatic interactions (Westall *et al.*, 1999). Under aerobic conditions all the LAS homologues, including LAS-C_{12}, are readily biodegraded by activated sludge. Many studies have demonstrated their primary biodegradation (parent compound removal) as well as ultimate biodegradation (for example Larson *et al.*, 1993).

Validation of activated sludge fate models requires accurate and reliable monitoring data. The fate of LAS in activated sludge plants already was investigated in several monitoring studies (Matthijs and de Henau, 1987; Brunner *et al.*, 1988; Rapaport and Eckhoff, 1990; Feijtel *et al.* , 1995; Waters and Feijtel, 1995; McAvoy *et al.*, 1998). However, all these studies were carried out in full-scale plants and (i) essential information about the plant characteristics and operational conditions was missing or (ii) the sets of LAS data were incomplete or lacked the required level of detail. Moreover, insufficient variation was applied to those parameters that activated sludge fate models are most sensitive to. In particular the sludge retention time (SRT) and (average) influent concentration of LAS have to be mentioned in this regard.

To obtain a set of LAS monitoring data which is more suitable for model validation, LAS-C_{12}

concentrations were determined in a pilot-scale municipal activated sludge plant under controlled and well-established conditions. Several experiments were carried out at various plant operational parameters (sludge and hydraulic retention time and COD loading rate) and influent concentrations of LAS. In each experiment the sludge in the plant was allowed to adapt to the new set of conditions.

Material and methods

Activated sludge plant and wastewater characteristics - A pilot-scale activated sludge plant (Figure 2) was employed which consisted of a completely mixed aeration tank (490 l) and a secondary settler (280 l). An equalisation tank (1000 l) and a contact tank (56 l) were installed, respectively to smooth out diurnal fluctuations in the wastewater composition and to prevent the occurrence of bulking sludge. The plant treated pre-settled municipal wastewater with the average composition given in Table 1. Dry compressed air was provided to the aeration tank at a constant flow rate through four fine pore diffusers. The dissolved oxygen concentration in the tank was not controlled but always kept above 3 mg·l^{-1}. The desired SRT was maintained by continuously wasting excess sludge from the aeration tank. The pH in the aeration tank was controlled in a range of 7.2-7.4 using automated 0.3 M NaOH and 0.3 M HCl additions.

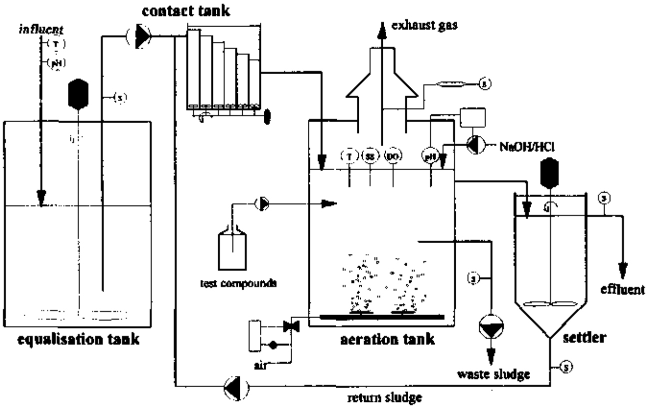

Fig. 2 Pilot-plant (S=sample points, T=temperature, DO=dissolved oxygen, SS=suspended solids).

Table 1 - Average composition of the pre-settled wastewater (determined in 24-h samples).

parameter	value
total COD (mgO$_2$·l^{-1})	500 ± 56*
soluble COD (mgO$_2$·l^{-1})	286 ± 48
NH$_4$-N (mg·l^{-1})	60 ± 9
PO$_4$-P (mg·l^{-1})	7.1 ± 2.1
suspended solids (mg·l^{-1})	83 ± 64

* standard deviation

Plant operation - The pilot-plant was operated in two periods, period A and period B, at two different sets of SRT, hydraulic retention time (HRT) and COD loading rate (Table 2). In both periods the plant initially received wastewater containing background concentrations of LAS-C$_{12}$ for at least four times the SRT (periods A1 and B1 in Table 2). After this, the plant was fed with enhanced concentrations of LAS-C$_{12}$ of about 5 mg·l^{-1} in period A and 10 mg·l^{-1} in period B (periods

A2 and B2 in Table 2). For this purpose stock solutions of a commercial LAS mixture (154.2 g LAS·l^{-1}, Isorchem 113/S-Na containing 31.4 % of LAS-C_{12}) in suprapur water were prepared in a 25 l PE container and were pumped directly into the aeration tank.

LAS analytical methodology - The LAS analytical methodology was adapted from Feijtel *et al.* (1995) and is presented schematically in Figure 3.

Table 2 - Operation of the pilot-scale activated sludge plant.

parameter	period A		period B	
sludge retention time (d)	10		27.3	
hydraulic retention time (h)	7.3		12.4	
COD loading rate (kg COD·kg^{-1}SS·d^{-1})	0.37		0.15	
influent flow rate (l·h^{-1})	67.4		39.6	
return sludge flow rate (l·h^{-1})	64.2		36.6	
air flow rate (l·h^{-1})	4884		2704	
waste sludge flow rate (l·h^{-1})	2.04		0.75	
	A1	A2	B1	B2
additional influent LAS-C_{12} (mg·l^{-1})	0	5	0	10

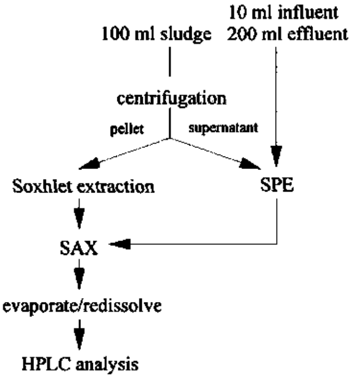

Fig. 3 Procedure for LAS analysis.

Sampling and preservation

At least every other day 24-h samples of 2.5 l influent and 2.5 l effluent were collected in polyethylene (PE) bottles for determination of total (sum of sorbed and dissolved) LAS-C_{12} concentrations. At least once a day 200 ml grab samples were taken from the aeration tank and return sludge and transferred into PE centrifuge tubes for determination of dissolved and sorbed LAS-C_{12}. The sludge samples were immediately centrifuged at 3500 rpm for 15 minutes. The supernatant was transferred into PE bottles. All the LAS samples were preserved by 3% (v/v) formalin (Merck, 35% formaldehyde solution) and stored for a maximum of 10 days at 4 °C until further analysis. Procedures for sampling, preservation and storage were validated in recovery experiments.

Isolation and concentration

Representative aliquots of pre-settled influent (10 ml), final effluent (200 ml) or supernatant of the centrifuged sludge samples (10-100 ml) were passed over 6 ml pre-conditioned C18 solid phase extraction (SPE) columns (Baker). These columns were pre-conditioned by passing 10 ml of methanol (99.8%, Boom), followed by 10 ml of water. The passing rate was not allowed to exceed 10 ml·min^{-1}. After passing the samples, the SPE columns were washed with 2 ml methanol/water (3:7 v/v) and eluted with 5 ml of methanol. Strong anion exchange (SAX) columns of 3 ml (Baker) were pre-conditioned by passing 10 ml of methanol. After passing the eluate of the SPE columns, the SAX columns were washed with 5 ml methanol and eluted with 2 ml of a 8:2 (v/v) methanol/HCl (>37%, Riedel-deHaën) mixture. The eluate was evaporated to dryness at 50 °C under a gentle flow of nitrogen gas. The dry residue was dissolved in 2-5 ml of HPLC mobile phase. Wet sludge pellets (0.6-1.2 g) were weighed and Soxhlet-extracted for a minimum of 4 hours with 80 ml of methanol. After the extract had been adjusted to a volume of 100 ml, 10-100 ml was treated according to the procedure described earlier for the liquid samples, starting with the SAX elution.

HPLC analysis and quantification

The HPLC mobile phase consisted of a 16:85 (v/v) suprapur water/methanol (99.9% HPLC quality, Lab-Scan) mixture containing 12.3 g·l^{-1} of sodium perchlorate (>99%, Merck). The HPLC column (250×6 mm Spherisorb 5 ODS-2, Chrompack) was operated at 20 °C and a flow rate of 1 ml·min^{-1}. The column was pre-conditioned by rinsing with suprapur water for 45 minutes followed by rinsing with mobile phase for another 45 minutes. Detection was by a fluorescence detector (1046A, Hewlett Packard) operated at an excitation wavelength of 232 nm and an emission wavelength of 290 nm. An autosampler (Marathon, Spark) was set to a pre-flush volume of 200 µl, a loop volume of 500 µl and an injection volume of 50 µl. Identification of the different LAS alkyl homologues and quantification were made against a commercial LAS mixture (Marlon A390) with an activity of 89.2% and a reported LAS-C_{12} content of 35-40%. A six point calibration curve was made from LAS stock solutions of 0 to 50 mg·l^{-1} in mobile HPLC phase. These stock solutions were renewed daily from a 1 g·l^{-1} stock solution in suprapur water which was stored at 4 °C in a PE bottle. Selected volumes of sludge supernatant and methanol extracts from sludge pellets (10-100 ml, see above) were aimed to obtain a target HPLC concentration of 2-20 mg·l^{-1}. Typical retention times for the LAS homologues LAS-C_{10}, LAS-C_{11}, LAS-C_{12} and LAS-C_{13} were 6.7, 8.5, 11.3 and 15.4 minutes, respectively.

Performance of the analytical methodology

Characteristics of the LAS-C_{12} analysis can be found in Table 3. After every tenth sample a blank sample and a 10 mg·l^{-1} standard were injected on the HPLC and were used to estimate the instrumental detection limit and instrumental precision, respectively.

Table 3 - Performance of the LAS-C_{12} analytical methodology.

instrumental precision	4%				
instrumental detection limit	136 µg·l^{-1}				
method recovery	92 ± 6%				
sample	influent	effluent	sludge		
fraction	total*	total*	total*	dissolved	sorbed
method precision	5%	47%	9%	not available	not available
method detection limit	27 µg·l^{-1}	4 µg·l^{-1}	15 µg·l^{-1}	2 µg·l^{-1}	5 µg·g^{-1} VSS

* sum of dissolved and sorbed LAS-C_{12}

Method recovery was determined in experiments where known amount of LAS were spiked to influent, effluent and sludge samples. The method detection limit was calculated from the instrumental detection limit using known concentration steps. Finally, method precision was determined from duplicate samples which comprised at least 20% of the total number of samples.

Other analysis - Separate 24-h influent and effluent samples were collected to determine the standard wastewater parameters COD, soluble COD, NH_4-N, NO_3-N and suspended solids (SS). These parameters were all determined according to Dutch standard practice (NEN).

Results

Standard water quality parameters - Average standard water quality parameters (COD, soluble COD and NH_4-N) in the influent and effluent of the pilot-plant were similar in all periods, indicating a stable performance of the pilot-plant with respect to COD removal and nitrification. Average effluent COD and NH_4-N concentrations were 60 and 0.1 $mg \cdot l^{-1}$, which corresponds to COD removal and nitrification efficiencies of 90 and 99%, respectively. The external LAS additions in periods A2 (5 $mg \cdot l^{-1}$) and B2 (10 $mg \cdot l^{-1}$) did not affect these removal efficiencies. Only in period B the average effluent suspended solids (SS) concentration (22 $mg \cdot l^{-1}$) was somewhat higher than in period A (8 $mg \cdot l^{-1}$).

LAS-C_{12} monitoring data - Figure 4 shows an example of background LAS-C_{12} concentrations in 24-h samples of the pre-settled wastewater that was used in the experiments. These samples were collected during a month without significant rainfall. The coefficient of variation of 10% is relatively low, which probably can be explained by the absence of preferred washing-days (Waters and Feijtel, 1995).

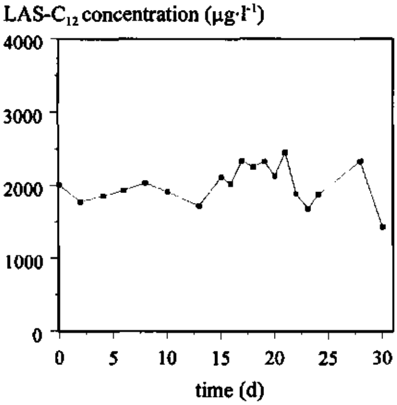

Fig. 4 LAS-C_{12} background concentration in 24-h samples of the pre-settled wastewater treated by the pilot-plant.

Table 4 presents total (sum of dissolved and sorbed) LAS-C$_{12}$ concentrations in 24-h samples of the influent and final effluent of the pilot-plant and dissolved, sorbed and total concentrations of LAS-C$_{12}$ in grab samples taken of the sludge in the aeration tank and of the return sludge. Dissolved LAS-C$_{12}$ concentrations in the sludge samples and total LAS-C$_{12}$ concentrations of the final effluent were extremely low and sometimes even close to their method detection limits (Table 3). Partly this explains the large standard deviation in these measurements.

Table 4 - LAS-C$_{12}$ monitoring data (influent and effluent are 24-h samples, aeration tank and return sludge are grab samples, σ = standard deviation).

		period A, SRT = 10 d				period B, SRT = 27.3 d			
		A1		A2		B1		B2	
		average	σ	average	σ	average	σ	average	σ
influent	total* ($\mu g\cdot l^{-1}$)	2039	72	6315	537	1908	132	11505	593
aeration tank	dissolved ($\mu g\cdot l^{-1}$)	20	3	15	12	9	8	11	5
	sorbed ($\mu g\cdot g^{-1}$ VSS)	69	7	52	11	46	7	37	3
	total* ($\mu g\cdot l^{-1}$)	251	21	211	34	249	32	218	14
return sludge	dissolved ($\mu g\cdot l^{-1}$)	17	5	6	4	14	4	8	5
	sorbed ($\mu g\cdot g^{-1}$ VSS)	75	19	64	18	42	13	34	2
	total* ($\mu g\cdot l^{-1}$)	400	77	322	117	420	130	390	28
effluent	total* ($\mu g\cdot l^{-1}$)	10	1	5	3	8	4	6	3

* sum of dissolved and sorbed concentrations

The concentrations of dissolved LAS-C$_{12}$ in the sludge samples were extremely low as compared to sorbed concentrations, confirming the high sorption potential of LAS-C$_{12}$ to activated sludge. Only 2-8% was present as dissolved LAS-C$_{12}$ whereas the remaining 92-98% was found to be sorbed by the sludge. Assuming linear sludge-water partitioning this corresponds with an average partition coefficient K_p of 3.7 ± 0.6 l·g^{-1} VSS or, given a VSS to SS ratio of 0.8, a K_p of 3.0 l·g^{-1} SS. The latter value is in good agreement with a K_p of 3.1 l·g^{-1} SS reported by Feijtel *et al.* (1995). It should be noted however that due to inaccuracies in the extremely low dissolved concentrations of LAS-C$_{12}$ variation of the calculated K_p among the different periods is large.

Average total LAS-C$_{12}$ concentrations in the different experimental periods were between 322 and 420 µg·l^{-1} in the return sludge and between 211 and 251 µg·l^{-1} in the aeration tank. (Table 4). The SRT (10 d in period A and 27.3 d in period B) did not seem to have a significant effect on these concentrations. Surprisingly, higher influent concentrations during the periods with the same SRT (6315 µg·l^{-1} in period A2 compared 2039 µg·l^{-1} in period A1 and 11505 µg·l^{-1} in period B2 compared to 1908 µg·l^{-1} in period B1) results in 7-19% lower concentrations in the aeration tank and return sludge. The effluent concentrations of LAS-C$_{12}$ is 5-10 µg·l^{-1} (Table 4) and seems to be more or less independent of the SRT and influent concentration. The effluent concentrations are rather inaccurate as they all are close to the method detection limit of 4 µg·l^{-1} and more detailed evaluation of these effects is not possible.

Distribution of LAS-C_{12} in the pilot-plant - The monitoring data of Table 4 were used to calculate the distribution of LAS-C_{12} in the pilot-plant (Table 5). The load of biodegraded LAS-C_{12} was calculated as the closure of the mass balance around the plant. During all periods more than 99% of the influent load of LAS-C_{12} was removed by biodegradation, less than 0.5% was discharged with the effluent and less than 0.4% was wasted with the excess sludge. As was mentioned earlier, the concentrations of LAS-C_{12} in the aeration tank tend to be somewhat lower when the pilot-plant received higher influent concentration of LAS-C_{12} (periods A2 and B2). As a result, the biodegradation efficiency in these periods also was somewhat higher.

Table 5 - Distribution of LAS-C_{12} in the pilot-plant.

	Period A, SRT = 10 d				period B, SRT = 27.3 d			
	A1		A2		B1		B2	
	load ($mg \cdot h^{-1}$)	% of influent	load ($mg \cdot h^{-1}$)	% of influent	load ($mg \cdot h^{-1}$)	% of influent	load ($mg \cdot h^{-1}$)	% of influent
influent	137.4	100	425.6	100	75.6	100	455.6	100
excess sludge	0.5	0.4	0.4	0.1	0.2	0.2	0.2	< 0.1
effluent	0.7	0.5	0.3	0.1	0.3	0.4	0.2	0.1
biodegradation*	131.6	99.2	424.9	99.8	75.1	99.3	455.2	99.9

* calculated as the closure of the mass balance around the pilot-plant

Homologue distribution - Analysis of a commercial LAS product (Isorchem 113/S-Na) showed a homologue distribution with 11% of LAS-C_{10}, 33.6% of LAS-C_{11}, 31.4% of LAS-C_{12} and 24% of LAS-C_{13} (Figure 5).

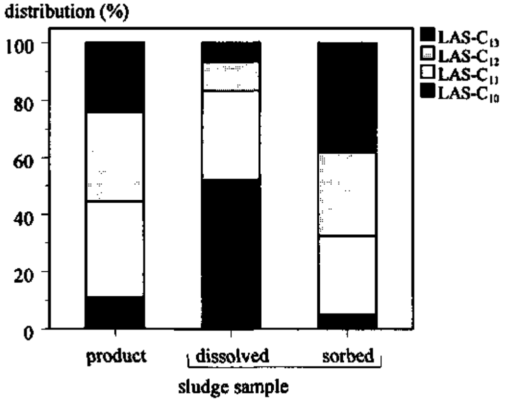

Fig. 5 Average homologue distribution of LAS in commercial LAS and of dissolved and sorbed LAS in activated sludge samples.

Although these homologues have comparable biodegradation rates (Larson *et al.*, 1993), their distribution may change significantly during wastewater treatment because higher homologues have a stronger tendency to sorb to the sludge (Rapaport and Eckhoff, 1990; Cavalli *et al.*, 1993). This effect is also illustrated in Figure 5 which shows the average homologue distribution of dissolved and sorbed LAS in sludge samples taken from the aeration tank of the pilot-plant. LAS-C_{10} has the lowest tendency to sorb to the sludge and makes up 52% of dissolved LAS but only 5% of sorbed LAS. The highest homologue, LAS-C_{13}, has the highest tendency to sorb to the sludge and with a contribution of 38% is the most important constituent of sorbed LAS and contributes only 7% to dissolved LAS. LAS-C_{12} accounted for 29% of sorbed LAS and 10% of dissolved LAS.

Discussion

Comparison to literature data - Using the homologue distribution of Figure 5 concentrations of total LAS (i.e., the sum of the C_{10}-C_{13} homologues) were calculated from the monitoring data in Table 4. In Figure 6 these calculated concentrations are compared to box-plots constructed from available literature data.

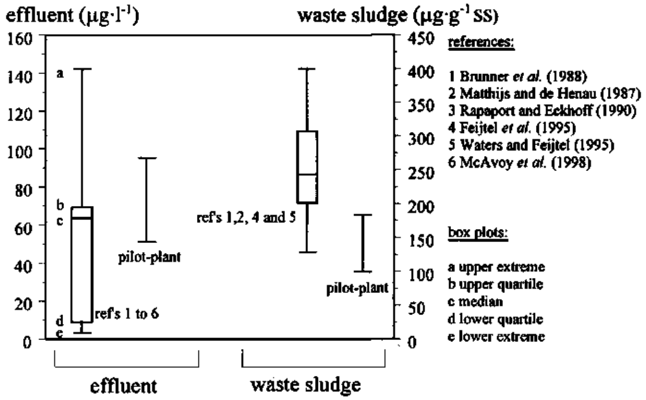

Fig. 6 Box-plots of literature data for waste sludge and effluent LAS concentrations (total of C_{10}-C_{13} homologues) compared to pilot-plant concentrations.

The concentration of LAS in the effluent of the pilot-plant seems to be somewhat higher than the literature data whereas the waste sludge concentration tends to be somewhat lower. This suggests that the conditions in the pilot-plant regarding sorption were relatively unfavourable. Westall *et al.* (1999) examined sorption of several LAS homologues (including LAS-C_{12}) to suspended sediments. They showed that sorption of LAS is promoted by the presence of calcium. They explained this by adsorption of Ca^{2+} onto the surface of the sediments, which reduces the negative surface charge and herewith the electrostatic repulsion of LAS. A similar phenomenon may also occur in activated sludge systems. Because the domestic wastewater that was used in our experiments contains relatively low calcium concentrations (40-50 mg·l^{-1}) this may explain the lower sorption potential of LAS compared to other studies.

Interaction between sorption and biodegradation - Because generally more than 99% of LAS-C_{12} could be removed by biodegradation (Table 5), sorption apparently did not result in an accumulation of LAS-C_{12} in the sludge phase. This can only be explained if sorbed LAS-C_{12} is directly accessible to biodegradation, or if sorption and desorption are rapid and largely reversible processes. The latter is supported by results of Matthijs and de Henau (1985) and Westall *et al.* (1999) and will also be confirmed by the experiments of chapter 4.2 of this thesis. Besides, sorption may have a positive effect on biodegradation of LAS-C_{12} because the sorbed fraction of LAS-C_{12} is not discharged with the final effluent but together with the settled sludge is retained in the system and in this manner is subjected to a longer (biological) treatment time (Cowan *et al.*, 1993).

Effect of the influent concentration and the SRT - Although average LAS-C_{12} concentrations in the aeration tank and return sludge were lower during periods of enhanced influent concentrations (period A2 compared to period A1 and period B2 compared to period B1), this effect is not very strong (Figure 7).

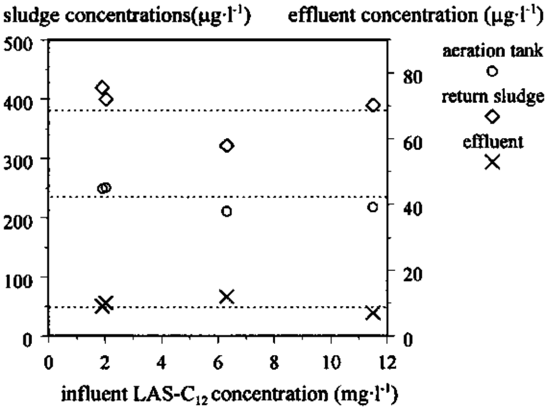

Fig. 7 Effect of influent LAS-C_{12} concentration on average LAS-C_{12} concentrations in the pilot-plant (dotted lines represent average concentrations).

The consistently high efficiency of biodegradation (Table 5) means that an increasing influent concentrations of LAS-C_{12} to the pilot-plant is compensated for by an increasing biodegradation rate of LAS-C_{12}. If this is caused by enrichment of LAS degraders, this would support application of the Monod model to describe biodegradation of LAS by activated sludge.

The Monod model also predicts that longer SRTs will yield lower effluent concentrations until a certain minimum concentration has been achieved. However, measured concentrations at SRTs of 10 and 27.3 d however were not significantly different (Table 4), indicating that this minimum already was achieved at a SRT of 10 d. Also results reported by Birch (1991), who investigated LAS removal in porous pot reactors, showed that SRTs exceeding 4-5 d no longer had an effect on the effluent concentration of LAS.

Conclusions

The monitoring data showed consistently low LAS-C_{12} concentrations of 5-10 $\mu g \cdot l^{-1}$ in the effluent of a pilot-scale municipal activated sludge plant, irrespective of the influent concentration of LAS-C_{12} (2000-11500 $\mu g \cdot l^{-1}$) and of the SRT (10 and 27.3 d). A large fraction (93-98%) of LAS-C_{12} in the plant was sorbed to the sludge. Nevertheless, in general more than 99% of the influent load of LAS-C_{12} could be removed by biodegradation, indicating that not only dissolved but also sorbed LAS-C_{12} is readily available for biodegradation.

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Chapter 4.2

Fate of Linear Alkylbenzene Sulfonates (LAS) in activated sludge plants

- sorption and primary biodegradation kinetics -

Abstract - Sorption and biodegradation of the C_{12}-homologue of linear alkylbenzene sulfonate (LAS-C_{12}) by municipal activated sludge were investigated. Sorption equilibration was within 5 to 10 minutes, showing that sorption of LAS-C_{12} by activated sludge is an extremely fast process. The equilibrium could be described by a linear sorption-isotherm with a sludge-water partition coefficient of 3.2 $l \cdot g^{-1}$ volatile suspended solids. The results of biodegradation kinetic tests showed that primary biodegradation of LAS-C_{12} cannot be described by a (growth) Monod model, but a (non-growth) secondary utilisation model should be used instead. The kinetics were described by a combined first- and zero-order model, related to the total concentration of LAS-C_{12} (i.e., the sum of dissolved and sorbed LAS-C_{12}). The concentration where the kinetics change from first- to zero-order decreased when the sludge in the plant was exposed to higher loading rates of LAS-C_{12}.

Introduction

In chapter 4.1 the results were described of a monitoring study to investigate the fate of the C_{12} homologue of linear alkylbenzene sulfonate (LAS-C_{12}) in a pilot-scale municipal activated sludge plant. Later, in chapter 4.3, these monitoring data will be compared to model predictions. The monitoring data obtained in chapter 4.1 reflect overall behaviour of LAS-C_{12}, which is determined by simultaneously operating sorption and biodegradation. In this chapter these processes will be investigated separately.

Sorption - Although LAS products (LAS-C_{10} - LAS-C_{13}) are highly soluble, they strongly partition to activated sludge. (Urano and Saito, 1984; Matthijs and de Henau, 1987; Feijtel, 1995). Generally, sorption of organic trace compounds by activated sludge is described by a linear sorption-isotherm (Bell and Tsezos, 1987; Dobbs *et al.*, 1989; Jacobsen *et al.*, 1993):

$$K_p = \frac{q}{S} \tag{1}$$

where K_p is the sludge-water partition coefficient ($l \cdot g^{-1}$ VSS) and q ($\mu g \cdot g^{-1}$ VSS) and S ($\mu g \cdot l^{-1}$) are sorbed and dissolved concentrations, respectively.

When hydrophobic interactions dominate sorption, the K_p can be estimated from an empirical relationship with the compound's octanol-water partition coefficient (K_{ow}) and the organic carbon content of the sludge (Schwarzenbach and Westall, 1985; Bell and Tsezos, 1987 and Dobbs *et al.*, 1989). However, in the case of LAS not only hydrophobic interactions, but also chemical and electrostatic interactions are involved (Berna *et al.*, 1989; Prats *et al.*, 1993; Westall *et al.*, 1999). Although mathematical models are available to describe these interactions (e.g., Di Toro *et al.*, 1990), their applicability is uncertain. Consequently, the K_p for LAS-C_{12} will have to be assessed in experiments.

For several organic trace compounds their sorption by activated sludge has been observed to be a fast process (e.g., Dobbs *et al.*, 1989; Jacobsen *et al.*, 1993; Kördel *et al.*, 1997). Similarly, for LAS reversible and rapid adsorption and desorption have been shown, albeit with suspended sediments and not with activated sludge (Hand and Williams, 1987, Westall *et al.*, 1999). Considering the large specific surface area of activated sludge and typical hydraulic retention times (HRTs) in treatment plants of 4 hours or more, it is expected that sorption of LAS-C_{12} also can be approximated as an instantaneous equilibration process. This still needs to be verified however.

Biodegradation - Under aerobic conditions all the LAS homologues (LAS-C_{10} to LAS-C_{13}) are readily biodegraded by activated sludge and many studies have demonstrated their primary biodegradation (parent compound removal) as well as mineralisation into inorganic molecules such as H_2O, CO_2 and SO_4^{2-} (e.g., Larson *et al.*, 1993).

Several kinetic models are available to describe biodegradation of organic trace compounds such as LAS by activated sludge (ECETOC, 1991; chapter 2). In most studies simple (pragmatic) first-order kinetics were adopted to describe biodegradation of the LAS homologues (e.g., Larson and Payne, 1981; Games *et al.*, 1982; Larson, 1983; Shimp and Larson, 1996; Federle and Itrich, 1997). Because it generally is assumed that micro-organisms do not have direct access to (ad)sorbed compounds, the rate of biodegradation usually is related to dissolved LAS alone:

$$r_{bio} = k_{1S}S \tag{2}$$

where r_{bio} is the (volumetric) rate of biodegradation ($\mu g \cdot l^{-1} \cdot h^{-1}$) and k_{1S} is a pragmatic first-order biodegradation rate constant related to dissolved LAS (h^{-1}).

Because the dissolved concentration S is determined by simultaneously operating biodegradation and sorption, k_{1S} cannot be estimated from measurements of S. A mineralisation variable such as O_2-consumption or CO_2-production should be measured instead. On the other hand, this may lead to erroneous results because for fate modelling the rate of primary biodegradation is required, which can be much faster than the rate of mineralisation.

To explain the extremely high removal efficiencies of surfactants, including LAS, in activated sludge plants, Cowan *et al.* (1993) included the possibility of direct biodegradation of sorbed compounds in their WWTreat model:

$$r_{bio} = k_{1S}S + k_{1q}qX \tag{3}$$

where k_{1q} is a first-order biodegradation rate constant for sorbed LAS (h^{-1}) and X is the concentration of activated sludge (g volatile suspended solids [VSS]$\cdot l^{-1}$).

This equation could be very useful if biodegradation is (partly) accomplished by extracellular enzymes that require a close association of LAS to the sludge. However, it may be impossible to separately assess the rate constants k_{1S} and k_{1q}. If instantaneous sorption equilibration is assumed, a more practicable approach may be to relate the rate of biodegradation to the sum of dissolved and sorbed LAS. Another serious disadvantage of equations (2) and (3) is that they ignore that the sludge concentration probably has a strong positive effect on the rate of biodegradation.

An entirely different approach was followed by Birch (1991) and by van Wijk *et al.* (1996) who used the Monod model (chapter 2) to describe biodegradation of LAS. Also the monitoring data of chapter 4.1 indicated that the Monod model may be valid for biodegradation of LAS. In contrast to the pragmatic models of equations (2) and (3) the Monod model assumes that biodegradation is accomplished by specific micro-organisms that use the target trace compound as their single, growth-limiting substrate. One of the consequences of this assumption would be that the effluent of activated sludge plants contain a LAS concentration which is independent of the influent concentrations of LAS. Remark that this is entirely different from the pragmatic equations above which predict a positive correlation between influent and effluent concentrations.

Purpose of this study - Sorption and primary biodegradation of LAS-C_{12} were investigated with biomass sampled from a pilot-scale municipal activated sludge plant. To be able to discriminate between non-growth biodegradation kinetics (equations 2 and 3) and specific growth as described by the Monod model, the biodegradation kinetics of LAS-C_{12} were determined when the sludge in the plant was acclimated to various influent concentrations of LAS-C_{12}.

Material and methods

Activated sludge plant and operation - A pilot-scale activated sludge plant was employed which consist of a completely-mixed aeration tank (490 l) and a secondary settler (280 l). The plant treated pre-settled municipal wastewater and was operated at different sludge retention times (SRTs) and influent LAS concentrations (Table 1). Each period lasted for at least four times the SRT before sludge was sampled from the plant to carry out sorption and biodegradation experiments. More details about the plant and its operation can be found in chapter 4.1.

Table 1 - Operation of the pilot-scale activated sludge plant.

Parameter	period A		period B	
sludge retention time (SRT) (d)	10		27.3	
hydraulic retention time (HRT) (h)	7.3		12.4	
COD loading rate (kg COD·kg^{-1}SS·d^{-1})	0.37		0.15	
	period A1	period A2	period B1	period B2
influent LAS-C_{12} concentration (mg·l^{-1})	2.0	6.3	1.9	11.5

Chemicals - LAS stock solutions were prepared from a commercial LAS mixture (Isorchem 113/S-Na with a concentration of 154.2 g·l^{-1} and 31.4% (w/w) of LAS-C_{12}) in suprapur water. Other LAS homologues in this stocksolution were LAS-C_{10} (11%), LAS-C_{11} (33.6%) and LAS-C_{13} (24%).

Sorption experiments - A sorption-isotherm and the kinetics of sorption and desorption were determined in batch experiments. Return sludge and final effluent were collected from the pilot-plant during period A1 (Table 1) and were mixed in 200 ml polyethylene (PE) centrifuge tubes for sorption-isotherm tests or in 3.5 l polyvinylchloride (PVC) jars for sorption kinetic tests to give sludge concentrations in a range of 2 to 4 g VSS·l^{-1}. Final effluent rather than tap water or suprapur water was used to maintain as much as possible the original water hardness and pH. The sludge in the centrifuge tubes and PVC jars was gently purged with nitrogen gas to provide mixing and to prevent aerobic biodegradation. Anaerobic biodegradation of LAS by activated sludge has not been found (Brunner *et al.*, 1988; Larson *et al.*, 1993 and Prats *et al.*, 1997). The PVC jars were mechanically stirred at 94 rpm. Known volumes of a LAS stock solution were added to give target initial LAS-C_{12} concentration in a range of 1-10 mg·l^{-1}. In the sorption-isotherm tests, after 12 h of equilibration time, the sludge was centrifuged for 5 minutes at 3500 rpm to determine sorbed and dissolved LAS-C_{12} concentrations. In the sorption kinetic tests a similar procedure was followed but this time 100 ml samples were collected at increasing time intervals (5 minutes - 1 h). After 6 h of equilibration time the sample was centrifuged at 3500 rpm for 5 minutes and 800 ml of the supernatant was discarded and replaced by (fresh) final effluent from the pilot-plant to lower the concentration of dissolved LAS-C_{12}. The sample was subsequently subjected to the same conditions and sampling regime described above. The pH in the tests was not controlled but measurements at the beginning and end of the tests showed that the average pH was 7.2 and did not change significantly during the tests.

Biodegradation tests - Three different types of biodegradation tests were carried out (Table 2), which differed in their operational mode (batch or continuous), substrate to biomass ratio and the type of measurement that was used to follow biodegradation (O_2-consumption or specific LAS-C_{12} analysis).

Table 2 - Biodegradation tests.

test	mode	measurement	sampling periods
ready	batch	O_2-consumption	A1
BAS	batch	LAS-C_{12}	A1
by-pass	continuous	LAS-C_{12}	A1, A2, B1 and B2

Ready biodegradability test

A ready biodegradability test was carried out according to the guidelines of OECD test method encoded 301F (OECD, 1993). An automated sapromate (Voith) was used to follow O_2-consumption in response to LAS mineralisation. An activated sludge sample was taken from the pilot-plant during period A1 (Table 1) and aerated for a period of 48 hours to remove as much as possible the remaining carbon sources and ammonia. The sludge, mineral media and stock solutions of the target compounds were mixed in the sapromate cells to give an initial sludge concentration of 30 mg of dry solids$\cdot l^{-1}$ and 80 mg$\cdot l^{-1}$ of LAS, 25.1 mg$\cdot l^{-1}$ of which is LAS-C_{12}. A test with a blank inoculum was included as well as a test with 109 mg$\cdot l^{-1}$ of the easily biodegradable potassium hydrogen phthalate (PHP) to serve as a method control. All the tests were carried out in duplicate over a period of 28 days at a temperature of 20 °C.

Batch activated sludge (BAS) test

BAS tests were carried out at initial target LAS-C_{12} concentrations of 2.5 and 7.5 mg$\cdot l^{-1}$ and sludge concentrations of approximately 1.5 and 3 g VSS$\cdot l^{-1}$. Return sludge and final effluent were collected from the pilot-plant during period A1 (Table 1) and were mixed in 3 l PVC jars to obtain a working volume of 2.5 l. The jars were mechanically stirred at 20 rpm and aerated with dry, compressed air provided through a diffuser stone. The jars were spiked with a known volume of a LAS stock solution. At increasing time intervals (10 min - 1 h) samples were taken for determination of dissolved and sorbed LAS-C_{12}.

By-pass test

This test was developed to mimic as closely as possible the conditions in the pilot-scale activated sludge plant. A detailed description of the test method can be found in chapter 3. In contrast to the ready and BAS tests, which were only carried out at the end of period A1, the by-pass test was used to determine the kinetics of the sludge when the plant was operated at various SRTs and influent LAS-C_{12} concentrations, i.e., at the end of periods A1, A2, B1 and B2 (Table 1).

Analysis - COD, NH_4-N, NO_3-N, suspended solids (SS) and VSS were all determined according to Dutch standard practice (NEN). The LAS analytical methodology was described earlier in chapter 4.1.

Results

Sorption - Figure 1 shows a typical example of one of the sorption kinetic experiments. Already within 5 to 10 minutes an equilibrium was achieved. Similarly, though less pronounced, desorption also resulted in a rapid equilibration. The sludge-water distributions at equilibrium were between 3.1 and 3.2 $l \cdot g^{-1}$ VSS.

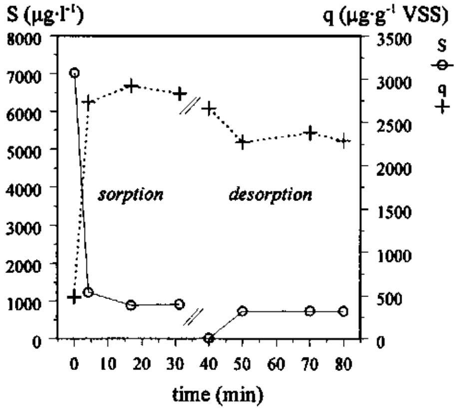

Fig. 1 Sorption and desorption of LAS-C_{12} by activated sludge.

Figure 2 combines the results of several sorption experiments. Each of the data-points was determined after 6 h of equilibration time and, based on the results of Figure 1, it can be safely assumed that an equilibrium was achieved. The analytical recovery, calculated from the sum of sorbed and dissolved LAS-C_{12}, varied between 86 and 108% and generally was somewhat higher in those tests with higher initial LAS-C_{12} concentrations. Nevertheless, the data in Figure 2 could be described by a linear sorption-isotherm (equation 1) with a sludge-water partition coefficient K_p of 3.2 $l\cdot g^{-1}$ VSS (correlation coefficient 0.991). This is similar to the K_p that was estimated from the data in Figure 1.

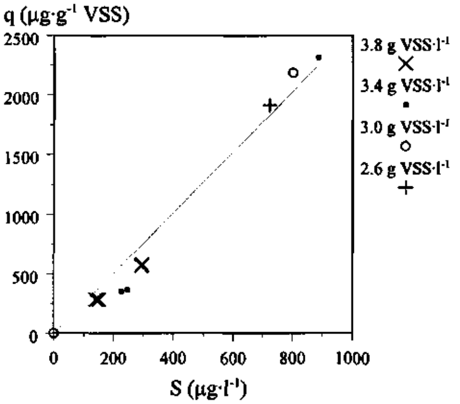

Fig. 2 Sorption-isotherm for LAS-C_{12} to activated sludge.

Ready biodegradability test - The O_2-consumption data generated in the ready biodegradability tests (data not shown) confirmed that LAS is readily biodegradable as defined by the OECD Guidelines (OECD, 1993) because (i) more than 60% of mineralisation was achieved within 28 days and (ii) this level was reached within a time window of 10 days from the start of mineralisation. It is assumed that all four homologues (LAS-C_{10} to LAS-C_{13}) are equally biodegradable (Larson *et al.*, 1993) and can be described by the same biodegradation kinetics. The results show that growth on LAS as the single carbon- and energy source is possible, which suggests that a (growth) Monod model (chapter 2) should be used to describe biodegradation of LAS. From the O_2-consumption data the Monod parameters biomass-yield Y and maximum specific growth rate μ_{max} were estimated according to a procedure described by Ellis *et al.* (1996). A yield-coefficient Y of 0.50 ± 0.02 mg $O_2\cdot mg^{-1}$ O_2 was found and a specific maximum growth rate μ_{max} of 0.050 ± 0.003 h^{-1}. This maximum specific growth rate is somewhat faster than a rate of 0.036 to 0.042 h^{-1} reported by Hrsak (1982). It was not possible to obtain a reliable estimate for another Monod parameter, the half-saturation concentration K_S.

BAS test - Figure 3 shows the concentrations of dissolved (S), sorbed (q) and total (C) LAS-C_{12} during one of the BAS tests. A rapid partitioning of LAS-C_{12} between sludge and water within the first 5 minutes supports the observation made earlier that sorption is an extremely fast process. After this initial distribution phase, dissolved and sorbed LAS-C_{12} disappear rapidly from the batch due to biodegradation.

Initially, the biodegradation kinetics were estimated using the results of each individual BAS test. However, even though the individual model fits were reasonably well, due to a lack of data-points in either the first-order or zero-order region, the parameters exhibited considerable variation among the batches. A different procedure therefore was followed in which the data were combined from those tests which were carried out at the same sludge concentration. For each data-point the rate of biodegradation was calculated and in Figure 4 these rates are plotted against their corresponding concentrations.

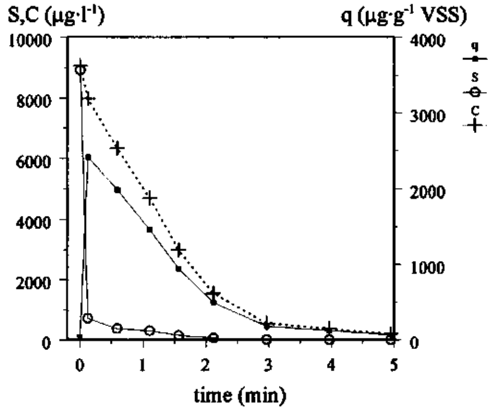

Fig. 3 Dissolved (S), sorbed (q) and total (C) concentration of LAS-C_{12} in one of the BAS tests.

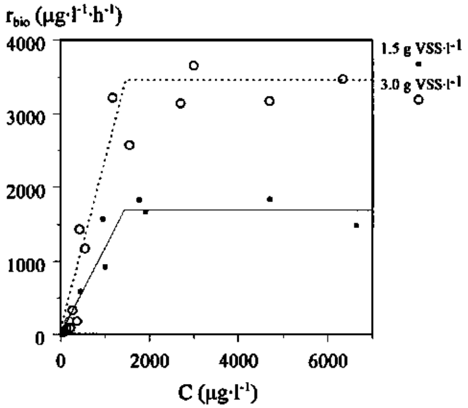

Fig. 4 Rate of biodegradation (r_{bio}) as a function of the total concentration of LAS-C_{12} (C) determined in BAS tests carried out at two different sludge concentrations.

From these plots the following combined first- and zero-order model was estimated:

$$r_{bio} = k_1 X C \quad \text{if} \quad C < C_{1/0} \tag{4a}$$

$$r_{bio} = k_0 X \quad \text{if} \quad C \geq C_{1/0} \tag{4b}$$

where k_1 is a first-order rate constant ($l \cdot g^{-1}$ $VSS \cdot h^{-1}$), k_0 is a zero-order rate constant ($\mu g \cdot g^{-1}$ $VSS \cdot h^{-1}$) and $C_{1/0}$ is the concentration where the kinetics change from first- to zero-order ($\mu g \cdot l^{-1}$). Estimated parameters for this combined first- and zero-order model can be found in Table 3.

By-pass tests - By-pass test were carried out when the pilot-plant was operated at different influent LAS-C_{12} concentrations and SRTs. The results of these tests are presented in Figure 5. Similar to the BAS tests, a combined first- and zero-order model (equations 4a and 4b) was estimated from the results and the model parameters are presented in Table 3.

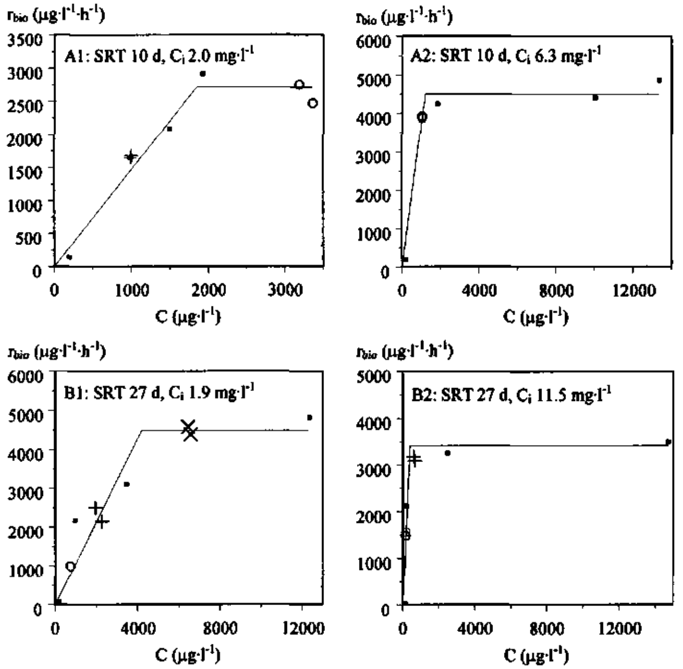

Fig. 5 Biodegradation kinetics of LAS-C_{12} at different SRTs and influent LAS-C_{12} concentrations (C_i) (• represents single measurements and +, o and × represent duplicate measurements).

Table 3 - Results from the BAS and by-pass tests with LAS-C_{12}. (C_i = influent LAS-C_{12} concentration. For the other symbols refer to the text).

	BAS tests	by-pass tests			
	period A1	period A1	period A2	period B1	period B2
plant operation					
SRT (d)	10	10	10	27.3	27.3
HRT (h)	7.3	7.3	7.3	12.4	12.4
X (g VSS·l⁻¹)	3.35	3.35	3.77	5.22	5.59
C_i (µg·l⁻¹)*	2039 ± 72**	2039 ± 72	6315 ± 537	1908 ± 132	11505 ± 593
kinetic parameters					
k_1 (l·g⁻¹VSS·h⁻¹)	0.68 (0.936***)	0.46 (0.974)	0.99 (0.999)	0.20 (0.888)	0.95 (0.833)
k_0 (µg·g⁻¹ VSS·h⁻¹)	1116 ± 85**	810 ± 68	1196 ± 86	860 ± 42	583 ± 31
$C_{1/0} = k_0/k_1$ (µg·l⁻¹)	1642	1753	1212	4369	613

* average from 24-h samples as reported in chapter 4.1.

** standard deviation

*** correlation coefficient

Discussion

Sorption - Sorption equilibration of LAS-C_{12} to activated sludge was practically instantaneous and could be described by a linear sorption-isotherm with a partition coefficient K_p of 3.2 $l \cdot g^{-1}$ VSS. However, these results may not be entirely realistic as the concentrations of LAS-C_{12} (1-10 $mg \cdot l^{-1}$) in these tests were 5 to 40 times higher than concentrations that prevail in an activated sludge plants (200 - 250 $\mu g \cdot l^{-1}$, chapter 4.1). Also, from sorbed and dissolved LAS-C_{12} concentrations in sludge samples taken from the pilot-plant a higher K_p was estimated of 3.7 $l \cdot g^{-1}$ VSS (chapter 4.1). Possibly, this difference can be attributed to a small fraction of sorbed LAS-C_{12} being less available for biodegradation. This fraction becomes increasingly important at lower total concentrations of LAS-C_{12}. Cowan *et al.* (1993) observed a similar phenomenon for the highly sorptive cationic surfactants C_{12}TMAC and DTDMAC. These compounds were detected at significantly higher sorbed concentrations than what could be expected from their K_p values.

Water hardness has a strong positive effect on sorption of LAS-C_{12} (Berna *et al.*, 1989; Prats *et al.*, 1993; Westall *et al.*, 1999). Because the municipal wastewater that was used in this study was relatively soft , the K_p that was found may be low compared to the K_p of sludge in other municipal activated sludge plants.

Biodegradation kinetics - The results from the BAS and by-pass tests all exhibited a more pronounced transition from first- to zero-order kinetics than for instance a Michaelis-Menten like equation would predict. This phenomenon has been frequently reported in the literature and several models, both empirical and mechanistic, have been proposed to describe this behaviour (Kovárová-Kovar and Egli, 1998). However, based on the experimental results a distinction between these models cannot be made and therefore it was decided to use the simple empirical first- and zero-order model of equations (4a) and (4b).

Because the BAS tests clearly demonstrated that the (non-specific) sludge concentration X, expressed as volatile suspended solids, has an effect on the rate of biodegradation (Figure 4), this concentrations was included in the biodegradation kinetics of equation (4). This infers that the parameters in pragmatic kinetic models such as equations (2) and (3), cannot be extrapolated to sludge concentrations other than those at which they were assessed.

Fate models which employ first-order kinetics ignore that the biomass has a maximum biodegradation capacity as represented by the zero-order biodegradation rate k_0. For LAS-C_{12} this seems to be a valid assumption since LAS-C_{12} concentrations in the completely mixed aeration tank of the pilot-plant (200-300 $\mu g \cdot l^{-1}$, chapter 4.1) were much lower than the concentration $C_{1/0}$ where the kinetics change from first- to zero-order (600-4500 $\mu g \cdot l^{-1}$, Table 3). However, at extremely short hydraulic retention times and for trace compounds which are not as readily biodegradable as LAS-C_{12} zero-order kinetics may become important.

The high rate of disappearance of sorbed LAS-C_{12} observed in the BAS tests showed that sorbed LAS-C_{12} is readily available for biodegradation. However, it remains unclear whether this is caused by (i) rapid desorption of LAS to replenish dissolved LAS-C_{12} that is removed by biodegradation or (ii) a combination of rapid desorption and direct biodegradation of sorbed LAS-C_{12}. All the same, for a practical application in activated sludge fate models a distinction between these two mechanisms is not relevant and the kinetics may as well be related to the sum of sorbed and dissolved LAS-C_{12}.

Extrapolation of the biodegradation kinetics to other plants - The biodegradation parameters of equations (4a) and (4b) are properties of the sludge at the time of sampling, i.e., given the operational characteristics such as the SRT and HRT and the influent concentration of LAS-C_{12} the sludge was acclimated to. To extrapolate to other conditions a choice has to be made between a (non-growth and non-specific) secondary utilisation model and the (specific and growth) Monod model. The Monod model dictates that the zero-order biodegradation rate constant k_0 is related to a maximum specific growth rate μ_{max} according to:

$$k_0 = \frac{\mu_{max}}{Y}\frac{B}{X} \qquad (5)$$

where B is the concentration of specific LAS-C_{12} degraders in the sludge (g VSS·l^{-1}). In case of a completely mixed bioreactor with biomass recycle B can be calculated from (chapter 2):

$$B = \frac{\theta_c Y(C_i - C_e)}{\theta_h(1 + k_d\theta_c)} \qquad (6)$$

where θ_c en θ_h are the SRT and HRT, respectively (h), C_i and C_e are the influent and effluent LAS-C_{12} concentrations (g·l^{-1}) and k_d is the decay rate of the specific biomass (h^{-1}).

The monitoring data in chapter 4.1 showed that C_e is negligible small as compared to C_i. Furthermore, neglecting decay and combining equations (5) and (6) gives the following linear relationship between k_0, the SRT and the sludge loading rate with LAS q_x (in µg·g^{-1} VSS·h^{-1}):

$$k_0 = \mu_{max}\theta_c q_x \qquad (7)$$

Using the μ_{max} of 0.05 h^{-1} determined in the ready biodegradability test, this theoretical relation between k_0 and $\theta_c q_x$ was calculated and in Figure 6 is compared to values for k_0 determined in the BAS and by-pass tests. This comparison shows that, in contrast to what the Monod model predicts, k_0 is independent of the SRT and LAS loading rate, and seems to be rather constant with a value of 920 ± 240 µg·g^{-1} VSS·h^{-1}.

There are several explanations for this deviation from the Monod model. First, at high loading rates LAS-C_{12} may exhibit a toxic effect. However, this is unlikely because (*i*) the concentrations in the pilot-plant were at least a factor 100 lower than a concentration of 35 mg·l^{-1} where LAS supposedly becomes toxic to activated sludge (Painter, 1992) and (ii) neither LAS-C_{12} removal nor removal of the standard water quality parameters (COD and NH_4-N) deteriorated due to enhanced influent LAS-C_{12} concentrations during periods A2 and B2. A second explanation could be a changing population of LAS-C_{12} degraders with slower specific growth rates at higher influent concentrations.

However, this would have to result in higher effluent LAS-C_{12} concentrations and cannot explain the consistently low effluent concentrations of LAS-C_{12} that were observed (<10 $\mu g \cdot l^{-1}$, chapter 4.1).

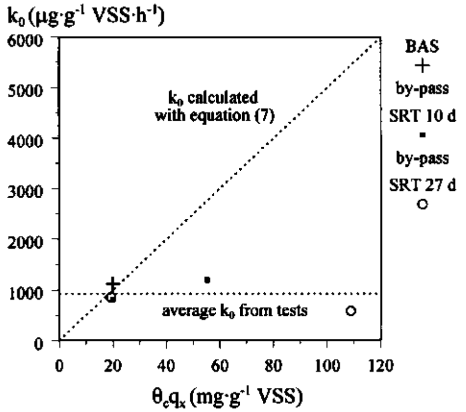

Fig. 6 Effect of the product of the SRT and the LAS-C_{12} loading rate $\theta_c q_x$ on the zero-order biodegradation rate constant k_0.

A third and most likely explanation is that a large and non-specific fraction of the biomass is capable to accomplish the first step in the biodegradation pathway of LAS-C_{12}, i.e., subsequent ω/β-oxidation of the alkyl chain (Jiménez *et al.*, 1991). This implies that primary biodegradation (parent compound removal) of LAS-C_{12} should be described by a secondary utilisation model rather than by the Monod model. It is important to recognise that this only applies to primary biodegradation, and not to ultimate biodegradation of LAS-C_{12}. In the biodegradation pathway a small group of specific micro-organisms is involved which is responsible for fission of the benzene ring of LAS-C_{12} (Jiménez *et al.*, 1991). Because ring-fission is considered to be the rate-limiting step (Larson *et al.*, 1993), ultimate biodegradation probably has to be described by Monod kinetics (van Ginkel, 1996).

Affinity - Similar to the half-saturation constant in the Monod model, $C_{1/0}$ is a complex parameter which combines the effect of (i) the population that is responsible for degradation, (ii) transport of the trace compound to the appropriate enzymes and (iii) the affinity of these enzymes for the trace compound, which may be subject to physiological adaptation (Templeton and Grady, 1988; Kuvárová-Kover and Egli, 1998). This complexity may explain why in the literature often a wide variety of values is reported for affinity constants, even in the case of pure cultures growing on a single substrate. Also in our experiments different values in a range of 600-4400 $\mu g \cdot l^{-1}$ were found for $C_{1/0}$ (Table 3). From Figure 7, in which $C_{1/0}$ is plotted as a function of the sludge loading rate with LAS (q_x), it appears that $C_{1/0}$ decreases with q_x. No obvious explanation is available for this phenomenon. More research is required to elucidate this relationship as it seems to control primary biodegradation of LAS-C_{12}.

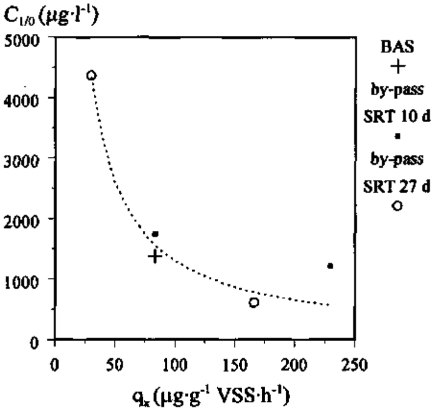

Fig. 7 Effect of sludge loading rate with LAS-C_{12} (q_x) on the affinity parameter $C_{I/O}$.

Conclusions

It was shown that sorption of LAS-C_{12} to activated sludge is an instantaneous equilibration process which can be described by a linear sorption isotherm. To describe primary biodegradation of LAS-C_{12} secondary utilisation kinetics should be used which (i) include the effect of the (non-specific) sludge concentration, (ii) are a combination of first- and zero-order kinetics and (iii) are related to the sum of dissolved and sorbed LAS-C_{12}. A serious problem in modelling the kinetics of primary biodegradation is that the concentration where the kinetics change from first- to zero-order is not a true constant but was found to decrease with an increasing loading rate of the sludge with LAS-C_{12}.

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Chapter 4.3

Fate of Linear Alkylbenzene Sulfonates (LAS) in activated sludge plants

- model validation and sensitivity analysis -

Abstract - The results of sorption and biodegradation experiments were used to develop a simple mathematical model predicting the fate of the C_{12}-homologue of linear alkylbenzene sulfonate (LAS-C_{12}) in municipal activated sludge plants. This model was validated against an extensive set of monitoring data collected in a pilot-scale plant when this plant was operated at various influent LAS-C_{12} concentrations and two sludge retention times. The results of this validation exercise showed that the model could accurately predict the fate of LAS-C_{12} in the plant. Only the effluent concentrations were slightly overestimated, probably because too low a sludge-water distribution coefficient was used in the model calculations. A sensitivity analysis demonstrated that LAS-C_{12} removal efficiency is favourable in plants which are operated at high sludge concentrations and have excellent sludge-water separation facilities.

Introduction

In chapter 4.1 monitoring data were collected to determine the behaviour of the C_{12} homologue of linear alkylbenzene sulfonate (LAS-C_{12}) in a pilot-scale municipal activated sludge plant, with the purpose to use these data to validate existing activated sludge fate models. However, from the results of several biodegradation tests it was suspected that existing activated sludge fate models employ incorrect biodegradation kinetics for LAS-C_{12} (chapter 4.2). Therefore, the results of these tests were used to develop a new model. In this chapter this model was corroborated with the monitoring data obtained in chapter 4.1. Also, a model sensitivity analysis was carried out to identify those parameters having the greatest impact on the fate of LAS-C_{12}.

Model algorithms

Sorption - In chapter 4.2 it was established that (i) sorption of LAS-C_{12} to activated sludge is an instantaneous equilibration process and that (ii) the equilibrium between sorbed and dissolved LAS-C_{12} can be described by a linear sorption-isotherm. Combining these observations means that the concentrations of dissolved and sorbed LAS-C_{12} can be calculated from the total concentration of LAS-C_{12} according to:

$$S = \frac{1}{1+K_p X} C \tag{1}$$

$$q = \frac{K_p}{1+K_p X} C \tag{2}$$

where K_p is the sludge-water partition coefficient ($l \cdot g^{-1}$VSS), C the total concentration of LAS-C_{12} (i.e., the sum of sorbed and dissolved LAS-C_{12}), q the sorbed concentration ($\mu g \cdot g^{-1}$ VSS), S the dissolved concentration ($\mu g \cdot l^{-1}$) and X the concentration of volatile suspended solids (g $VSS \cdot l^{-1}$).

Primary biodegradation - The results of several biodegradation tests (chapter 4.2) were compiled into the biodegradation kinetics schematically shown in Figure 1. These kinetics reflect primary biodegradation, i.e., parent compound removal rather than ultimate biodegradation into inorganic molecules. Because no distinction can be made between biodegradation of sorbed and dissolved LAS-C_{12}, the kinetics are related to the total concentration of LAS-C_{12}, C. The rate of biodegradation r_{bio} (in $\mu g \cdot l^{-1} \cdot h^{-1}$) could be described by a simple combined first- and zero-order model according to:

$$r_{bio} = k_1 X C \quad \text{if} \quad C < C_{1/0} \tag{3a}$$

$$r_{bio} = k_0 X \quad \text{if} \quad C \geq C_{1/0} \tag{3b}$$

where k_1 is a first-order rate constant ($l \cdot g^{-1} VSS \cdot h^{-1}$), k_0 is a zero-order rate constant ($\mu g \cdot g^{-1} VSS \cdot h^{-1}$) and $C_{1/0}$ is the concentration C where the kinetics change from first- to zero-order ($\mu g \cdot l^{-1}$). Remark that the biodegradation parameters in equations (3a) and (3b) are related according to:

$$C_{1/0} = \frac{k_0}{k_1} \qquad (4)$$

and that $C_{1/0}$ is a measure of the affinity of the biomass to scavenge LAS-C_{12}.

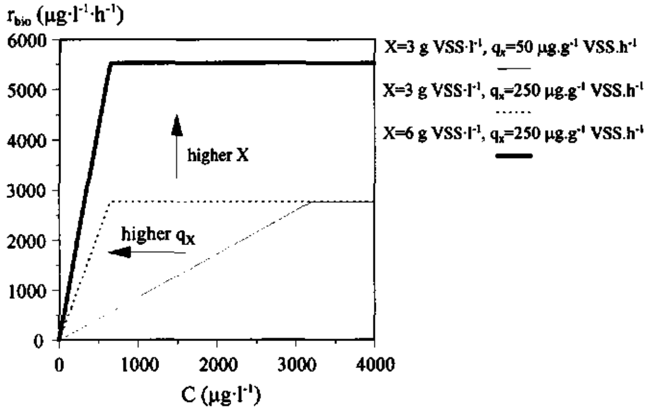


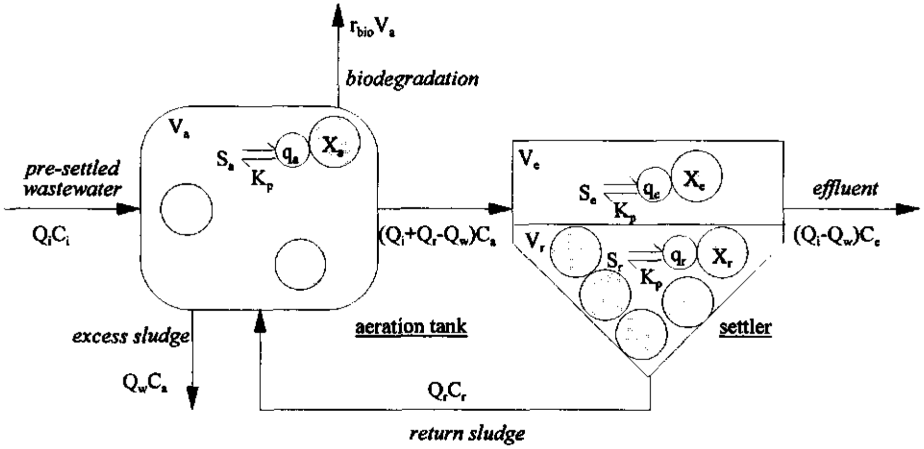
Fig. 1 Biodegradation rate of LAS-C_{12} (r_{bio}) as a function of the (total) concentration of LAS-C_{12} (C) at two different sludge loading rates with LAS-C_{12} (q_x) and at two different sludge concentrations (X).

In chapter 4.2 it was observed that the maximum capacity of the sludge to biodegrade LAS-C_{12}, expressed by the zero-order biodegradation rate constant k_0, was more or less independent of the influent concentration of LAS-C_{12}. This indicates that biodegradation of LAS-C_{12} follows secondary utilisation kinetics, i.e., growth of the biomass that is responsible for LAS-C_{12} degradation mainly depends on the supply of another (primary) substrate. The concentration $C_{1/0}$ where the kinetics change from first- to zero-order on the other hand was not a constant but was found to decrease with an increasing influent load of LAS-C_{12} (chapter 4.2). The underlying mechanisms for this dependency are not understood and in the model the following empirical relationship will be used:

$$C_{1/0} = \frac{\alpha}{q_x} \qquad (5)$$

where α is an empirical constant ($\mu g^2 \cdot l^{-1} \cdot g^{-1} VSS \cdot h^{-1}$) and q_x is the (sludge) loading rate with LAS-C_{12} ($\mu g \cdot g^{-1} VSS \cdot h^{-1}$).

Model activated sludge plant - Similar to the pilot-scale activated sludge plant in which the monitoring data were collected (chapter 4.1), the model activated sludge plant (Figure 2) consists of a completely mixed aeration tank and a secondary settler. The plant treats pre-settled domestic wastewater. A constant sludge retention time (SRT) is maintained by continuously wasting excess sludge from the aeration tank. The settler is divided into two completely mixed compartments: a compartment for clarified effluent and a compartment containing settled (return) sludge. Furthermore, it is assumed that biodegradation can only take place in the aeration tank.



symbols		subscripts	
C	total concentration of LAS-C_{12} ($\mu g \cdot l^{-1}$)	a	aeration tank
K_p	sludge-water partition coefficient ($l \cdot g^{-1}$ VSS)	e	effluent
Q	flow rate ($l \cdot h^{-1}$)	i	influent
q	sorbed concentration of LAS-C_{12} ($\mu g \cdot g^{-1}$ VSS)	r	return sludge
r_{bio}	biodegradation rate ($\mu g \cdot l^{-1} \cdot h^{-1}$)	w	waste sludge
S	dissolved concentration of LAS-C_{12} ($\mu g \cdot g^{-1}$ VSS)		
V	volume (l)		
X	sludge concentration (g VSS$\cdot l^{-1}$)		

Fig. 2 Model activated sludge plant and fluxes of LAS-C_{12} in this plant.

Model calculations - Mass balances for LAS-C_{12} were written around every compartment and these were solved to yield the concentrations of total, dissolved and sorbed LAS-C_{12} in the different compartments.

Model validation

The model parameters that were used are presented in Tables 1 and 2. The biodegradation and sorption parameters of Table 1 were obtained in chapter 4.2. The sludge-water partition coefficient K_p was determined in separate sorption experiments. The zero-order biodegradation rate constant k_0 was calculated as the average of several values for k_0 determined in biodegradation tests carried out with sludge sampled from the pilot-plant when the sludge was acclimated to different influent LAS-C_{12} concentrations. The parameter α was estimated according to equation (5) from a relation between the concentration where the kinetics change from first-order to zero-order $C_{1/0}$ and the sludge loading rate with LAS-C_{12} q_x. The plant operational parameters of Table 2 were reported earlier in chapter 4.1.

Table 1 - Sorption and biodegradation parameters and plant characteristics.

Parameter	value
sorption	
K_p ($l \cdot g^{-1}$ VSS)	3.2
biodegradation	
α ($\mu g^2 \cdot l^{-1} \cdot g^{-1}$ VSS)	160000
k_0 ($\mu g \cdot g^{-1}$ $VSS \cdot h^{-1}$)	760
plant characteristics	
V_a (l)	490
V_e (l)	50
V_r (l)	150

Table 2 - Plant operational parameters.

parameter	period A		period B	
X_e ($gVSS \cdot l^{-1}$)	0.008		0.022	
SRT (d)	10		27.3	
Q_i ($l \cdot h^{-1}$)	67.4		39.6	
Q_r ($l \cdot h^{-1}$)	64.2		36.6	
Q_w ($l \cdot h^{-1}$)	2.08		0.76	
	period A1	period A2	period B1	period B2
X_a ($gVSS \cdot l^{-1}$)	3.35	3.77	5.22	5.59
X_r ($gVSS \cdot l^{-1}$)	5.11	4.93	9.66	11.24
C_i ($\mu g \cdot l^{-1}$)	2039*	6315**	1908*	11505**

* background concentrations in the wastewater

** sum of background concentrations and external addition

Figure 3 compares observed and model predicted distribution of LAS-C_{12} in the pilot-plant. The model agrees with the monitoring data indicating that more than 99% of the influent load of LAS-C_{12} is removed by biodegradation and less than 1% of this load is discharged from the plant with the final effluent or with the excess sludge. Although the effluent and excess sludge fluxes of LAS-C_{12} were extremely low, the model still is able to match the observed trend of a higher discharge in the order period A1 > period B1 > period A2 ≈ period B2.

Figure 4 compares measured and model predicted concentrations of LAS-C_{12} in the aeration tank (C_a, S_a and q_a), return sludge (C_a, S_a and q_a) and final effluent (C_e). The predictions generally are in good agreement with the monitoring data, that is, they are within the range of standard deviation of the measurements. Only the predicted effluent concentrations (C_e) systematically are 40 to 50% higher than observed effluent concentrations. Except for the dissolved concentration of LAS-C_{12} in the return sludge (S_r) in period A2, the model output follows the observed trend in concentrations among the different periods very well.

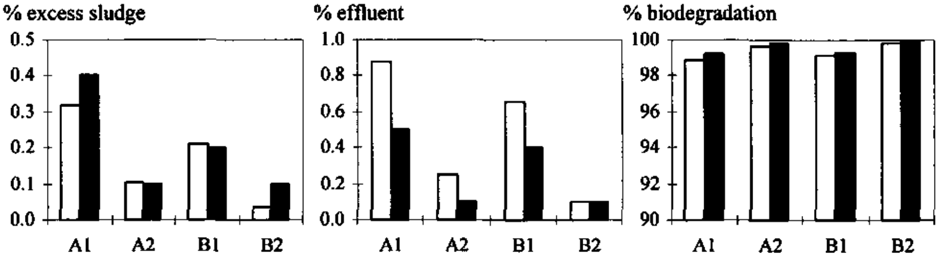


Fig. 3 Observed (white) and model predicted (black) distribution of LAS-C_{12} in a pilot-scale activated sludge plant.

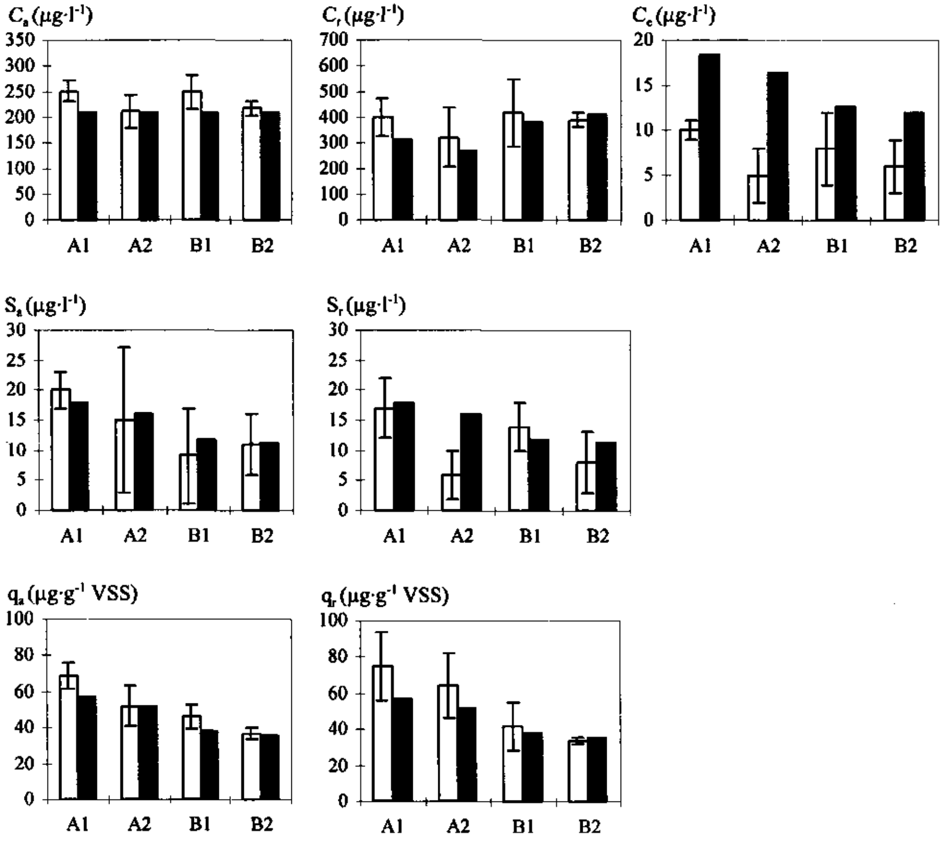


Fig. 4 Observed (white) and predicted (black) LAS-C_{12} concentrations in a pilot-scale activated sludge plant (C, S and q are total, dissolved and sorbed concentrations respectively; subscripts a, e and r denote aeration tank, final effluent and return sludge respectively; error bars represent standard deviations in monitoring data).

Sensitivity analysis

A model sensitivity analysis was carried out to identify those parameters that have the greatest impact on the fate of LAS-C_{12}. Each time one of the parameters of Table 3 was varied whereas the other parameters were kept constant. The operational parameters of period A1 were taken from Table 2 to describe the "average" plant.

Table 3 - Parameter variation applied in the model sensitivity analysis.

parameter	average	variation min	variation max
C_i ($\mu g \cdot l^{-1}$)	2000	500	10000
hydraulic retention time (h)	7.3	1.5	25
sludge retention time (d)	10	0.5	40
X_a ($g\ VSS \cdot l^{-1}$)	3.35	1	12
K_p ($l \cdot g^{-1}\ VSS$)	3.2	2.5	4.2
X_e ($mg \cdot l^{-1}$)	8	0	200
α ($\mu g^2 \cdot l^{-1} \cdot g^{-1}\ VSS \cdot h^{-1}$)	160000	100000	210000
k_0 ($\mu g \cdot g^{-1}\ VSS \cdot h^{-1}$)	920	530	970

Graphic presentations of the most important results can be found in Figure 5. The effect of the HRT, influent LAS-C_{12} concentration and SRT are not shown as these only begin to have an effect below extremely low and unrealistic values of 1.5 h, 500 $\mu g \cdot l^{-1}$ and 2 d, respectively. The insensitivity to the HRT and influent LAS-C_{12} concentration can be explained by a higher affinity of the biomass for LAS-C_{12} at higher loading rates (lower $C_{1/0}$ in equation 5), which is accompanied by a faster rate of biodegradation. The SRT determines the amount of excess sludge that is produced. From this, the SRT could be expected to have an impact on the load of (sorbed) LAS-C_{12} leaving the system with this excess sludge and herewith on the average concentration of LAS-C_{12} in the plant itself. However, compared to biodegradation this removal pathway has a negligible contribution (Figure 3), explaining the insensitivity of the model to the SRT. Only at SRTs below 2 d removal of LAS-C_{12} with the excess sludge gradually becomes more important.

Sludge concentrations in the system X_a between 1 and 12 g VSS$\cdot l^{-1}$ and sludge-water partition coefficients K_p between 2.5 and 4.2 exhibit no effect on the concentration of LAS-C_{12} in the aeration tank, but have a strong impact on effluent LAS-C_{12} concentrations (Figures 5a and 5b). Higher concentrations of effluent suspended solids X_e cause higher effluent LAS-C_{12} concentrations (Figure 5c) because sorbed LAS-C_{12} which is associated with these solids escapes biodegradation.

The biodegradation parameters α en k_0 have strong but opposing effects on the concentrations of LAS-C_{12} (Figures 5d en 5e). It is recognised that these effects may be unrealistic as no information whatsoever is available about the variation of α and k_0 that can be expected in reality.

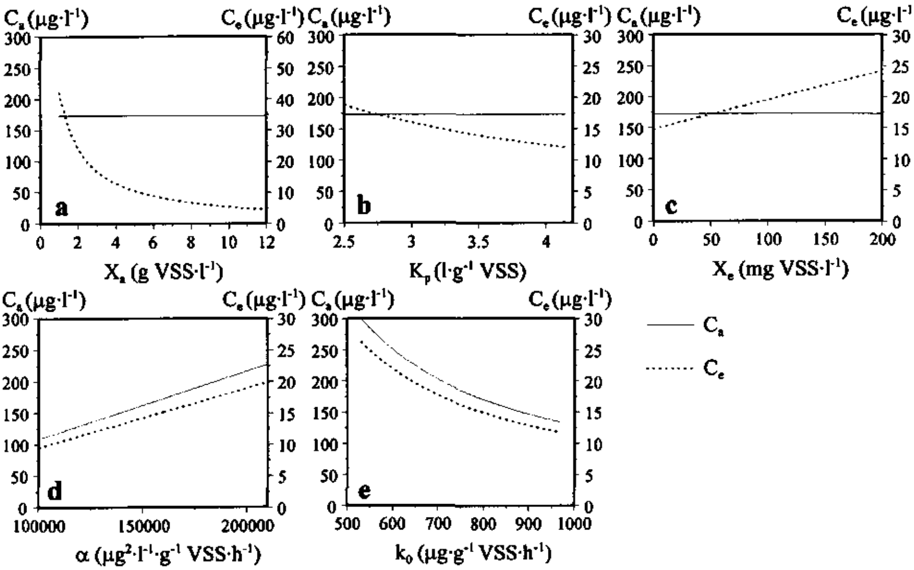


Fig. 5 Effect of the sludge concentration in the aeration tank X_a (**a**), sludge-water partition coefficient K_p (**b**), effluent suspended solids concentration X_e (**c**) and the biodegradation parameters α (**d**) and k_0 (**e**) on the concentrations of LAS-C_{12} in the aeration tank C_a (solid lines) and final effluent C_e (dotted lines).

Discussion

Overestimation of effluent LAS-C_{12} concentrations - The model systematically overestimates effluent concentrations of LAS-C_{12} by 40-50% (Figure 4). Partly this can be explained by too low a sludge-water distribution coefficient K_p of 3.2 $l \cdot g^{-1}$ VSS. This parameter was determined in separate sorption tests at relatively high (initial) LAS-C_{12} concentrations (chapter 4.2). However, in the pilot-plant the LAS-C_{12} concentrations are 5 to 40 times lower due to biodegradation and a significantly higher K_p of 3.7 $l \cdot g^{-1}$ VSS was estimated from these concentrations (chapter 4.1). As was shown by the sensitivity analysis a higher K_p would yield significantly lower effluent concentrations (Figure 5b). Consequently, to obtain accurate model predictions an accurate value for the K_p should be assessed.

Insensitivity to the influent concentration of LAS-C_{12} - The model output is insensitive to the influent concentration of LAS-C_{12} because a change in this influent concentration is compensated for by a change of the concentration where the biodegradation kinetics change from first-order to zero-order $C_{1/0}$. This phenomenon of "affinity-controlled" biodegradation has not only been observed for LAS-C_{12}, but also for nitrilotriacetic acid (chapter 5). It is recognised however that the model output relies strongly on the empirical relation that was established between influent loading rate and $C_{1/0}$ in equation (5), whereas such a relation is not in any way supported by the literature. Partly this may be explained by a lack of literature data on the effect of the influent concentration on the biodegradation kinetics of activated sludge and partly by a lack of suitable methods to assess these kinetics. It could be interesting to further investigate the underlying mechanisms, which may have a microbiological as well as a chemical character.

Effect of the sludge concentration - The sensitivity analysis shows that higher (non-specific) sludge concentrations in the plant result in lower effluent concentrations of LAS-C_{12} (Figure 5a). This is caused by a shift from dissolved to sorbed LAS-C_{12} at higher sludge concentrations. As a result, a larger fraction of LAS-C_{12} can be retained in the system and in this manner is subjected to a longer treatment time. Higher effluent suspended solids concentrations (Figure 5c) have the opposite effect because LAS-C_{12} associated with these solids will escape biodegradation.

Although the SRT does not have a (direct) effect, indirectly longer SRTs promote LAS-C_{12} elimination as these are accompanied by higher (non-specific) sludge concentrations. This effect of the SRT was not incorporated in the model but can easily be implemented, for instance with the IAWQ model No. 1 (Henze *et al.*, 1987) which can predict the (non-specific) sludge concentration from the total organic load (expressed as chemical oxygen demand) to a plant.

Biodegradation parameters - The biodegradation parameters α and k_0 have a considerable impact on the fate of LAS-C_{12} (Figures 5d and e). However, it is not known to what extent these parameters vary in practice. Most likely, wastewater composition and strength will be important factors determining these parameters.

Reliability of existing activated sludge fate models - This validation exercise demonstrates that existing activated sludge fate models will give erroneous predictions of the fate of LAS-C_{12} in municipal activated sludge plants.

In accordance with the model described in this paper, the WWTreat model proposed by Cowan *et al.* (1993), considers LAS-C_{12} to be a secondary substrate. Another similarity is that the WWTreat also takes a longer retention time of sorbed LAS-C_{12} into account. However, changing biodegradation parameters in response to a changing influent concentration of LAS-C_{12} is not considered. As a result, WWTreat will fail to predict that the effluent concentration of LAS-C_{12} is insensitive to the influent concentration of LAS-C_{12}.

Models which are based on Monod kinetics (Birch, 1991; van Wijk *et al.*, 1996) do agree with the observations that the effluent concentration is independent of the influent concentration, even though the Monod model is based on entirely different assumptions. However, the Monod model fails to predict that higher non-specific sludge concentrations will result in lower effluent LAS concentrations.

Conclusions

A model was developed which could accurately predict the fate of LAS-C_{12} in a municipal pilot-scale activated sludge plant. The most important characteristics of this model are that biodegradation is the dominant elimination process and increasing influent concentrations of LAS-C_{12} are compensated for by an increasing biodegradation rate due to a higher affinity of the biomass to scavenge LAS-C_{12}. Final effluent concentrations of LAS-C_{12} that can be achieved are mainly determined by sludge-water partitioning. High sludge concentrations in the plant and low effluent suspended solids concentrations are favourable conditions to achieve a large degree of LAS elimination.

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Chapter 5

Fate of nitrilotracetic acid (NTA) in activated sludge plants

Abstract - Nitrilotriacetic acid (NTA) was one of the test compounds in a study towards the reliability of activated sludge fate models. For this purpose the behaviour of NTA in a pilot-scale municipal activated sludge plant was investigated. The plant was operated at sludge retention times of 10 and 27.3 d and received influent concentrations of NTA between 0.2 and 35 $mg \cdot l^{-1}$. More than 99% NTA removal was achieved, irrespective of the operational conditions. Effluent concentrations of NTA always were lower than the method detection limit. Sorption and subsequent discharge with the excess sludge was found to be an insignificant removal pathway and the high removal efficiencies for NTA could be attributed entirely to biodegradation. Most activated sludge fate models either employ secondary utilisation kinetics or Monod kinetics to describe biodegradation of trace compounds by activated sludge. However, the results of several biodegradation tests combined with information from the literature, showed that neither of these models can be used to accurately predict the biodegradation rate of NTA. Although at high influent NTA concentrations the Monod model could be valid, at low influent concentrations this model may have to be replaced by a different, more complex model including enzyme induction. Furthermore, the potential of the biomass to scavenge NTA was found to depend on the influent concentration of NTA. Only little is known about the underlying mechanisms that cause these deviations from existing activated sludge fate models.

Introduction

Nitrilotriacetic acid (NTA) was one of the test compounds in a study towards the reliability of models that can be used to predict the distribution of organic trace pollutants in municipal activated sludge plants. NTA is used in large quantities as a substitute for polyphosphates in washing powders, and as a chelating agent for Ca^{2+} and Mg^{2+} in water treatment where control of water hardness is required, for instance in the textile, paper and galvanizing industry (BUA, 1986). Monitoring studies in several countries have shown that NTA is present in (pre-settled) domestic wastewater at concentrations between 0.2 and 3.6 $mg \cdot l^{-1}$ (Woodiwiss and Walker, 1979; Perry *et al.*, 1984; BUA, 1986; Giger *et al.*, 1987; Alder *et al.*, 1990). These concentrations generally are in good agreement with what can be expected from average data on the NTA content of detergents, detergent consumption and wastewater production (Woodiwiss and Walker, 1979; BUA, 1986).

Because of its carcinogenicity and potential to remobilise heavy metals from polluted soils and sediments, NTA removal in (municipal) activated sludge plants already has received considerable attention. The behaviour of NTA in these plants is determined by two processes: sorption and biodegradation. Contradictory results have been reported regarding sorption. While Shumate *et al.* (1970) and Bernhardt (1984) reported that sorption is a negligible process, other investigations have shown that 5 to 30% of the NTA-load to an activated sludge plant can be accumulated by the sludge and in this manner is removed with the excess sludge (e.g. Rossin *et al.*, 1982; Stephenson *et al.*, 1983; Alder *et al.*, 1990). In general, it is believed that sorption only is important in the absence of biodegradation, which is the case at influent concentrations too low to obtain growth of NTA-degraders or during a period of acclimation when a NTA-degrading population still has to develop (Hunter *et al.*, 1985). Otherwise, at higher influent concentrations and after this period of acclimation, biodegradation commences and the contribution of sorption to overall removal becomes negligibly small (Rossin *et al.*, 1982 and Hunter *et al.*, 1985).

Several standard tests, carried out according to the guidelines of the Organisation for Economic Co-operation and Development (OECD), indicate that NTA is inherently biodegradable (e.g., Hunter, 1985). This means that NTA only can be biodegraded under "favourable" conditions. An important characteristic of NTA is that it forms complexes with metals, including those present in domestic wastewater. Warren (1974) and Madsen and Alexander (1985) have shown that relatively weak calcium and iron complexes with NTA can be more easily biodegraded than stronger metal complexes with cadmium, zinc, copper and nickel. In spite of the "inherent" biodegradability of NTA, once biodegradation has been established more than 98% NTA removal can be accomplished in activated sludge plants (BUA, 1986). In particular the temperature (van 't Hof *et al.*, 1983; Heide, 1984 and Birch, 1991), the SRT (Cech and Chudoba, 1988 and Birch, 1991) and the COD loading rate (Giger *et al.*, 1987) have a strong impact on the actual removal efficiency that can be achieved. As was mentioned previously, a certain period of acclimation is required before biodegradation of NTA commences. This period ranges from a few days to a few weeks (Shumate *et al.*, 1970; Rossin *et al.*, 1982, Van 't Hof *et al.*, 1983; Larson, 1983; Perry *et al.*, 1984 and Hunter *et al.*, 1985). Usually, it is assumed that this acclimation corresponds with enrichment of NTA-degrading micro-organisms to a degree at which biodegradation can be detected. This also explains why several workers consider the (growth) Monod model (chapter 2) the appropriate model to describe the biodegradation kinetics of NTA by activated sludge (Cech and Chudoba, 1988; Chudoba *et al.*, 1989; Alder *et al.*, 1990 and Birch, 1991). However, more recent results published by Bally *et al.* (1994) and Bally and Egli (1996) have demonstrated that acclimation not only is determined by enrichment, but partly may be caused by an increased production of NTA-degrading enzymes by micro-organisms which are indigenous members of the original population in municipal activated sludge plants. These NTA-degraders can simultaneously consume NTA and other easily biodegradable carbon sources and the availability of these carbon sources may dramatically reduce the period required to produce the NTA degrading enzymes.

Validation of models that predict the fate of trace pollutants in municipal activated sludge plants requires accurate monitoring data. Unfortunately, most of the data sets concerning the behaviour of NTA during activated sludge treatment miss the required level of detail. Also, they are often limited to short periods of time (12-48 hours) and reflect short-term variations rather than the long-term averages which are required to validate steady-state models. Finally, insufficient information is available about the effects of the sludge retention time (SRT) and influent NTA concentration whereas both may be important parameters determining the behaviour of NTA. For these reasons it was decided to collect a new set of NTA monitoring data in a pilot-scale municipal activated sludge plant. Using the monitoring data and the results of several biodegradation tests the validity of existing activated sludge fate models to predict the fate of NTA was investigated.

Material and methods

Activated sludge plant operation - A pilot-scale activated sludge plant was employed which consisted of a completely mixed aeration tank of 490 l and a final settler with a volume of 280 l. The plant was fed with pre-settled domestic wastewater. Average wastewater concentrations were 500 mg·l^{-1} of total COD, 286 mg·l^{-1} of soluble COD, 60 mg·l^{-1} of NH_4-N, 7.1 mg·l^{-1} of PO_4-P and 85 mg·l^{-1} of suspended solids (SS). The plant was operated at two different sets of a SRT, hydraulic retention time (HRT) and COD loading rate (periods A and B in Table 1 and Figure 1).

Table 1 - Operational parameters of the pilot-scale activated sludge plant.

parameter	period A		period B	
sludge retention time (d)	10		27.3	
hydraulic retention time (h)	7.3		12.4	
COD loading rate (kg COD·kg^{-1} VSS·d^{-1})	0.46		0.19	
recirculation factor (-)	1		1	
average wastewater temperature (°C)	22.8		17.2	
	A1	A2	B1	B2
influent NTA concentration (mg·l^{-1})	0.3*	28**	0.3*	35**

* background concentration in the wastewater

** enhanced concentration by external addition

To maintain the desired SRT, sludge was wasted directly from the aeration tank. The pH was controlled in a range of 7.2-7.4 by 0.3 M NaOH and 0.3 M HCl additions. The aeration tank was aerated at a constant air flow rate provided through fine-pore bubble diffusers. The oxygen concentration was not controlled but always was above 3 mg·l^{-1}. More details about the plant can be found in chapter 4.1. For each of the periods A and B the pilot-plant was operated for at least four times the SRT and a background (wastewater) concentration of NTA before the influent concentration was enhanced (Table 1 and Figure 1). For this purpose a stock solution of NTA ($C_6H_6NO_6Na_3$, Sigma ~ 99.5%) in suprapur water was prepared, stored in a 25 l polyethylene (PE) container and pumped directly into the aeration tank.

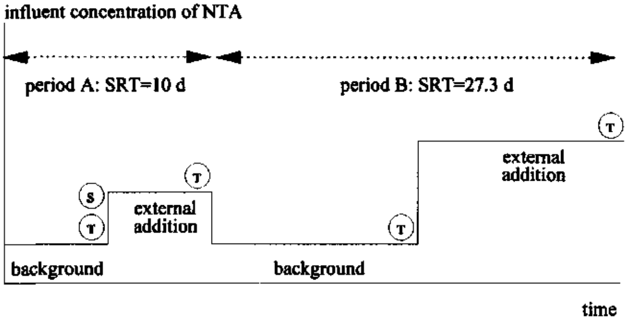

Fig. 1 Pilot-plant operation (T=biodegradation tests, S=sorption tests).

Sampling - Daily, 24-h composite samples from the (pre-settled) influent and final effluent were collected in 3 l polyethylene (PE) containers for determination of NTA and the standard water quality parameters COD, soluble COD, NH_4-N, NO_3-N and suspended solids (SS). The samples for determination of NTA were preserved by pre-addition of 3% (v/v) formalin (Merck, 35% formaldehyde solution) and the samples for determination of the standard water quality parameters received 5 ml of H_2SO_4. All the NTA samples were stored in a refrigerator at 4 °C for a maximum of 2 days until further analysis. Also, daily grab samples were taken from the aeration tank and the return sludge to determine total and dissolved concentrations of NTA and concentrations of suspended and volatile suspended solids.

Sorption tests - Several sorption tests were carried out during period A1 (Figure 1). Return sludge and final effluent were collected from the pilot-plant and were mixed in 200 ml PE centrifuge tubes to give target sludge concentrations in a range of 3-5 g VSS·l^{-1}. The mixed-liquor was gently purged with nitrogen gas to exclude oxygen and in this manner prevent biodegradation of NTA. A stock solution of NTA was added to give initial NTA concentrations in a range of 1-10 mg·l^{-1}. To determine background NTA concentrations also two blank tests without NTA addition were included. After 12 hours of equilibration time samples were taken and subsequently analysed for total and dissolved NTA.

Biodegradation tests- The kinetics of primary biodegradation of NTA were determined at the end of each of the periods A1 to B2 (Figure 1). For this purpose a so-called by-pass test was employed. A detailed description of the experimental procedure for this test can be found in chapter 3.

Analysis - Standard water quality parameters COD, NH_4-N, NO_3-N, SS and VSS were all determined according to Dutch standard practice (NEN). The analytical procedure for NTA was adapted from a method to determine ethylenediaminetetraacetate (EDTA) as described by Bergers and Groot (1994). Table 2 summarises the main characteristics of the analytical methodology.

Table 2 - Characteristics of the NTA analytical methodology.

method analytical recovery	93%		
instrumental precision	7%		
instrumental detection limit	150 $\mu g \cdot l^{-1}$		
	influent	effluent	sludge
method precision	12.7%	4%	8.3%
method detection limit	30 $\mu g \cdot l^{-1}$	30 $\mu g \cdot l^{-1}$	30 $\mu g \cdot l^{-1}$

To determine total NTA concentrations 100 ml samples of influent, final effluent and sludge immediately received 2 ml of a 5 $g \cdot l^{-1}$ $Cu(NO_3)_2.3H_2O$ solution (Merck, pro analysis) and were mixed on a rotary shaker for at least 6 hours to extract NTA and to form a stable Cu-NTA complex. To determine concentrations of dissolved NTA, 100 ml samples were immediately centrifuged for 5 minutes at 3500 rpm before adding $Cu(NO_3)_2.3H_2O$. After 15 minutes of centrifugation at 3500 rpm, 80 ml of the supernatant was transferred to a rotavap and evaporated under vacuum to a residual volume of 10-20 ml. From this residual volume, 1.8 ml was transferred into an Eppendorf tube and centrifuged for 5 minutes at 11000 rpm. The supernatant was transferred into a 2 ml HPLC vial and stored at 4 °C. Instrumental analysis was by reverse-phased ion-pair HPLC equipped with a 25 cm 8×4 mm Nuclosil 10-SB column (Chrompack) and UV detection at 280 nm (Spectraflow 783, Kratos).

Results and discussion

NTA monitoring data - Figure 2 shows an example of background NTA concentrations in 24-h samples of the (pre-settled) domestic wastewater that was used in the experiments. The daily variation is relatively low (coefficient of variation of 24%). Similar to what was found for linear alkylbenzene sulfonate (LAS, chapter 4.1), this indicates the absence of a preferred "washing day" (Waters and Feijtel, 1995). Because biodegradation of NTA requires a long period of acclimation (Shumate *et al.*, 1970; Rossin *et al.*, 1982, van 't Hof *et al.*, 1983, Larson, 1983; Perry *et al.*, 1984;

Hunter *et al.*, 1985) it can be expected that this relatively stable supply will favour biodegradation of NTA in (municipal) activated sludge systems.

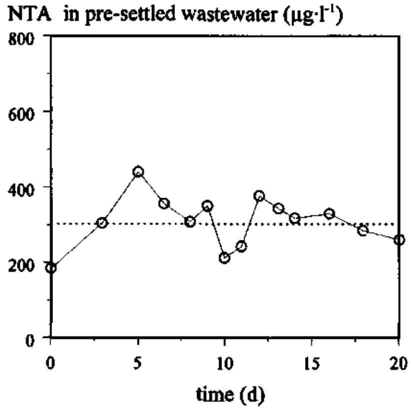

Fig. 2 NTA concentrations in 24-hour samples of pre-settled domestic wastewater (--- average).

Table 3 provides an overview of the performance of the pilot-plant with respect to NTA removal. Average (background) influent concentrations of NTA during periods A1 and B1 were approximately 300 µg·l⁻¹. This agrees fairly well with concentrations of 200-300 µg·l⁻¹ that can be calculated from NTA consumption and wastewater production data (BUA, 1986).

Table 3 - Average NTA monitoring data in the pilot-scale activated sludge plant.

		period A, SRT = 10 d temperature 22.8 ± 0.5 °C		period B, SRT = 27.3 d temperature 17.2 ± 1.3 °C	
NTA concentrations		A1	A2	B1	B2
influent	total (µg·l⁻¹)	304 ± 68*	28410 ± 2148	302 ± 72	35508 ± 1013
	% of influent COD	0.04	4	0.06	5.4
	load (mg·h⁻¹)	20.4	1907	11.9	1403
aeration tank	dissolved (µg·l⁻¹)	all below method detection limit of 30 µg·l⁻¹			
	total (µg·l⁻¹)				
return sludge	dissolved (µg·l⁻¹)				
	total (µg·l⁻¹)				
effluent	total (µg·l⁻¹)				

* standard deviation

Average concentrations of dissolved and total NTA in the aeration tank, return sludge and final effluent were all below the method detection limit of 30 µg·l⁻¹, irrespective of the influent concentration of NTA and the operational conditions in the plant. This corresponds to NTA removal efficiencies of at least 90% in periods A1 and B1 at low influent concentrations of NTA, and efficiencies greater than 99% in periods A2 and B2 when the pilot-plant was fed with enhanced concentrations of NTA. Similar high removal efficiencies have also been observed in other studies (van 't Hof *et al.*, 1983; Heide, 1984; Giger *et al.*, 1987).

Sorption - After 12 hours of equilibration time 96 to 110% of the NTA that was spiked to the sludge samples was recovered as dissolved NTA. This recovery was randomly distributed among the different tests. From this, it can be concluded that sorption of NTA in the pilot-plant was a negligible removal process. This agrees with findings by Shumate *et al.* (1970) and Bernhardt (1984), but is in conflict with other investigations in which sorption capacities of 6 to 2400 $\mu g \cdot g^{-1}$ suspended solids (SS) were found (Stephenson *et al.*, 1983; Rossin *et al.*, 1982 and Hunter *et al.*, 1985). According to Rossin *et al.* (1982) this wide range of sorption capacities indicates that relatively weak (sorptive) forces are involved. Once biodegradation of NTA in a plant has commenced, sorption will become insignificant as a removal mechanism and probably only serves as an intermediate stage in the passage of NTA into the bacterial cells.

Biodegradation - Figure 3 shows the results of the biodegradation tests which were carried out at the end of periods A1 to B2.

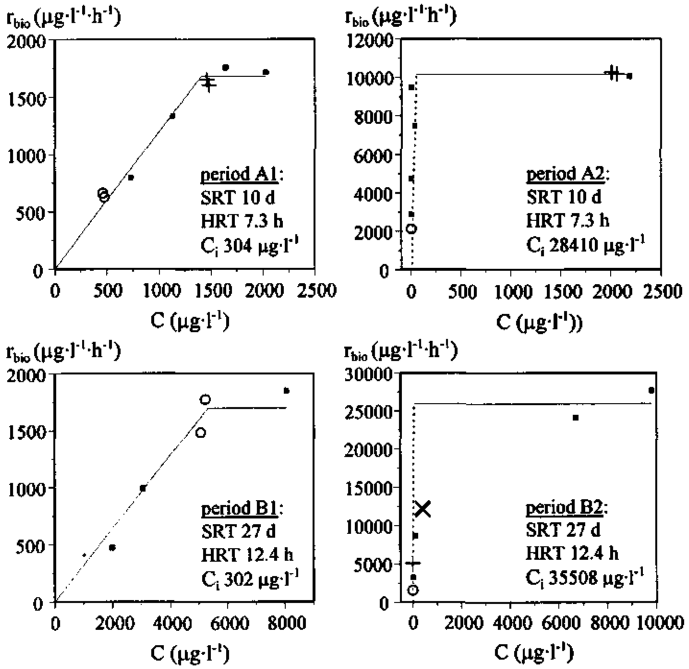

Fig. 3 Kinetics of primary biodegradation of NTA by activated sludge cultivated at different SRTs and influent NTA concentrations to the pilot-plant (C_i) (• represents single measurements and + and o represent duplicate measurements).

Similar to another test compound, the C_{12}-homologue of linear alkylbenzene sulfonate (LAS-C_{12}, chapter 4.1), a combined first- and zero-order model is used to describe the results:

$$r_{bio} = k_1 X C \quad \text{if} \quad C < C_{1/0} \tag{1a}$$

$$r_{bio} = k_0 X \quad \text{if} \quad C \geq C_{1/0} \tag{1b}$$

with r_{bio} the biodegradation rate ($\mu g \cdot l^{-1} \cdot h^{-1}$), C the concentration of (the sum of dissolved and sorbed) NTA, $C_{1/0}$ the concentration C where the biodegradation kinetics change from first- to zero-order ($\mu g \cdot l^{-1}$), k_1 a first-order rate constant ($l \cdot g^{-1}$ $VSS \cdot h^{-1}$), k_0 a zero-order rate constant ($\mu g \cdot g^{-1}$ $VSS \cdot h^{-1}$) and X the sludge concentration (g $VSS \cdot l^{-1}$).

Estimated parameters for this combined first- and zero-order model are presented in Table 4. It is appreciated that the estimated biodegradation parameters are inaccurate. In particular the tests in periods A2 and B2 with sludge that had been exposed to high influent concentrations of NTA exhibited a $C_{1/0}$ close to, or even below the method detection limit of 30 $\mu g \cdot l^{-1}$. As a result, for the first-order rate constant k_1 ($=k_0/C_{1/0}$) only a minimum value could be calculated. Also, the zero-order rate constants k_0 are inaccurate because only a limited number of data-points is available in the zero-order region. Despite these inaccuracies it is believed that at least a qualitative evaluation of the effects of the SRT and the influent concentration of NTA on the biodegradation kinetics is possible.

Table 4 - Results from the by-pass tests with NTA (C_i influent NTA concentration to pilot-plant, X_a sludge concentration in aeration tank, for the other symbols refer to the text).

	period			
	A1	A2	B1	B2
plant operation				
SRT (d)	10	10	27.3	27.3
HRT (h)	7.3	7.3	12.4	12.4
X_a (g $VSS \cdot l^{-1}$)	3.35	3.77	5.22	5.59
C_i ($\mu g \cdot l^{-1}$)	304 ± 68*	28410 ± 2148	302 ± 72	35508 ± 1013
kinetic parameters				
k_1 ($l \cdot g^{-1} VSS \cdot h^{-1}$)	0.36 (0.995***)	≥ 338	0.06 (0.963)	≥ 864
k_0 ($\mu g \cdot g^{-1}$ $VSS \cdot h^{-1}$)	501 ± 20*	2650 ± 90	348 ± 11	4641 ± 447
$C_{1/0} = k_0/k_1$ ($\mu g \cdot l^{-1}$)	1392	≤ 30**	5800	≤ 30**

* standard deviation

** method detection limit of 30 $\mu g \cdot l^{-1}$

*** correlation coefficient

Verification of the biodegradation kinetics - In each period, using the biodegradation kinetics of equations (1a) and (1b) and the biodegradation parameters of Table 4, effluent NTA concentrations can be calculated from the (steady-state) mass balance for NTA around the pilot-plant. Except for period B1 calculated effluent concentrations agree with the monitoring data that they are below the method detection limit of 30 $\mu g \cdot l^{-1}$ (Figure 4). Why the calculated effluent concentration in period

B1 is higher than in the other periods is not clear. Obviously, a more detailed verification would require a more sensitive analytical technique than the one that was used in this study.

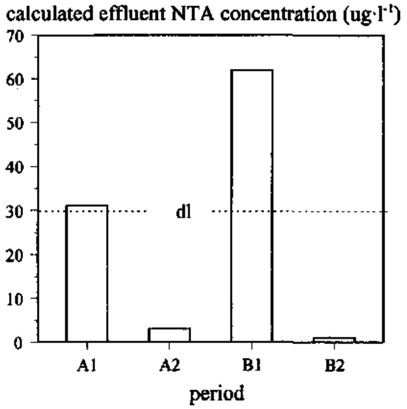

Fig. 4 Effluent NTA concentrations of the pilot-plant, calculated with the biodegradation parameters of Table 4 (dl = method detection limit for NTA).

The biodegradation parameters of Table 4 are properties of the sludge, given the operational parameters of the plant (SRT, HRT, etc.) and in particular the influent concentration of NTA the sludge was acclimated to. These parameters cannot be exchanged between the different periods because this would give erroneous predictions of the effluent concentration of NTA. For example, using the biodegradation parameters of period A1 an effluent concentration of more than 1000 $\mu g \cdot l^{-1}$ would be predicted for period B2, which is at least a factor 30 higher than the real effluent concentration in this period. This means that for a generic application biodegradation kinetics should be employed in which this aspect is included (see below).

Monod kinetics versus secondary utilisation kinetics - Activated sludge fate models differ in the biodegradation kinetics they employ. It is often assumed that trace compounds are degraded as a secondary substrate by (non-specific) micro-organisms which utilise another (primary) substrate as their carbon and energy source. In contrast, growth models, such as the Monod model, assume that specific micro-organisms utilise the trace compound itself as their single, growth-limiting substrate. A distinction between secondary utilisation and Monod kinetics can be made from the zero-order biodegradation rate constant k_0 as a function of the influent load of the target compound. For instance, for the C_{12}-homologue of linear alkylbenzene sulfonate (LAS-C_{12}) it was found that k_0 was independent of the influent load of LAS and from this it was concluded that biodegradation of this compound should be modelled by secondary utilisation kinetics (chapter 4.2). In contrast, the k_0 for NTA increases with increasing influent concentrations of NTA (Table 4) and therefore a Monod model probably is more appropriate. If decay of NTA-degrading biomass is neglected and the effluent concentration of NTA is negligibly small as compared to the influent concentration, the following relationship can be derived from the Monod model (see chapter 4.2):

$$k_0 = \mu_{max}\theta_c q_x \tag{2}$$

where μ_{max} is the maximum specific growth rate of the NTA-degraders (h^{-1}), θ_c is the SRT (h) and q_x is the sludge loading rate with NTA ($\mu g \cdot g^{-1} VSS \cdot h^{-1}$).

Figure 5 shows that in accordance with equation (2) k_0 increases with $\theta_c q_x$. Together with results reported by other workers (e.g., Cech and Chudoba, 1988; Birch, 1991 and Alder *et al.*, 1990) this strongly supports the validity of the Monod model. However, the experimental data deviate from equation (2) that already at extremely low values for $\theta_c q_x$ a minimum NTA-degrading capacity can be detected of 300-500 $\mu g \cdot g^{-1}$ $VSS \cdot h^{-1}$ (Figure 5). A possible explanation is given by Egli and Bally (1996) who discussed that NTA-degrading micro-organisms are indigenous members of the activated sludge population, which produce a low constitutive level of NTA-degrading enzymes and which can simultaneously utilise NTA and other carbon sources such as glucose and acetate. According to Bally *et al.* (1994) a higher influent concentration of NTA in the influent does not result in enrichment of NTA-degraders but in an increased production of the key-enzymes by the degraders already present in the sludge. A combination of induction at low loading rates and enrichment at higher loading rates may explain the (hypothetical) hyperbolic-like increase of k_0 with $\theta_c q_x$ in Figure 5. From the two highest values of $\theta_c q_x$ a maximum specific growth rate μ_{max} of 0.023 h^{-1} can be estimated. This (rather slow) μ_{max} approaches a μ_{max} of 0.030 ± 0.004 h^{-1} determined in a ready biodegradability test with NTA (Temmink and Klapwijk, 1998).

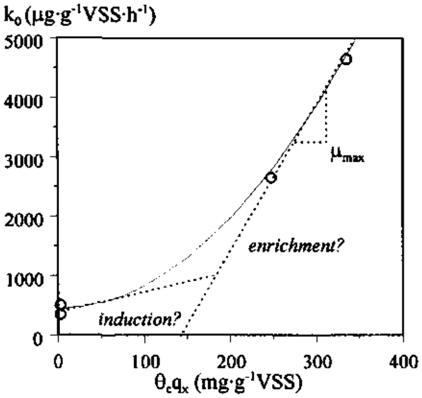

Fig. 5 Zero-order biodegradation rate k_0 as a function of the product of the SRT θ_c and sludge loading rate with NTA q_x.

Obviously, the number of data-points in Figure 5 is limited and a detailed analysis is impossible. Besides, such an analysis would require measurements of the number of NTA-degraders, enzyme concentrations and the type and concentration of other carbon sources in the wastewater that can be consumed by the NTA-degraders. This particularly is important because in practice $\theta_c q_x$ is expected to be much smaller than 100 mg NTA·g^{-1}VSS, and this is the range where according to Figure 5 enzyme induction supposedly is the dominant mechanism of NTA biodegradation.

For steady-state situations, models based on enrichment and models based on enzyme induction may yield similar predictions for effluent NTA concentrations because in both cases an increase of the influent concentration of NTA is compensated for by an increase of the NTA-degrading activity of the sludge. A distinction between these mechanisms based on effluent concentrations probably only is possible for dynamic situations because enzyme induction is in the order of minutes to hours and is a much faster process than enrichment of NTA-degraders which may take days (also see chapter 7).

Concentration $C_{1/0}$ where the kinetics change from first- to zero-order - The concentration $C_{1/0}$ where the kinetics change from first- to zero-order reflects the affinity of the biomass to scavenge NTA. Despite the inaccurate estimates of $C_{1/0}$, a clear dependency on the influent concentration of NTA still can be recognised with high values for $C_{1/0}$ at low influent concentrations and extremely low values for $C_{1/0}$ at high influent concentrations (Table 4). A similar result, although less dramatically, was also found for LAS-C_{12} (chapter 4.2) but no obvious explanation was available.

For NTA this variation in $C_{1/0}$ possibly can be related to the metal chelating capacity of NTA in combination with the biodegradability of the different metal complexes. Several investigations have shown that the relatively weak calcium and iron complexes with NTA (Ca-NTA and Fe-NTA) can more easily be biodegraded than stronger metal complexes with cadmium, zinc, copper and nickel (Warren, 1974; Madsen and Alexander, 1985). Most likely, these differences are related to the mechanism with which the metal complexes are transported across the cell membrane (Madsen and Alexander, 1985) and this in turn may have an effect on the affinity with which these complexes can be scavenged by the biomass.

Figure 6 shows the simulated distribution between the easily biodegradable Ca-NTA and a stronger but less easily biodegradable NTA metal complex (Me-NTA). Sorption of Me to activated sludge was included in the calculations and an influent concentration of Me of 500 μg·l^{-1} was taken as the sum of cadmium, copper, nickel, lead and zinc concentrations in domestic wastewater as reported by Heide (1984). For equilibration between NTA and Me and between Me and sludge, respectively a logK of 13.7 and 6.3 were taken from Alder *et al.* (1990). These values are based on the strongest Me-NTA complex which is Cu-NTA, but lower values, for instance those based on zinc or lead, would give similar results. Furthermore, it was assumed that calcium is present in excess (approximately 40-50 mg·l^{-1} in the domestic wastewater that was used) and that all the NTA not fixed by Me is available to form a Ca-NTA complex. Figure 6 shows that at the low influent concentrations of NTA during periods A1 and B1 (< 500 μg·l^{-1}) practically all the NTA is present as Me-NTA. If, as was mentioned earlier, transport of Me-NTA across the cell membrane is difficult, this may explain the relatively high values of $C_{1/0}$ during these periods. In contrast, at the high influent concentrations of NTA during periods A2 and B2 (>25000 $\mu g \cdot l^{-1}$), practically all the NTA is present as the easily biodegradable Ca-NTA complex, which may explain the extremely low values for $C_{1/0}$ observed in these periods.

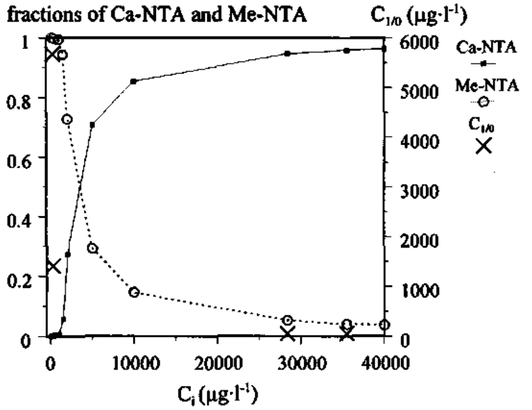

Fig. 6 Concentration of NTA where the biodegradation kinetics change from first- to zero-order ($C_{1/0}$), and calculated fractions of easily (Ca-NTA) and less easily (Me-NTA) biodegradable NTA complexes as a function of the influent NTA concentration to the pilot-plant (C_i).

Reliability of existing models to describe the fate of NTA - To predict the rate of biodegradation, existing activated sludge fate models generally employ (non-growth) secondary utilisation kinetics (e.g. Namkung and Rittmann, 1987; Govind *et al.*, 1991; Struijs *et al.*, 1991; Cowan *et al.*, 1993) or (growth) Monod kinetics (e.g. Birch, 1991; van Wijk *et al.*, 1996; McAvoy *et al.*, 1999). However, the results in this chapter, combined with literature information, strongly indicate that neither of these models can accurately describe biodegradation of NTA.

First of all, existing activated sludge fate models do not take enzyme induction into account which at low influent NTA concentrations may very well be regulating biodegradation of NTA. Even though models are available to describe enzyme induction (e.g., Ballo and Ramkrishna, 1991a and 1991b), these today are not applicable to mixed-cultures such as activated sludge.

Secondly, the (indigenous) concentration of NTA-degraders is extremely important. The number of NTA-degraders probably is determined by the availability of carbon sources other than NTA and competition for these carbon sources between NTA-degraders and other micro-organisms. Competition for carbon sources is not considered in activated sludge fate models, but could easily be incorporated. However, this would require identification of the carbon sources in domestic wastewater that can be utilised by the NTA-degraders and knowledge about the concentration of these carbon sources in the wastewater.

Finally, existing activated sludge fate models assume that biodegradation parameters are independent of the operational conditions in a plant. However, the fact that the concentration where the kinetics change from first- to zero-order ($C_{1/0}$) varies with the influent concentration of NTA clearly illustrates that this not necessarily is the case.

Conclusions

More than 99% NTA removal was achieved in a pilot-scale municipal activated sludge plant and effluent NTA concentrations were always below the method detection limit of 30 $\mu g \cdot l^{-1}$, irrespective of the SRT (10 and 27.3 d) and influent NTA concentration which varied between background concentrations of 0.3 $mg \cdot l^{-1}$ and enhanced concentrations of 28-35 $mg \cdot l^{-1}$. Biodegradation was the only removal mechanism as sorption of NTA to activated sludge was found to be an insignificant process. These results suggest that activated sludge fate models rightly employ a Monod model to describe the biodegradation kinetics of NTA. However, measurements of the biodegradation kinetics with sludge that was acclimated to different influent concentrations of NTA, supported by literature results, indicated that for an accurate prediction of the biodegradation rate probably a more complex model is needed because (i) at low influent concentrations of NTA the Monod model seems to be invalid and may have to be replaced by a different, more complex model including enzyme induction and (ii) the potential of the biomass to scavenge NTA is dependent on the influent concentration of NTA and this dependency may very well be related to water hardness and types and concentrations of metals in the wastewater. Only little is known about the underlying mechanisms that cause these deviations from the Monod model.

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Chapter 6

Fate of toluene in activated sludge plants

Abstract - The fate of toluene in a pilot-scale municipal activated sludge plant was investigated. This plant was operated at two sludge retention times (10 and 27.3 d) and was fed with domestic wastewater containing various concentrations of toluene (4 to 1800 $\mu g \cdot l^{-1}$). Biodegradation of toluene was absent when the sludge was exposed to background concentrations of toluene in the wastewater of 4 $\mu g \cdot l^{-1}$. When the influent concentration was raised above 100 $\mu g \cdot l^{-1}$ biodegradation was established and could eliminate more than 97% of the influent load. Toluene concentrations in the effluent and off-gas were low: less than 2 $\mu g \cdot l^{-1}$ and 0.3 $\mu g \cdot l^{-1}$, respectively. Predictions with a simple fate model could follow the observed trend in the observations if Monod kinetics were employed to describe biodegradation of toluene. It is expected that more reliable model predictions are feasible, but this would require more accurate values for the Monod parameters (maximum specific growth rate and half-velocity constant) and for the liquid-gas mass-transfer coefficients of toluene.

Introduction

Toluene is an important constituent in gasoline's, crude oil and oil derivates and is widely used as a solvent (Rippen, 1996). It therefore is not surprising that it is a common hydrocarbon pollutant in many industrial wastewaters. Toluene can also be found in the influent of municipal wastewater treatment plants at concentrations between 0.5 and 500 $\mu g \cdot l^{-1}$, depending on the contribution of industrial wastewater to the total wastewater volume (Russell and Jenkins, 1982; Hannah and Rossman, 1982; Namkung and Rittmann, 1987; Melcer *et al.*, 1988; DBW/RIZA, 1990 and Bell *et al.*, 1993).

Municipal wastewater often is treated biologically in activated sludge systems. The physical-chemical properties of toluene (solubility, volatility, hydrophobicity, etc.), its biodegradability and plant operational characteristics such as the sludge retention time (SRT) and air flow rate determine whether toluene in these plants (i) is removed from the wastewater by sorption to the biomass and in this manner is wasted with the excess sludge, (ii) is removed by air stripping and/or surface volatilisation, (iii) together with the final effluent is discharged to the surface water or (iv) is effectively eliminated from the environment by biodegradation. According to the literature data in Table 1, an average of 6% of the toluene load to activated sludge plants will appear in the effluent of these plants. This means that the remaining 94% is removed from the wastewater by combined sorption, volatilisation and biodegradation.

Table 1 - Literature data on the removal of toluene in municipal activated sludge plants.

	removal (% of influent load)			
reference	effluent	off-gas	biodegradation*	total removal**
Namkung and Rittmann (1987)	7	nd	nd	93
Weber *et al.* (1987)	<1	17	83	>99
DBW/RIZA (1990)	6.7	nd	nd	93
Melcer *et al.* (1991)	nd	nd	nd	99.1
Melcer *et al.* (1992)	0.2	1.2	98.6	99.8
Bell *et al.* (1993)	16	25	59	84
Parker *et al.* (1993)	2	15	81	98
Clapp *et al.* (1994)				
- low loaded plants	2-11	20	69-78	79-98
- high-loaded plants	0-10	14	74-87	90-100
average removal	6 ± 5***	15 ± 8	79 ± 13	94 ± 5

nd no data reported

* calculated as closure of the mass balance around the plant

** calculated from influent and effluent concentration

*** standard deviation

Sorption of trace compounds to activated sludge can be related to its hydrophobicity, expressed by the octanol-water partition coefficient K_{ow} (Schwarzenbach and Westall, 1985; Bell and Tsezos, 1987 and Dobbs *et al.*, 1989). Although a log(K_{ow}) of 2.75 (Rippen, 1996) would suggest a considerable sorption potential of toluene to sludge, several studies have demonstrated the absence of toluene in municipal sludges (Kincannon *et al.*, 1982; Weber *et al.*, 1987; Parker *et al.*, 1993 and Thompson *et al.*, 1993). Apparently sorption of toluene is a negligible process compared to volatilisation and biodegradation.

Because toluene is a volatile compound, it is subject to air stripping in the (aerated) bioreactor of activated sludge plants and to surface volatilisation. From the data in Table 1 an estimated 15% of toluene removal can be attributed to these processes.

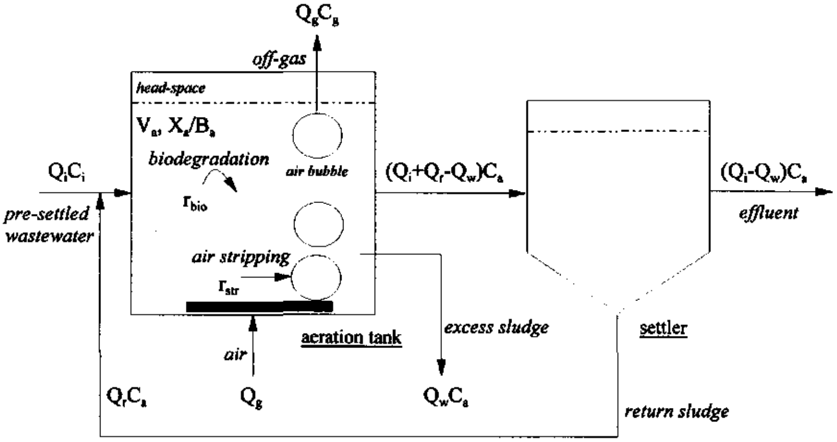
The average closure of the mass balances around the plants in Table 1 corresponds to 79% toluene removal. Assuming that no other destructive processes take place, this removal must have been accomplished by biodegradation. This shows that toluene can be readily biodegraded by activated sludge, which is also demonstrated by the many positive results obtained in (standardised) biodegradation tests (e.g. Davis *et al.*, 1981; Tabak *et al.*, 1981, Blackburn *et al.*, 1984, Tabak *et al.*, 1990).

The last decade several models have been developed in which mathematical equations for volatilisation, biodegradation and sorption are combined to predict the fate of organic trace contaminants in activated sludge plants (Blackburn *et al.*, 1984; Namkung and Rittmann, 1987; Govind *et al.*, 1991; Struijs *et al.*, 1991 and Cowan *et al.*, 1993; McAvoy *et al.*, 1998). The reliability of these models was the subject of a study involving several test compounds, including toluene. Toluene was selected to investigate the competition between biodegradation and volatilisation. For this purpose monitoring data were collected in a pilot-scale municipal activated sludge plant. To include the effects of exposure history and operational characteristics, the pilot-plant was operated at various influent toluene concentrations and at two different SRTs. Based on the equations used by existing models, a simple fate model was developed for the pilot-plant and predictions with this model were compared with the monitoring data.

Model development

The pilot-scale plant treats pre-settled wastewater and consists of a completely mixed aeration tank with a (fine) bubble aeration system, and a secondary settler to retain the biomass (Figure 1). To control the SRT excess sludge is wasted directly from the aeration tank. It is assumed that sorption of toluene to the sludge can be ignored and only biodegradation and air stripping are important removal processes. Their occurrence is restricted to the aeration tank. The aeration tank contains a small head-space volume but the retention time of the off-gas in this head-space is negligibly small.

Air stripping - Volatile trace compounds can escape to the atmosphere by air stripping and by surface desorption. In aeration tanks of activated sludge plants surface desorption only becomes important at small air flow rates and for trace compounds of low volatility (Kyosai and Rittmann, 1991). Under the experimental conditions that were applied air stripping is the dominant process and the contribution of surface desorption will not be taken into account.



symbols		subscripts	
C	concentration of toluene ($\mu g \cdot l^{-1}$)	a	aeration tank
Q	flow rate ($l \cdot h^{-1}$)	e	effluent
r_{bio}	biodegradation rate ($\mu g \cdot l^{-1} \cdot h^{-1}$)	g	gas
V	volume (l)	i	influent
X	non-specific sludge concentration (g $VSS \cdot l^{-1}$)*	r	return sludge
B	specific sludge concentration (g $VSS \cdot l^{-1}$)*	w	waste sludge

* VSS=volatile suspended solids

Fig. 1 Model activated sludge plant and toluene fluxes in this plant.

A mechanistic model describing the transfer of a compound to an air bubble as it rises to the surface has been described by Matter-Müller *et al.* (1981). If it is assumed that (i) the bulk liquid is completely mixed, (ii) a series of air bubbles rises through the liquid, (iii) pressure and volume changes in these bubbles can be neglected and (iv) the inlet air is free of toluene, the rate of air stripping is first-order with respect to the concentration of toluene in the bulk liquid:

$$r_{str} = k_{str} C \tag{1}$$

where r_{str} is the volumetric rate of stripping ($\mu g \cdot l^{-1} \cdot h^{-1}$), C is the toluene concentration in the bulk liquid ($\mu g \cdot l^{-1}$) and k_{str} is the (first-order) stripping rate constant (in h^{-1}) which equals:

$$k_{str} = \frac{Q_g H}{V} f_{sat} \tag{2}$$

with H Henry's law constant, the concentration of toluene in the gas phase in equilibrium with the concentration of toluene in the liquid phase (-), Q_g the air flow rate ($l \cdot h^{-1}$), V the liquid volume and f_{sat} the fractional saturation of toluene in the air bubbles as they escape from the liquid surface (-).
For compounds with a low Henry's law constant and/or at long retention times of the air bubbles in the liquid phase, an equilibrium will be approached between the liquid phase and the gas phase and

f_{sat} equals 1. Otherwise, f_{sat} is smaller than 1 and has to be calculated from:

$$f_{sat} = 1 - \exp(-\frac{K_{ol}aV}{HQ_g}) \tag{3}$$

where a is the interfacial gas-liquid area per unit of volume ($m^2 \cdot m^{-3}$) and K_{ol} is the overall mass-transfer coefficient ($m \cdot h^{-1}$). Usually, the parameters K_{ol} and a are substituted by a combined parameter $K_{ol}a$ because this parameter can be correlated to a (known) $K_{ol}a$ for oxygen $K_{ol}a_{O2}$ according to:

$$K_{ol}a = K_{ol}a_{O2}\left(\frac{D}{D_{O2}}\right)^n \tag{4}$$

where D and D_{O2} are the diffusion coefficients of toluene and oxygen in water, respectively ($m^2 \cdot h^{-1}$) and n is a power constant.

Equation (4) is only valid if liquid-gas transfer is controlled by the resistance of the liquid film. According to Munz and Roberts (1989) this is a valid assumption for compounds with a $H \geq 0.1$, which includes toluene with H=0.25 (Matter-Müller *et al.*, 1981). However, according to Hsieh *et al.* (1993) equation (4) would only be valid for compounds with a $H \geq 1.2$ and this does not include toluene. The power constant n is determined by the hydrodynamic conditions, with n = 1.0 for laminar conditions and n = 0.5 for turbulent conditions. For diffused aeration systems a value of n = 1.0 can be applied (Matter-Müller *et al.*, 1981; Bielefeldt and Stensel, 1999b).

Biodegradation - In the literature the rate of biodegradation of toluene by activated sludge is described by (non-growth) secondary utilisation kinetics (e.g., Blackburn *et al.*, 1984; Kemp *et al.*, 2000) or by (growth) Monod kinetics (e.g., Kincannon *et al.*, 1982; Weber *et al.*, 1987; Tabak *et al.*, 1990; Bielefeldt and Stensel, 1999b).

Secondary utilisation

When toluene is supplied at concentrations which are too low to support growth of specific toluene degrading micro-organisms, it still may be biodegraded as a secondary substrate, i.e., by biomass which depends on the supply of another (primary) substrate for growth (Rittmann, 1992). Often biodegradation in this case is described by a saturation function for the trace compound and a linear relation with the concentration of total (non-specific) biomass:

$$r_{bio} = k_0 \frac{C}{K_C + C} X \tag{5}$$

with r_{bio} the rate of biodegradation ($\mu g \cdot l^{-1} \cdot h^{-1}$), k_0 a maximum (zero-order) rate of biodegradation ($\mu g \cdot g^{-1}$ VSS$\cdot h^{-1}$), X the total (non-specific) concentration of biomass (g VSS$\cdot l^{-1}$) and K_C a half-velocity constant ($\mu g \cdot l^{-1}$).

Monod kinetics

When, on the other hand, the wastewater contains a sufficiently large concentration of toluene such that a specific toluene degrading population can develop, classic Monod kinetics (chapter 2) would be more appropriate. An equation for the biodegradation kinetics similar to equation (5) can be used. Only this time the maximum biodegradation rate k_0 is related to the maximum growth rate of these toluene degraders and only a small (specific) fraction of the total biomass is able to degrade toluene:

$$r_{bio} = \frac{\mu_{max}}{Y} \frac{C}{K_C + C} B \tag{6}$$

where μ_{max} is the maximum specific growth rate of the toluene degraders (h^{-1}), Y is the biomass yield (g VSS$\cdot\mu g^{-1}$ toluene) and B is the concentration of the toluene degraders (g VSS$\cdot l^{-1}$).

The fundamental difference with equation (5) is that the non-specific biomass concentration X in equation (5) is more or less a constant, whereas the concentration of toluene degraders B in equation (6) is determined by the feed of toluene. Consequently, equation (6) has to be solved in combination with mass balances for B and toluene (see below).

Mass balances - Because it was assumed that no removal processes take place in the secondary settler, the concentrations of toluene in the final effluent and in the return sludge are equal to the concentration of toluene in the (effluent of) the completely mixed aeration tank. Under steady-state conditions, the following mass balances can now be written around the aeration tank for dissolved toluene and for toluene in the gas phase (also see Figure 1):

$$Q_i(C_i - C_a) - V_a(r_{str} + r_{bio}) = 0 \tag{7}$$

$$V_a r_{str} - Q_g C_g = 0 \tag{8}$$

with C_i and C_a the influent concentration of toluene and concentration of toluene in the (effluent) of the aeration tank, respectively ($\mu g \cdot l^{-1}$), Q_i the influent (wastewater) flow rate ($l \cdot h^{-1}$) and C_g the concentration of toluene in the air bubbles when they escape from the liquid phase ($\mu g \cdot l^{-1}$).

When Monod kinetics are used (equation 6), an additional mass balance around the pilot-plant is required for the (specific) toluene degraders. This balance includes growth and sludge wasting, but wash-out of sludge with the final effluent and biomass decay are neglected:

$$Y r_{bio} V_a - Q_w B = 0 \tag{9}$$

with Q_w the waste flow rate of sludge ($l \cdot h^{-1}$).

Material and methods

Activated sludge plant and operation - A pilot-scale activated sludge plant was employed which consisted of an aeration tank and a secondary settler. The aeration tank (Figure 2) had a liquid depth of 1 m and a working volume of 490 l. The tank was covered and the head-space volume was 50 l. Pulse tracer experiments confirmed that the liquid phase was completely mixed. A constant flow rate of dry, compressed air was maintained by two mass-flow controllers and was introduced at the bottom of the tank through four fine pore diffusers. The dissolved oxygen (DO) concentration was not controlled but always exceeded 3 mg·l^{-1}. To maintain the desired SRT sludge was wasted continuously from the aeration tank with a peristaltic pump. The pH of the mixed-liquor was controlled in a range of 7.2-7.4 using automated 0.3 M NaOH and 0.3 M HCl additions. More details about the pilot-plant can be found in chapter 4.1.

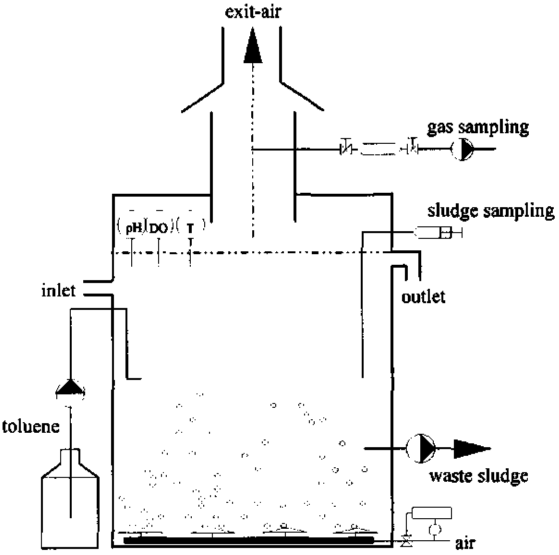


Fig. 2 Aeration tank of the pilot-scale activated sludge plant (secondary settler and return sludge are not shown, DO=dissolved oxygen, T=temperature).

The pilot-plant treated pre-settled municipal wastewater with average COD and NH_4-N concentrations of 500 mg·l^{-1} and 60 mg·l^{-1}, respectively. The plant was operated during two separate periods A and B according to the parameters of Table 2. The sludge was acclimated to an (enhanced) influent concentration of 100 µg·l^{-1} (periods A1 and B1 in Table 2). For this purpose stock solutions of toluene (Merck, analytical grade) were prepared with 24 l of suprapur water and were pumped directly into the aeration tank. The stock solutions were replenished every 4-5 days and were stored in a covered 25 l polyethylene (PE) container. After a period of at least four times the SRT, the influent concentration of toluene was further increased to target concentrations of 2000 $\mu g \cdot l^{-1}$ in period A2 and to 1000 $\mu g \cdot l^{-1}$ in period B2 (Table 2).

Table 2 - Operational parameters of the pilot-scale activated sludge plant.

parameter	symbol	period A		period B	
influent flow rate ($l \cdot h^{-1}$)	Q_i	67.1		39.5	
hydraulic retention time (HRT) (h)	θ_h	7.3		12.4	
waste sludge flow rate ($l \cdot h^{-1}$)	Q_w	2.04		0.75	
sludge retention time (SRT) (d)	θ_c	10		27.3	
COD loading rate (kg COD·kgVSS·d^{-1})	-	0.46		0.19	
return sludge flow rate ($l \cdot h^{-1}$)	Q_r	64.2		36.6	
air flow rate ($l \cdot h^{-1}$)	Q_g	4884		2704	
average wastewater temperature (°C)	T	22.3		17.2	
		A1	A2	B1	B2
target influent toluene concentration ($\mu g \cdot l^{-1}$)	C_i	100	2000	100	1000

Sampling - Every other day 24-h samples were taken from the influent of the plant to determine toluene concentrations and the standard water quality parameters SS, VSS, COD, NH_4-N and NO_3-N. The samples were collected in 3 l covered PE containers which were stored at 4 °C. To prevent biodegradation, prior to sampling the containers received 5 ml of concentrated H_2SO_4. Also every other day, grab samples were taken from the aeration tank and off-gas to determine toluene concentrations. Sludge samples from the aeration tank were taken with a glass syringe through a ¼-inch stainless steel pipe with the outlet located 50 cm below the liquid surface (Figure 2). The sample was gently transferred into 40 ml glass vials (Chrompack, purge and trap specific) which already contained 2 drops of concentrated HCl. The vials were filled without a head-space and were immediately sealed with a screw cap provided with a polytetrafluorethylene (TFE) faced silicon septum (Chrompack). All the samples were stored in the dark at 4 °C for a maximum of 2 days until subsequent instrumental analysis.

Off-gas samples were taken with a vacuum-pump at a constant flow rate from the hood above the aeration tank (Figure 2). The air was guided through 50 or 100 ml glass sample bulbs provided with a TFE-faced silicone septum. The actual sample was taken by subsequently closing the outlet and inlet valves of these sample bulbs. The samples were stored for a maximum of 6 hours until instrumental analysis.

Analytical procedures - COD, soluble COD, suspended solids, volatile suspended solids, NH_4-N and NO_3-N were all determined according to Dutch standard practice (NEN). Influent and sludge samples for determination of toluene were analysed by successive purge and trap (Tekmar, 3000) and GC (Chrompack, CB Sil 13 CB column) with flame ionisation detection (Fisons, FID-80). Although in this manner concentrations of total toluene are determined, i.e., dissolved as well as sorbed toluene, it is assumed that sorption is a negligible process and the measurements therefore reflect dissolved toluene. Air samples were injected on another GC (Hewlet-Packard, CP Sil 5 CB column) equipped with the same detector. The most important characteristics of the analytical procedure for toluene are summarised in Table 3. Analytical recovery was determined in abiotic batch stripping tests, instrumental precision by injecting known standards (every tenth sample) and method precision was calculated as the standard deviation in duplicate samples which were taken every fifth sample.

Table 3 - Characteristics of the toluene analytical methodology.

parameter	water/sludge samples	air samples
analytical recovery	98%	103-128%
instrumental precision	10.6%	14.6%
method precision	16.5%	25%
detection limit	0.1 $\mu g \cdot l^{-1}$	0.02 $\mu g \cdot l^{-1}$

Biodegradation tests - Three different biodegradation tests were carried out which differed in operational mode (batch or continuous), substrate to biomass ratio and the type of measurement that was used to follow biodegradation (O_2-consumption or specific toluene analysis).

Batch activated sludge (BAS) tests

In these BAS tests, return sludge and final effluent were collected from the pilot-plant and were mixed in covered 3 l jars to obtain a working volume of 2.5 l and target sludge concentrations of 2 and 4 g VSS$\cdot l^{-1}$. The jars were mechanically stirred at 20 rpm and aerated at 25 $l \cdot h^{-1}$ with dry, compressed air provided through a sparger stone. The jars were situated in a temperature controlled room at 20 °C. With a glass syringe a known volume of a toluene stock solution was injected below the liquid surface to give target concentrations between 0 and 1100 $\mu g \cdot l^{-1}$. Abiotic control tests were carried out at the same target concentrations, but with 2.5 l of suprapur water instead of activated sludge. At regular intervals 40 ml samples were taken with a glass syringe for determination of dissolved toluene. Off-gas samples were taken according to a procedure similar to the pilot-plant.

Ready biodegradability test

A ready biodegradability test was carried out according to the guidelines of OECD test method 301F (OECD, 1993). An automated sapromate (Voith) was used to follow oxygen consumption in response to toluene mineralisation. A (non-acclimated) activated sludge sample was sampled from the pilot-plant before period A and was aerated for a period of 48 hours to remove as much as possible the remaining carbon sources and ammonia. The sludge, mineral media and stock solutions were mixed in the sapromate cells to give an initial sludge concentration of 30 mg dry solids$\cdot l^{-1}$ and an initial toluene concentration of 26 mg$\cdot l^{-1}$ on COD basis. A test with a blank inoculum was included as well as a test with 109 mg$\cdot l^{-1}$ of the readily biodegradable compound potassium hydrogen phthalate (PHP) to serve as a method control. All the tests were carried out in duplicate over a period of 30 days at a temperature of 20 °C. From the oxygen consumption data the maximum specific growth rate μ_{max} was estimated according to a procedure described by Ellis *et al.* (1996).

By-pass test

In period B2, when the pilot-plant was operated at a sludge retention time of 27.3 days and a target influent toluene concentration to the pilot-plant of 1000 $\mu g \cdot l^{-1}$, a so-called by-pass test was carried out with the sludge from the pilot-plant to determine the biodegradation kinetics of toluene. This test was designed to mimic as closely as possible the conditions in the plant and to obtain a good approximation of the real biodegradation kinetics. The procedure for this test was described earlier in chapter 3.

Mass-transfer coefficient for oxygen ($K_{ol}a_{O2}$) - Usually the $K_{ol}a_{O2}$ is estimated from the response in dissolved oxygen (DO) concentration upon re-aeration in clean water (ASCE, 1984). However, the presence of wastewater contaminants and sludge may have an impact on the $K_{ol}a_{O2}$. To account for this effect, we used DO measurements in combination with an on-line measurement of the oxygen utilisation rate (OUR) as proposed by Spanjers and Klapwijk (1990). The completely mixed aeration tank was operated in batch mode for a period of 24 h to deplete as much as possible the external carbon sources and ammonia. After the aeration was switched off and the DO concentration had dropped to zero, the contents were re-aerated and both the DO and oxygen consumption rate were followed in time. For this situation the following mass balance can be written:

$$\frac{dS_{O2}}{dt} = K_{ol}a_{O2}(S_{O2s} - S_{O2}) - r_{O2} \qquad (10)$$

where S_{O2} is the DO concentration ($mg \cdot l^{-1}$), S_{O2s} is the DO concentration at saturation ($mg \cdot l^{-1}$) and r_{O2} is the OUR ($mg \cdot l^{-1} \cdot h^{-1}$).

The DO measurement has a certain response time which also was taken into account by modelling the reading of the probe that was used (WTW, TriOxmatic 600) as a first-order response to the real DO concentration (Spanjers and Olsson, 1992):

$$\frac{dS_{O2p}}{dt} = \frac{1}{\tau_p}(S_{O2} - S_{O2p}) \qquad (11)$$

where S_{o2p} is the DO probe reading ($mg \cdot l^{-1}$) and τ_p is the DO probe response time (h).

The τ_p was determined in a step response experiment and was equal to 7.5 sec. The $K_{ol}a_{O2}$ was estimated by calibration of equations (10) and (11) against the measured responses of the DO concentration and the OUR.

Results

Monitoring data - Figure 3 shows (background) toluene concentrations in 24-h samples of the pre-settled wastewater. The average daily wastewater concentration of 4 ± 1 $\mu g \cdot l^{-1}$ is relatively low compared to concentrations of 0.5 to 500 $\mu g \cdot l^{-1}$ found in other monitoring studies (Russell and Jenkins, 1982; Hannah and Rossman, 1982; Namkung and Rittmann, 1987; Melcer *et al.*, 1988; DBW/RIZA, 1990 and Bell *et al.*, 1993). Probably this can be explained by the small contribution of industrial wastewater, originating from a hospital and accounting for less than 5% of the total wastewater volume.

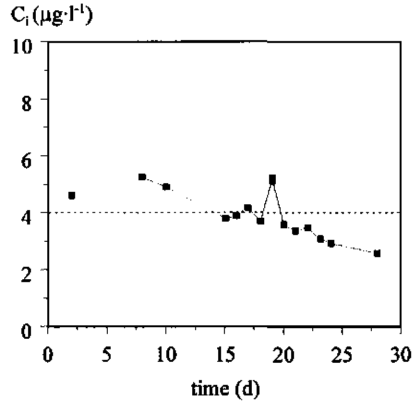


Fig. 3 Toluene concentrations in 24-h samples of pre-settled domestic wastewater (C_i) (--- = average concentration).

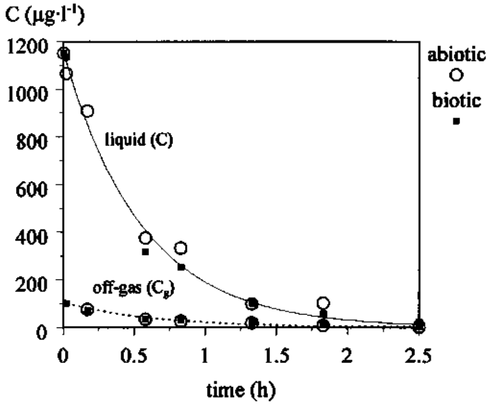


Fig. 4 Dissolved (C) and off-gas (C_g) toluene concentrations in an abiotic batch tests with suprapur water and in a biotic tests with activated sludge.

Figure 4 shows typical examples of liquid and off-gas concentrations of toluene in an abiotic control test with suprapur water and in a biotic test with activated sludge. The sludge in these experiments was sampled from the pilot-plant before period A when it was acclimated to an average background toluene concentration of 4 $\mu g \cdot l^{-1}$ (see above). In both batches toluene disappeared at similar rates. Together with the observation that all the toluene that was spiked to the batches could be recovered from the off-gas, this leads to the conclusion that the sludge did not have a significant "indigenous" toluene degrading capacity.

Average toluene concentrations with their standard deviations, emission rates from the pilot-plant and relative distributions of toluene during periods A1 to B2 are presented in Table 4. Toluene concentrations in the aeration tank varied between 0.15 and 2.05 $\mu g \cdot l^{-1}$. This corresponds well with concentrations of 0.1 to 6.2 $\mu g \cdot l^{-1}$ found in other monitoring studies carried out in pilot-scale as well as in full-scale plants (Blackburn *et al.*, 1984; Namkung and Rittmann, 1987; Melcer *et al.*, 1991; Melcer *et al.*, 1992; Bell *et al.*, 1993; Parker *et al.*, 1993 and Clapp *et al.*, 1994). Also, the off-gas toluene concentrations of 0.02 to 0.30 $\mu g \cdot l^{-1}$ that were found are similar to monitoring data between 0.1 and 1.3 $\mu g \cdot l^{-1}$ reported by Bell *et al.* (1993) and by Parker *et al.* (1993).

Table 4 - Toluene concentrations, emission rates and distribution in the pilot-plant.

	period A		period B	
operational parameters				
SRT θ_c (d)	10		27.3	
HRT θ_h (h)	7.3		12.4	
air flow rate Q_g ($l \cdot h^{-1}$)	4884		2704	
influent flow rate Q_i ($l \cdot h^{-1}$)	67.1		39.6	
waste sludge flow rate Q_w ($l \cdot h^{-1}$)	2.04		0.75	
temperature (°C)	22.3		17.2	
	period A1	period A2	period B1	period B2
sludge concentration				
aeration tank X_a (g VSS$\cdot l^{-1}$)	3.35	3.77	5.22	5.59
toluene concentrations ($\mu g \cdot l^{-1}$)				
influent C_i ($\mu g \cdot l^{-1}$)	100 ± 10*	1880 ± 160	98 ± 42	730 ± 67
aeration tank C_a ($\mu g \cdot l^{-1}$)	2.05 ± 0.66	1.23 ± 0.33	0.15 ± 0.03	0.20 ± 0.05
off-gas C_g ($\mu g \cdot l^{-1}$)	0.3 ± 0.06	0.23 ± 0.07	0.02 ± 0.02	0.02 ± 0.01
C_g/C_a (-)	0.15	0.19	0.13	0.10
toluene load ($mg \cdot h^{-1}$)				
influent = Q_iC_i	6.7	126.9	3.1	29.0
effluent = $(Q_i-Q_w)C_a$	0.27	0.16	0.01	0.02
waste sludge = Q_wC_a	< 0.01	< 0.01	< 0.01	< 0.01
off-gas = Q_gC_g	1.47	1.12	0.05	0.05
biodegraded = $Q_iC_i-Q_iC_a-Q_gC_g$	4.95	125.6	3.03	28.9
distribution (%)				
effluent	3.9	0.1	0.4	0.1
waste sludge	<0.1	<0.1	<0.1	<0.1
off-gas	21.7	0.9	1.8	0.2
biodegraded	74.3	99.0	97.9	99.8

* standard deviation

Three important observations can be made with respect to the concentrations of toluene. First, a comparison between periods A1 and A2 and between periods B1 and B2, at equal SRTs and air flow rates but at different influent toluene concentrations, suggests that the influent concentration of toluene did not have a significant effect on the concentration of toluene in the aeration tank or on the concentration of toluene in the off-gas of this tank. Second, in period B these concentrations were about 10 times lower than in period A, most likely due to the longer SRT and/or lower air flow rate in period B. Finally, the ratio of the concentration of toluene in the off-gas over the concentration in the liquid phase of the aeration tank varies between 0.10 and 0.19, but appears to be somewhat higher in period A than in period B.

The load of toluene that was wasted with the excess sludge, calculated from the waste sludge flow rate Q_w and the (total) concentration of toluene in the completely mixed aeration tank C_a, was negligibly small (< 0.01 $mg \cdot h^{-1}$) in all the periods. Only in period A1 a small amount of toluene was discharged with the effluent (4%) or was removed with the off-gas (22%). The remaining 74% of toluene removal can be attributed to biodegradation. In the other three periods (A2, B1 and B2) more than 97% of the influent load was removed by biodegradation and only extremely small amounts of toluene were discharged with the effluent (<0.5%) or escaped with the off-gas (<2%). These extremely high toluene degradation efficiencies by municipal activated sludge are in agreement with results published by Melcer *et al.* (1992), but are higher than efficiencies in a range of 59 to 83% reported by others (e.g., Bell *et al.*, 1993; Parker *et al.*, 1993 and Clapp *et al.*, 1994).

Model parameters - A linear relationship could be established between the mass-transfer coefficient for oxygen $K_{ol}a_{O2}$ and the air flow rate Q_g (Figure 5) with a slope of 0.0046 l^{-1} and an intercept of -7.9 h^{-1} (r^2=0.999). The (first-order) stripping rate constant for toluene k_{str}, calculated from $K_{ol}a_{O2}$ with equations (2) to (4), is presented in Table 6 together with the other air stripping parameters. A temperature effect on Henry's law constant for toluene was accounted for by a relationship given by Staudinger and Roberts (1996).

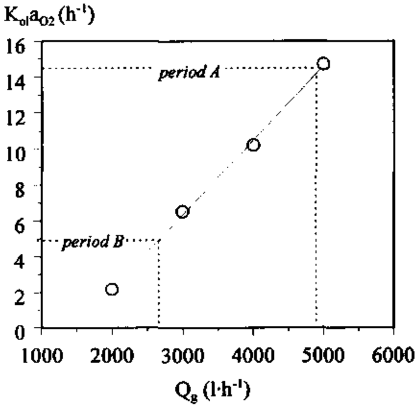


Fig. 5 Effect of the air flow rate (Q_g) on the oxygen mass-transfer coefficient ($K_{ol}a_{O2}$).

Table 5 - Model input parameters.

parameter	period A	period B
stripping		
temperature (°C)	22.3	17.2
H (-)*	0.25	0.19
$K_{ol}a/K_{ol}a_{O2}$**	0.53	0.53
Q_g ($l{\cdot}h^{-1}$)	4884	2704
$K_{ol}a_{O2}$ (h^{-1})	14.8	4.7
$K_{ol}a$ (h^{-1})	7.9	2.5
f_{sat} (-)	0.96	0.84
k_{str} (h^{-1})	2.39	1.15
biodegradation		
secondary utilisation (eq. 5)		
K_C ($\mu g{\cdot}l^{-1}$)***	376	
k_0 ($\mu g{\cdot}g^{-1}VSS{\cdot}h^{-1}$)***	545	
Monod kinetics (eq. 6)		
K_C ($\mu g{\cdot}l^{-1}$)***	376	
μ_{max} (h^{-1})****	0.125	

* taken from Staudinger and Roberts (1996)
** taken from Matter-Müller *et al.* (1981)
*** determined in by-pass test (see chapter 3)
**** determined in ready biodegradability test

Table 6 also includes the biodegradation parameters for secondary utilisation kinetics (equation 5) and for Monod kinetics (equation 6). A half-velocity constant K_C of 376 $\mu g{\cdot}l^{-1}$ and a maximum biodegradation rate constant k_0 of 545 $\mu g{\cdot}g^{-1}$ $VSS{\cdot}h^{-1}$ were determined in the by-pass test (chapter 3).

The maximum specific growth rate μ_{max} for the Monod model of 0.125 ± 0.016 h^{-1} was estimated from the results of the ready biodegradability test (data not shown).

Model validation - Based on the plant operational parameters of Table 2 and the air stripping and biodegradation parameters of Table 6, toluene concentrations in the aeration tank of the pilot-plant (C_a) and in the off-gas of this aeration tank (C_g) were calculated with equations (1) to (9) using the solver of Microsoft Excel®. In Figure 6 these calculated concentrations are compared to the monitoring data of Table 4.

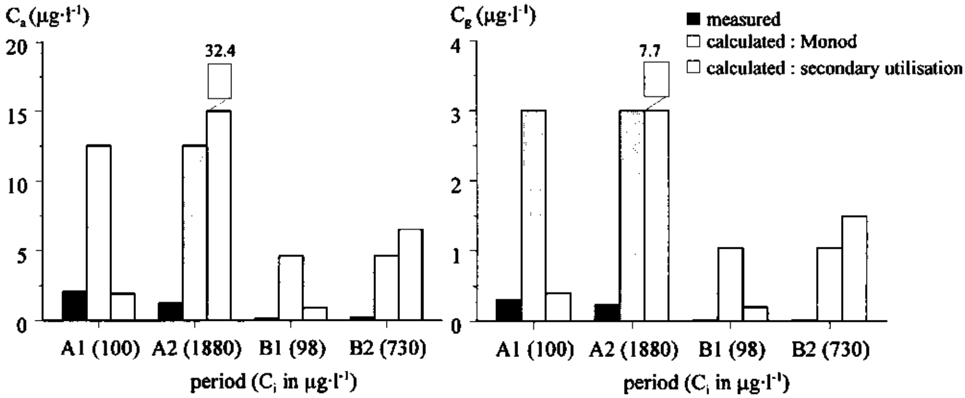


Fig. 6 Measured (black) and calculated (grey: Monod; white: secondary utilisation) toluene concentrations in the aeration tank of the pilot-plant (C_a) and in the off-gas of this aeration tank (C_g).

Model predictions based on secondary utilisation kinetics are reasonably accurate for periods A1 and B1 when the pilot-plant was operated at low influent toluene concentrations. This may not be very surprising as the biodegradation parameters for this model (K_C and k_0) were determined with sludge that was acclimated to an equally low influent toluene concentration. However, at higher influent concentrations in periods A2 and B2 secondary utilisation kinetics predict concentrations that are 30 to 60 times higher than the measurements.

Model predictions based on Monod kinetics exceed the monitoring data in all four periods. However, in contrast to secondary utilisation kinetics, Monod kinetics correctly predict the observed trend (i) that an increase of the influent concentration (from period A1 to A2, and from period B1 to B2) has no significant effect on the toluene concentrations and (ii) that in period B at a longer SRT and lower air flow rate these concentrations are consistently lower than in period A.

Finally, the model predicted ratio between the off-gas concentration of toluene and the liquid concentration of toluene C_g/C_a approximately was a factor 2 higher than observed values for this ratio (Table 4).

Discussion

Biodegradation kinetics - The model validation exercise showed that the observed trend in the monitoring data could be predicted by Monod kinetics but not by secondary utilisation kinetics. However, the concentrations of toluene in the aeration tank and in the off-gas of this tank were systematically overestimated. Probably this can be explained by inaccuracies in the Monod parameters maximum specific growth rate μ_{max} and half-velocity constant K_C. In particular this applies to μ_{max} which was determined in a ready biodegradability test. The conditions in this test were entirely different from the conditions in the pilot-scale plant and this may have resulted in a changing composition and/or physiological state of the toluene degrading population during the test. Also, in this test toluene mineralisation was followed over time whereas the model predictions were based on primary biodegradation (parent compound removal) which may be a much faster process than mineralisation. Finally, although the biodegradation parameters μ_{max} and K_C supposedly are constants, results with other test compounds such as the C_{12}-homologue of linear alkylbenzene sulfonate (chapter 4) and nitrilotriacetic acid (chapter 5) have shown that biodegradation parameters may depend on the operational characteristics and influent concentration the activated sludge is acclimated to. For toluene these effects were not investigated.

The results apply to influent toluene concentrations between 100 and 1880 $\mu g \cdot l^{-1}$. According to Nyholm and Ingerslev (1996) and Namkung and Rittmann (1987) 100 $\mu g \cdot l^{-1}$ could be the lower limit for the applicability of the Monod model because lower concentrations may not support growth of (specific) toluene degraders. Note that this minimum concentration to obtain specific growth also is determined by air stripping because toluene which escapes to the atmosphere otherwise would have been available for growth. Although at a (background) influent concentration of 4 $\mu g \cdot l^{-1}$ no biodegradation could be detected, results reported by Parker *et al.* (1993), Clapp *et al.* (1994), and Smith *et al.* (1993) suggested that already at influent concentrations of 7-30 $\mu g \cdot l^{-1}$ biodegradation becomes the major removal mechanism for toluene. It remains unclear however whether at these concentrations Monod kinetics should be applied or biodegradation will be dominated by secondary utilisation.

Air stripping - The ratio between the off-gas and liquid concentrations of toluene in the aeration tank C_g/C_a appeared to be somewhat lower in period B than in period A (Table 4). Most likely this can be attributed to the lower temperature in period B which reduces the tendency of a compound to escape to the air. Although in principle this effect can easily be accounted for in fate models, this requires knowledge about the effect of the temperature on Henry's law constant. For toluene this knowledge was available but for many other trace pollutants this is not the case.

Model predicted values for C_g/C_a were approximately a factor 2 higher than observed values for C_g/C_a. This can only be explained if the ratio between the mass-transfer coefficients for toluene and oxygen $K_{ol}a/K_{ol}a_{O2}$ of 0.53 that was taken from Matter-Müller *et al.* (1981) was too high. A model calibration showed that accurate predictions of C_g/C_a would require a much lower value for $K_{ol}a/K_{ol}a_{O2}$ of 0.16. This may not be entirely unrealistic as Hsieh *et al.* (1993) reported values in a range of 0.17-0.37. Furthermore, they showed that $K_{ol}a/K_{ol}a_{O2}$ was dependent on the operating conditions, particularly on the gas retention time $\tau_g=V/Q_g$ with a decreasing trend in $K_{ol}a/K_{ol}a_{O2}$ at increasing values for τ_g. In our experiments τ_g was 6 and 10 minutes in periods A and B, respectively. These values are close to a τ_g of 8 minutes Hsieh *et al.* (1993) found a $K_{ol}a/K_{ol}a_{O2}$ of 0.17 for, but much longer than the τ_g of 1.5 minutes that was applied by Matter-Müller *et al.* (1981) who found a $K_{ol}a/K_{ol}a_{O2}$ of 0.53. It seems that to obtain accurate predictions of air emission rates this dependency of $K_{ol}a/K_{ol}a_{O2}$ on τ_g should be examined in more detail.

Competition between biodegradation and air stripping - Toluene was selected as a test pollutant to investigate competition between biodegradation and volatilisation. The results showed that biodegradation is the dominant removal mechanism, provided that toluene is supplied at sufficiently high influent concentrations. For less easily biodegradable trace compounds, for example volatile chlorinated compounds, a stronger competition between biodegradation and air stripping can be expected (Kincannon *et al.*, 1983; Dunovant *et al.*, 1986; Melcer *et al.*, 1992 and Bell *et al.*, 1993). It therefore is recommended to use such compounds to further investigate the competition between biodegradation and air stripping in activated sludge plants.

Conclusions

The monitoring data indicated that activated sludge fate models which employ Monod kinetics to describe the rate of biodegradation can give accurate predictions of effluent and off-gas concentrations of toluene provided that (i) accurate values are available for the biodegradation parameters (μ_{max} and K_C) (ii) an accurate value is available for the ratio between the mass-transfer coefficients for toluene and oxygen $K_{ol}a/K_{ol}a_{O2}$ and (iii) the influent concentration of toluene is sufficiently high to support growth of specific toluene degraders. In the experiments the pilot-plant was fed at a minimum concentration of 100 $\mu g \cdot l^{-1}$. It remains unclear whether the Monod model also is valid at lower influent concentrations or a different kinetic model would be required.

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Chapter 7

Activated sludge response to a step increase of the influent concentration of selected organic trace pollutants

Abstract - Activated sludge fate models could be a useful tool in the environmental exposure assessment of organic priority compounds. The majority of these models only consider steady-state situations, i.e., they assume that a constant load of these compounds is supplied with the wastewater. In reality activated sludge plants have to deal with time-variable loads and this may have a significant impact on their removal efficiencies. The dynamic behaviour of three readily biodegradable test pollutants (linear alkylbenzene sulfonate, nitrilotriacetic acid and toluene) was investigated in a pilot-scale municipal activated sludge plant. For all three compounds the sludge adapted to a step increase of the influent concentration of these compounds by increasing their rate of biodegradation. However, major differences were observed in the transient response, indicating that completely different adaptation mechanisms were involved. It was concluded that existing activated sludge fate models cannot be used to accurately predict the effect of highly variable influent concentrations.

Introduction

During the last two decades several activated sludge fate models have been developed (Namkung and Rittmann, 1987; Govind, 1991; Cowan *et al.*, 1993 and Struijs *et al.*, 1996; McAvoy *et al.*, 1998). These models can be used (i) to predict the environmental exposure to specific organic trace compounds expected to appear in municipal wastewater's, (ii) to optimise the design and control of treatment plants with respect to the removal of these pollutants and (iii) to establish limits on treatment plant influent loads based upon allowable effluent loads (Kemp *et al.*, 2000). In general, these activated sludge fate models assume a constant supply of these trace compounds with the wastewater and this supply is estimated from annual production or consumption data. However, widely time-varying wastewater concentrations of trace compounds have been demonstrated in several monitoring studies. Usually this variation is dominated by diurnal fluctuations. Examples are given by Alder *et al.* (1990) for nitrilotriacetic acid (NTA) and by Feijtel *et al.* (1996) for linear alkyl benzene sulfonates (LAS). Particularly when industrial effluents contribute to the (municipal) wastewater, daily fluctuations and pulse loads also become important (e.g. Melcer *et al.*, 1989).

The most important removal processes determining the distribution of organic compounds in activated sludge plants are biodegradation and the mass-transfer processes sorption and air stripping. In contrast to the mass-transfer processes that are expected to show relatively simple dynamic behaviour in response to influent perturbations, the biodegradation response may be extremely complicated. Similar to the well-known activated sludge model No. 1 (Henze *et al.*, 1987), which describes the removal of the standard water quality parameters COD (chemical oxygen demand) and ammonia, a Monod model can be used to explain that variations in the influent concentration of trace compounds are balanced by growth and sludge wastage of specific micro-organisms that utilise these compounds as their source of energy. However, the transient response is not determined by growth and sludge wastage alone, but also other microbial adaptation processes may be involved such as physiological adaptation (changes in the biochemical composition of the micro-organisms), induction of specific enzymes, synthesis of storage polymers and excretion of intermediate metabolic products (Daigger and Grady, 1982).

In this chapter, the dynamic behaviour of three test compounds (the C_{12}-homologue of LAS, NTA and toluene) in response to a step increase of their influent concentration was followed in a pilot-scale municipal activated sludge plant. The main objective of this exercise was to investigate whether this behaviour can be modelled by a classic growth response or that a more complicated model would be required.

Material and methods

Pilot-plant and operation - A pilot-scale activated sludge plant was employed which consisted of a completely mixed aeration tank and a secondary settler. A detailed description of this plant can be found in chapter 4.1 and the most important characteristics are given in Table 1. The background concentration of toluene in the (pre-settled) domestic wastewater was 4 ± 1 $\mu g \cdot l^{-1}$, but this was too low to initiate biodegradation of toluene (chapter 6). Therefore, starting two months before the experiments, with an external addition the influent concentration of toluene was increased to 98 $\mu g \cdot l^{-1}$. The concentrations of NTA and LAS-C_{12} in the wastewater, 302 and 1908 $\mu g \cdot l^{-1}$ respectively, were sufficiently high to obtain biodegradation (chapters 4.1 and 5). After an operating period of more than 4 times the sludge retention time (SRT), a step increase of the influent concentrations of LAS-C_{12}, NTA and toluene was initiated (Table 2). For this purpose stock solutions of LAS-C_{12} (154.2 g LAS$\cdot l^{-1}$, Isorchem 113/S-Na containing 31.4% of LAS-C_{12}), NTA ($NTANa_3$, Sigma 99.5%) and toluene (Merck, analytical grade), were prepared in 24 l of suprapur water and stored in a 25 l polyethylene container.

Table 1 - Operational characteristics of the pilot-scale activated sludge plant.

characteristic	value
aeration tank volume (l)	490
settler volume (l)	200
sludge retention time (SRT) (d)	27.3
hydraulic retention time (HRT) (h)	12.4
influent flow rate ($l \cdot h^{-1}$)	39.6
return sludge flow rate ($l \cdot h^{-1}$)	36.6
waste sludge flow rate ($l \cdot h^{-1}$)	0.75
air flow rate ($l \cdot h^{-1}$)	2704
volatile suspended solids (VSS) aeration tank ($g \cdot l^{-1}$)	5.59
VSS effluent ($mg \cdot l^{-1}$)	22
COD loading rate (kg $COD \cdot kg^{-1}$ $VSS \cdot d^{-1}$)	0.19

Table 2 - Average influent concentrations of the test compounds.

	influent concentration ($\mu g \cdot l^{-1}$)	
compound	background	step increase
LAS-C_{12}	1908	11505
NTA	302	35508
toluene	98	730

Sampling and analysis - Figure 1 gives an overview of the sampling strategy that was followed. Sampling for LAS-C_{12}, NTA and toluene started 12 days before the start of the step response experiment. For all three test compounds 24-h samples were taken from the influent. In addition, 24-h samples were taken from the influent and final effluent to determine the standard water quality parameters COD, soluble COD, NH_4-N and suspended solids (SS).

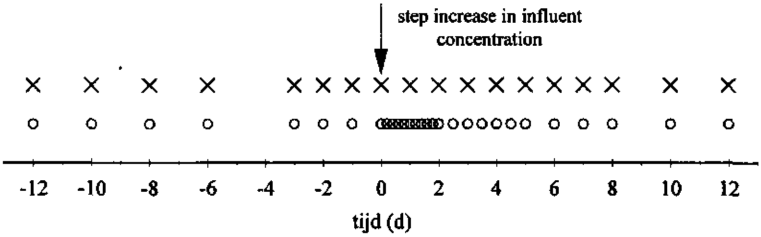

Fig. 1 Sampling strategy for the test compounds (× 24-h composite samples of the influent, o grab samples of the aeration tank, return sludge, final effluent and off-gas).

Grab samples for determination of LAS-C_{12} and NTA were taken from the aeration tank, return sludge and final effluent. Grab samples for toluene were taken from the aeration tank and the off-gas of this tank. Sampling continued until a steady-state was achieved with respect to the concentration of the test compounds in the aeration tank. Approximately 20-30% of all the samples was taken in duplicate. COD, NH_4-N, SS and VSS were determined according to Dutch standard practice (NEN). Detailed information about the sampling, preservation and analytical procedures can be found in chapters 4.1 (LAS-C_{12}), 5 (NTA) and 6 (toluene).

Results

Figures 2 and 3 show LAS-C_{12} and NTA concentrations in grab samples taken from the aeration tank, return sludge and final effluent, just before and immediately following the step increases in the influent concentration. Figure 4 shows toluene concentrations in grab samples taken from the aeration tank and off-gas of this tank. Although the measurements were already available starting 12 days before the step increase and exhibited a relatively stable behaviour, for reasons of clarity, Figures 2 to 4 only show concentrations starting 3 days before the step increases.

All three test compounds responded similarly to the step increase of their influent concentration with a rapid increase in concentration to reach a peak, followed by a more gradual decrease to a concentration which was also observed before the start of the step disturbance. The random pattern in the concentration of LAS-C_{12} in the final effluent (Figure 2) probably was due to a low accuracy of the measurements as these were all are close to the method detection limit of 4 $\mu g \cdot l^{-1}$ (chapter 4.1). Besides, because LAS-C_{12} strongly sorbs to the sludge, the effluent concentration is extremely sensitive to variations in the concentration of suspended solids in the final effluent.

Focusing on the concentrations in the aeration tank, the behaviour of the test compounds was characterised by the following parameters: (i) the time lag between the increase of influent concentration and the occurrence of a peak concentration, (ii) the height of this peak in comparison with the maximum "abiotic" response if biodegradation would have been absent (see the appendix for calculations) and (iii) the time it took to recover from the peak and to achieve a new steady-state. Values for these parameters are presented in Table 3.

Table 3 - Characteristics of the response of the aeration tank concentration to a step increase of the influent concentration.

	time to reach peak (d)	maximum abiotic response ($\mu g \cdot l^{-1}$)	observed peak concentration		time to recover from peak (d)
			concentration $\mu g \cdot l^{-1}$	% of maximum abiotic response	
LAS-C_{12}	≤ 0.2	154380	≥ 395*	≥ 0.3	> 3
NTA	~ 1	35508	17677	50	< 2
toluene	≤ 0.2	48	≥ 3.1	≥ 6	2 - 3

* sum of dissolved and adsorbed concentrations

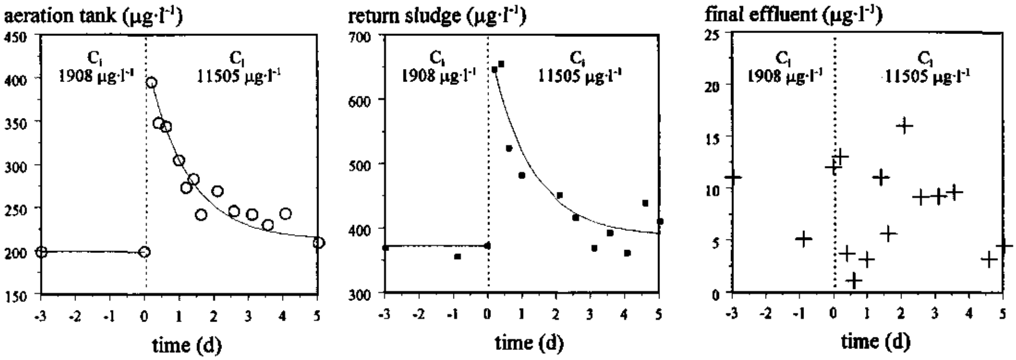

Fig. 2 Sum of dissolved and sorbed LAS-C_{12} concentrations in the aeration tank, return sludge and final effluent in response to a step increase of the influent concentration (C_i).

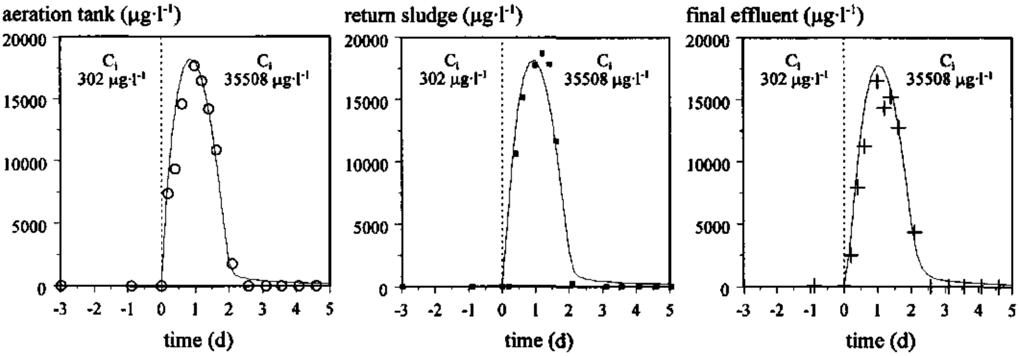

Fig. 3 NTA concentrations in the aeration tank, return sludge and final effluent in response to a step-increase of the influent concentration (C_i).

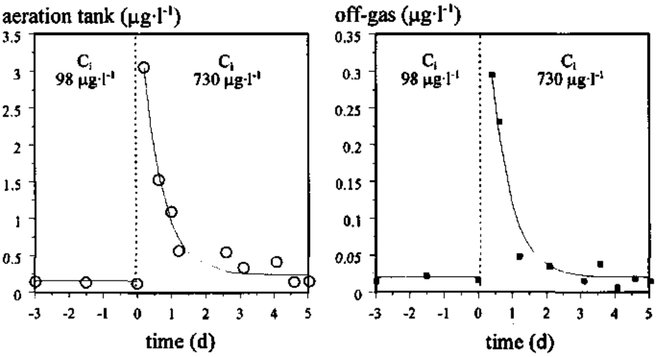

Fig. 4 Toluene concentrations in the aeration tank and off-gas in response to a step-increase of the influent concentration (C_i).

A clear difference can be detected between the behaviour of LAS-C_{12} and toluene as compared to the behaviour of NTA. It took less than 0.2 d before the concentration of LAS-C_{12} and toluene reached a peak. Although the real peak cannot be observed because it may have occurred before the first sampling instant at 0.2 d, this peak most likely has been much lower than the maximum abiotic response, showing the ability of the activated sludge system to attenuate the input disturbance that was imposed upon it. For toluene a similar result was reported by Melcer *et al.* (1991). In contrast to LAS-C_{12} and toluene, it took more than 24 hours before the concentration of NTA reached a peak. This peak amounted to 50% of the calculated maximum abiotic response, which for NTA is equal to the influent concentration. Recovery after the peak was somewhat slower for LAS-C_{12} than for toluene and NTA.

The dynamic behaviour of the aeration tank concentration in the absence of biodegradation (the abiotic response) was simulated using the equations of the appendix. Figure 5 shows this abiotic response for all three test compounds. LAS-C_{12} is a highly sorptive compound and 93 to 98% of LAS-C_{12} present is sorbed to the sludge (chapter 4). Therefore, in the absence of biodegradation LAS-C_{12} would accumulate at a much higher concentration than the influent concentration (Figure 5). Sorption also slows down the abiotic response because sorbed LAS-C_{12} is removed with the small flow of waste sludge rather than with the much higher flow of final effluent. Because it is a non-sorptive and non-volatile compound the abiotic response of NTA is mainly determined by the hydraulic retention time. Removal of NTA with the waste sludge and off-gas can be neglected and the new abiotic steady-state concentration equals the influent concentration. For toluene this abiotic end concentration is much lower than the influent concentration because a large part of the influent load of toluene is removed by air stripping. Because air stripping is a relatively fast process, the abiotic response of toluene also is faster than the abiotic responses of NTA and LAS-C_{12}.

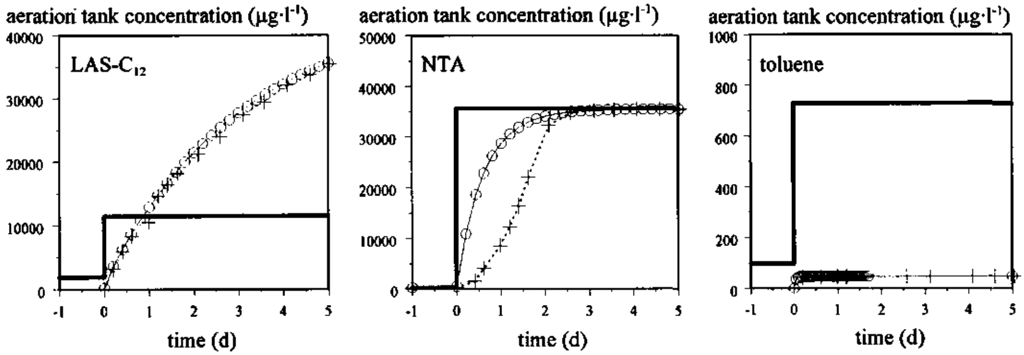

Fig. 5 Simulated abiotic (o) and calculated biodegraded (+) concentration in the aeration tank in response to a step increase of the influent concentration (bold line) of LAS-C_{12}, NTA and toluene.

Assuming that for all three test compounds biodegradation is the only relevant conversion process, the biodegradation response was calculated as the difference between the simulated abiotic response and the measured response. The results of these calculations are also presented in Figure 5. For LAS-C_{12} and toluene the concentration that was biodegraded more or less coincides with the abiotic concentration, indicating that the sludge can immediately biodegrade the increased influent load of these compounds. In contrast, biodegradation of NTA lags behind the abiotic response by 1-2 days. This is also explains the much higher gain (% of abiotic response) of NTA compared to LAS-C_{12} and toluene (Table 3).

Discussion

For all three test compounds the sludge in the pilot-plant responded to the enhanced loading rate with an increase of the rate of biodegradation. This is in agreement with previous observations that all three compounds are readily biodegraded by activated sludge and that their effluent concentration is more or less independent of their influent concentration (chapters 4, 5 and 6 for LAS-C_{12}, NTA and toluene, respectively). However, there are significant differences in the transient response of the rate of primary biodegradation, i.e., in the rate at which the parent compounds are removed (Figure 6).

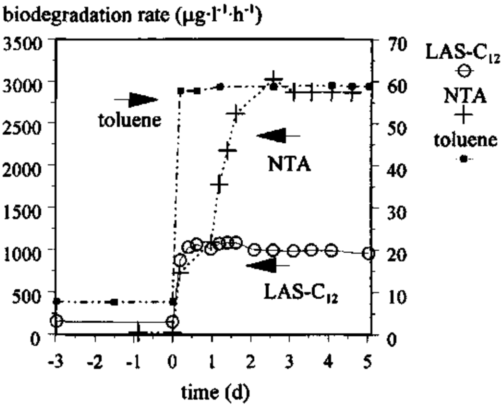

Fig. 6 Calculated rate of biodegradation rate of LAS-C_{12}, NTA and toluene in response to a step increase of their influent concentrations.

The immediate response of the biodegradation rate of LAS-C_{12} could be explained by growth, assuming that the micro-organisms responsible for the initial steps in the biodegradation pathway, hydroxylation of the alkyl-chain from the terminal methyl-group(s) and β-oxidation (Jiménez *et al.*, 1991), possess an extremely fast (maximum) specific growth rate. However, measurements of the primary biodegradation kinetics of LAS-C_{12} (chapter 4.2) strongly suggested that enrichment of

LAS-C_{12} degraders is an unlikely explanation. An alternative explanation could be that the initial steps in the biodegradation pathway are widespread features among the micro-organisms in (municipal) activated sludge and that these micro-organisms can easily switch between LAS-C_{12} and other carbon sources supplied by the municipal wastewater. Remark that the fast response of primary biodegradation not necessarily means that ultimate biodegradation of LAS is equally fast. Soluble sulfophenyl alkanoates (SPCs) are intermediate products in the biodegradation pathway that have to be metabolised via ring-opening and desulfonation by a second group of micro-organisms. It is known that this second step in the biodegradation pathway is rate-limiting and during the transient period a significant peak of SPCs can be expected in the effluent (van Ginkel, 1996).

In contrast to LAS-C_{12}, kinetic experiments have shown that an increase of the influent load of NTA results in an almost proportional increase of the NTA degrading capacity (chapter 6), indicating that enrichment of specific NTA degraders is the dominant adaptation mechanism, at least at very high influent concentrations of NTA. In fact, the model fits in Figure 3 were obtained with a Monod model using a relatively slow maximum specific growth rate μ_{max} of 0.05 h^{-1} which is not far from an equally slow μ_{max} of 0.03 ± 0.004 h^{-1} found in an oxygen consumption test with NTA as the sole carbon source (Temmink and Klapwijk, 1998). Also the literature mentions that NTA degraders are slow growing micro-organisms (Egli, 1994). The assumption of a growth response seems to be contradictory with Bally *et al.* (1994) who state that an increased enzyme production is the dominant biodegradation response. However, the (enhanced) influent concentration of NTA contributed significantly to the wastewater COD (~5%) and enrichment therefore may have been more important than enzyme induction.

Similar to LAS-C_{12}, the rate of primary biodegradation of toluene showed an extremely fast response to a step increase of the influent concentration. Attempts to model this behaviour with a Monod model failed. Although no data are available to support this, at least not for activated sludge, it seems that a much faster adaptation process is involved, for example induction of toluene degrading enzymes by members of the existing microbial population.

It seems that the behaviour of activated sludge plants in case of time-variable loading with organic trace compounds is highly complex and is not determined by growth and sludge wasting alone, but also by other adaptation mechanisms. Moreover, this behaviour not only depends on the compound that is involved, but probably also on the exact nature (time pattern and strength) of the influent variations. Because microbial adaptation processes are still not very well understood, reliable dynamic models are not yet available and more research in this direction is desirable. It is also possible to use a less mechanistic approach, for instance black-box models based on time series analysis (Melcer *et al.*, 1989). However, such models have to be calibrated and this requires an extensive set of accurate monitoring data which usually is not available.

Conclusions

The results showed that for all three compounds a step increase in the influent concentration is accompanied by an increase of the biodegradation rate for these compounds. However, differences were observed in the transient response, indicating that different adaptation mechanisms are involved. It can be concluded that existing activated sludge fate models cannot be used to accurately predict the effect of highly variable influent concentrations.

Appendix - Abiotic response

A.1 Nomenclature

symbols			subscripts	
C	total concentration of a compound*		e	clarified effluent
K_p	sludge-water partition coefficient	$l \cdot g^{-1}$ VSS	i	influent
k_{str}	stripping rate constant	h^{-1}	r	return sludge
Q	flow rate	$l \cdot h^{-1}$	w	waste sludge
V	volume	l		
X	volatile suspended solids	g VSS$\cdot l^{-1}$		

* sum of sorbed and dissolved concentrations

A.2 Model

The pilot-plant consist of a completely mixed aeration tank and a secondary settler (Figure A1). The latter consists of a compartment containing clarified effluent and a compartment with settled return sludge.

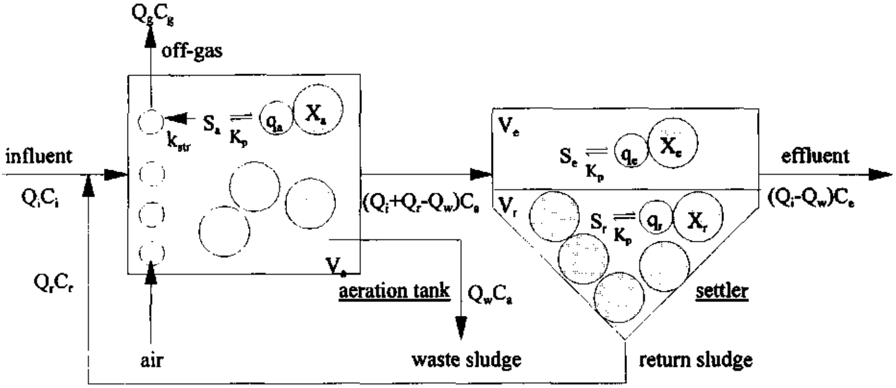

Fig. A1 Model activated sludge plant.

Assuming that sorption of a compound is an instantaneous process which follows linear sludge-water partitioning and air stripping is limited to the dissolved fraction of a compound, the following mass balances can be written around the aeration tank, clarified effluent compartment and settled return sludge compartment, respectively:

$$V_a \frac{dC_a}{dt} = Q_i C_i + Q_r C_r - (Q_i + Q_r) C_a - k_{str} V_a \frac{1}{1 + K_p X_a} C_a \qquad (A.1)$$

$$V_e \frac{dC_e}{dt} = (Q_i - Q_w)(\frac{1 + K_p X_e}{1 + K_p X_a} C_a - C_e) \qquad (A.2)$$

$$V_r \frac{dC_r}{dt} = Q_r (\frac{1 + K_p X_r}{1 + K_p X_a} C_a - C_r) \qquad (A.3)$$

For K_p a value of 3.2 $l \cdot g^{-1}$ VSS was taken from chapter 4.2 and for k_{str} a value of 1.15 h^{-1} from chapter 6. The other parameters can be found in Table 2. Steady-state concentrations were evaluated from the equations above by setting the accumulation terms dC/dt to zero. Dynamic responses of these concentrations to a step increase of the influent concentration were obtained with the simulation software Simnon®.

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Chapter 8

General discussion

Introduction

In the literature several models have been reported that can be used to predict the distribution of (organic) trace compounds in municipal activated sludge plants. These models consist of mathematical equations for the most important processes determining this distribution: sorption, volatilisation and biodegradation. The literature review of chapter 2 showed that there are many similarities between these models, but also some fundamental differences. The most important difference concerns the biodegradation kinetics, which can range from simple pragmatic first-order to secondary utilisation and Monod kinetics. Because activated sludge fate models are extremely sensitive to the rate of biodegradation, these differences in the biodegradation kinetics can also be expected to give completely different model predictions.

Another important conclusion of the literature review of chapter 2 was that none of the available activated sludge fate models has been thoroughly validated and therefore their application remains uncertain. This can be attributed to a lack of accurate monitoring data or because these monitoring data are not accompanied by essential information about plant operation at the time of sampling. Even more important is that insufficient variation was applied to those parameters that activated sludge models are most sensitive to. In particular the sludge retention time (SRT) and influent trace pollutant concentration have to mentioned in this regard.

In this thesis the reliability of existing activated sludge fate models was explored. For this purpose monitoring data were collected in a pilot-scale municipal activated sludge plant for a number of selected test compounds. The pilot-plant was operated at two SRTs and was fed with various influent concentrations of the test compounds. For each of these conditions the kinetics of (primary) biodegradation were determined using a new biodegradation test method (chapter 3). This test was developed because the literature review of chapter 2 indicated that existing (standardised) biodegradation tests do not yield reliable kinetic data. Based on the experimental results and supported by information from the literature, in this chapter the reliability of existing activated sludge fate models is discussed and some of their shortcomings will be highlighted. Based on these shortcomings some recommendations for future research activities will be given.

Selection of test compounds

The most important parameters in activated sludge fate models are the physical-chemical and biodegradation properties of the organic trace compounds. Because these properties vary strongly among trace pollutants, a large number of test compounds should be investigated. Initially, 17 test compounds were selected whose physical-chemical and biodegradation properties are presented in Table 1 and are visualised in Figure 1. The distinction that was made in the biodegradation properties "readily", "slowly" or "non-biodegradable" was rather arbitrary because for many compounds different literature sources gave conflicting results. Partly this can be attributed to differences in the test conditions that were imposed upon the target compound. For example, ethylenediaminetetraaceticacid (EDTA) usually is given the label "non-biodegradable" (e.g., Alder *et al.*, 1990), but nevertheless can be readily biodegraded by activated sludge as long as extremely favourable conditions are applied, that is, long SRTs (Cech and Chudoba, 1988), long adaptation times and alkaline conditions (van Ginkel *et al.*, 1999).

Table 1- Selected test compounds for the validation of activated sludge fate models.

compound	acronym	$\log(K_{ow})$	log(H)*	biodegradability	reference
methyl-tert-butyl ether	MTBE	1.04	1.78	unknown	Rippen (1996)
chloroethane	CA	1.46	3.05	none	BUA (1986-1993)
nitrobenzene	NB	1.59	0.38	slowly	BUA (1986-1993)
1-amino-2-chlorobenzene	1A2C	1.76	-0.3	none	BUA (1986-1993)
1,1-dichloroethane	11DCA	1.79	2.67	slowly	Rippen (1996)
linear alkylbenzene sulfonate	LAS	1.96	**	easily	Rippen (1996)
4-nitrophenol	4NP	2.04	-4.44	slowly	BUA (1986-1993)
diethylphthalate	DEP	2.35	-1.95	slowly	BUA (1986-1993)
2,6-dimethylphenol	26DMP	2.51	-0.42	easily	Shiu *et al.* (1994)
toluene	TOL	2.75	2.69	easily	Rippen (1996)
dipropylphtalate	DPP	3.27	-1.51	slowly	Staples *et al.* (1997)
diphenylamine	DPA	3.62	-1.04	none	BUA (1986-1993)
2,6-di-tert-butyl-p-cresol	BHT	4.17	1.78	none	BUA (1986-1993)
di-n-butylphthalate	DnBP	4.45	-1.2	slowly	Staples *et al.* (1997)
fluoranthene	FA	5.13	-0.18	none	Rippen (1996)
ethylenediaminetetraacetic acid	EDTA	***	**	none	Alder *et al.* (1990)
nitrotriacetic acid	NTA	***	**	easily	BUA (1986-1993)

* H in $Pa \cdot m^3 \cdot mol^{-1}$

** non-volatile compound, no value for available for H.

*** non-sorptive compound, no value available for K_{ow}.

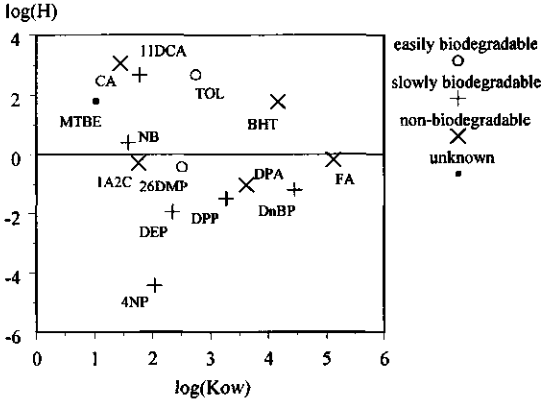

Fig. 1 Properties of test compounds for validation of activated sludge fate models (for explanation of acronyms see Table 1).

Several analytical problems made it impossible to collect monitoring data for each of the 17 selected compounds. The most important of these problems was the sometimes extremely low analytical recovery as determined in abiotic control experiments. As a result, for these compounds the contribution of biodegradation to overall compound removal could not be calculated. Eventually it was decided to restrict the experiments to three compounds for which consistent and accurate analytical results could be obtained: the C_{12}-homologue of linear alkylbenzene sulfonate (LAS-C_{12}), nitrilotriacetic acid (NTA) and toluene. Apart from biodegradation, LAS-C_{12} is subject to sorption and toluene to air stripping. The behaviour of NTA is affected by neither of these abiotic processes. This means that together these test compounds represented all three possible elimination routes in an activated sludge plant, i.e., excess sludge, off-gas and final effluent.

Model activated sludge plant

In analogy with most activated sludge fate models, the pilot-plant in which the validation experiments were carried out had a very simple layout. It was fed with pre-settled municipal wastewater and consisted of a completely mixed bioreactor with a diffused aeration system and a secondary settler. In reality wastewater treatment plants obviously can be much more complex. Physical pre-treatment steps such as primary settling and grit removal, but also sludge treatment facilities can contribute significantly to the fate of trace pollutants, in particular if these are amenable to sorption. Some fate models include equations to describe the occurrence of sorption in primary settlers (Struijs *et al.*, 1991; Melcer *et al.*, 1993 and Siegrist, 1996) and in sludge treatment units (Siegrist, 1996). However, none of these equations have been validated. In addition, sludge treatment often consists of (anaerobic) digestion, which for some compounds can constitute a destructive process.

Before domestic wastewater enters a municipal activated sludge plant, the sewer system already may have had an effect on the trace pollutants present in this wastewater. For example, 10 to 60% of the total load of LAS that is discharged by households already disappears in the sewer system, probably due to biodegradation (e.g., Moreno *et al.* 1998). The actual removal efficiency of the sewer system depends on a complex interaction between the type of sewer, the (hydraulic) retention time in the sewer, the composition of the wastewater and the redox conditions (Boeije, 1999). Unfortunately, hardly any information is available about the behaviour of trace compounds in sewer systems. A "worst-case" approach with zero removal therefore seems logic, unless monitoring data can provide more realistic removal capacities.

Apart from activated sludge systems, municipal wastewater sometimes is treated in biofilm systems, trickling filters for example. Although some models have been developed to describe the behaviour of trace compounds in trickling filters (e.g., Melcer *et al.*, 1995), the available knowledge still is limited. More knowledge certainly is required because biofilm systems are becoming increasingly popular.

Most of the available activated sludge fate models employ completely mixed bioreactors whereas in reality plug-flow or intermediate hydraulic regimes are more common. Although mathematical representations are available to describe these hydraulic regimes, little is known about the impact they have on the composition of the microbial population, and herewith on the microbial processes and biodegradation kinetics.

For practical reasons no surface aerators were used in the validation experiments whereas in practice these are applied on a large scale. It is expected however that mathematical models for surface aeration are similarly robust and reliable as models that are used for diffused aeration (e.g., Roberts *et al.*, 1984 and Hsieh *et al.*, 1993a) and therefore do not need further validation.

A very important restriction of existing activated sludge fate models is that they only consider aerobic conditions while modern plants often include anaerobic and anoxic compartments to accommodate nitrogen and phosphorus removal. Depending on the nature of a compound, this may have a positive, negative or no effect on its biodegradation. Although anaerobic and anoxic compartments as such can easily be implemented in a model (Boeije *et al.*, 1998), determination of the proper biodegradation rates under these conditions may be extremely complicated, in particular because exposure to alternating redox (and substrate) conditions will further increase the diversity and complexity of the microbial population.

Sorption

In particular hydrophobic, non-biodegradable pollutants can partition to the sludge and accumulate at concentrations exceeding those in the wastewater. Typical examples of such pollutants are pesticides, phthalates and polycyclic aromatic hydrocarbons. The literature review of chapter 2 showed that in most activated sludge fate models sludge-water partitioning is assumed to be an instantaneous equilibration process and the equilibrium is described by a linear sludge-water partition coefficient K_p. These assumptions are supported by many experimental results reported in the literature and further validation experiments are thought superfluous. The K_p usually is estimated from an empirical relationship with the compound's octanol-water partition coefficient K_{ow} and literature data show that this is a valid strategy as long as sorption is dominated by hydrophobic interactions. However, for certain compounds also other (chemical or electrostatic) sorptive forces are involved. In these cases the K_p cannot be estimated but will have to be specifically assessed in sorption experiments. A decision as to whether the K_p can be predicted or has to be assessed must be taken based on the molecular structure of a compound.

For some compounds a strong interaction between sorption and biodegradation may exist. For example, the validation experiments of chapter 4 showed that in spite of a high degree of sorption (92-98%), more than 99% LAS-C_{12} removal could be achieved in a pilot-scale municipal activated sludge plant. This shows that not only the dissolved, but also the sorbed fraction of a compound may be readily available for biodegradation. Still, this is ignored in many activated sludge fate models, which can result in a dramatic overestimation of the environmental exposure to LAS-C_{12} and other sorptive, biodegradable compounds. The (bio)availability of sorbed compound means that sorption and desorption are rapid and fully reversible processes or that the sorbed fraction is directly accessible to the micro-organisms. For some compounds this may not be the case. For instance, Cowan *et al.* (1993) suspected that part of the sorbed fraction of the cationic surfactants C_{12}TMAC and DTDMAC was not available for biodegradation. Also the results for LAS-C_{12} (chapter 4) gave a slight indication for the same phenomenon, although the effect on the biodegradation efficiency was minimal.

Volatilisation

Volatile organic compounds are frequently detected in the off-gas of industrial and municipal wastewater treatment plants. They may constitute an environmental hazard once they are transferred to the atmosphere, which in aerated bioreactors is caused by air stripping. The literature provides ample evidence that the air stripping equations employed by activated sludge fate model are reliable. However, the validation experiments with toluene (chapter 6) led to the suspicion that more attention is required towards an accurate assessment of the mass-transfer coefficient of trace compounds $K_{ol}a$, which is an essential parameter in air stripping equations. Usually this $K_{ol}a$ is estimated from a fixed relationship with the $K_{ol}a$ for oxygen. However, according to Hsieh *et al.* (1993b) this relationship depends on the air flow rate, which can vary significantly among activated sludge plants. It seems that for an accurate assessment of air emission rates this dependency should be examined in more detail.

Biodegradation

The mass transfer processes sorption and volatilisation merely distribute organic trace pollutants among the environmental compartments water, sludge and air whereas biodegradation can effectively reduce the rate at which they are released into the environment. The biodegradation potential of municipal activated sludge plants is large, both quantitatively and qualitatively. Hence, biodegradation is a very important process which should be accurately described by activated sludge fate models. However, at the same time biodegradation is a very complicated process involving many factors and sub-processes.

Selection of the biodegradation kinetics - Much information is available about biodegradation of single compounds by pure cultures. However, knowledge about degradation of mixtures of organic compounds by mixed cultures such as (municipal) activated sludge still is limited. This also explains why many equations exist to describe the biodegradation kinetics of organic trace pollutants by activated sludge. An overview of these kinetics was presented in chapter 2. Most activated sludge fate models employ (growth) Monod kinetics or (non-growth) secondary utilisation kinetics (Figure 2). A proper choice between these two kinetic regimes is essential for the reliability of the model predictions. According to Nyholm and Ingerslev (1996) secondary utilisation will dominate at influent (wastewater) concentrations lower than 100 $\mu g \cdot l^{-1}$ because this is the minimum concentration to provide sufficient energy and carbon sources for growth of specific degraders.

However, the results with LAS-C_{12} (chapter 4) and NTA (chapter 6) have shown that a distinction between these two kinetic regimes probably cannot be made from the influent concentration alone. Despite the fact that LAS-C_{12} supposedly was supplied at high enough influent concentrations to obtain growth (≥ 2 mg·l^{-1}), the Monod model could not explain the behaviour of LAS-C_{12} in the pilot-scale activated sludge plant and a secondary utilisation model seemed more appropriate (chapter 4). Apparently a large group of micro-organisms, pre-dominantly growing on substrates other than LAS-C_{12}, accomplishes primary biodegradation of LAS-C_{12}. In this manner the contribution of LAS-C_{12} itself to the total number of LAS-C_{12} degraders becomes insignificantly small. This also could mean that the first step in the biodegradation pathway of LAS-C_{12}, i.e., degradation of the (linear) alkyl chain, is a wide-spread feature among the micro-organisms in municipal activated sludge systems.

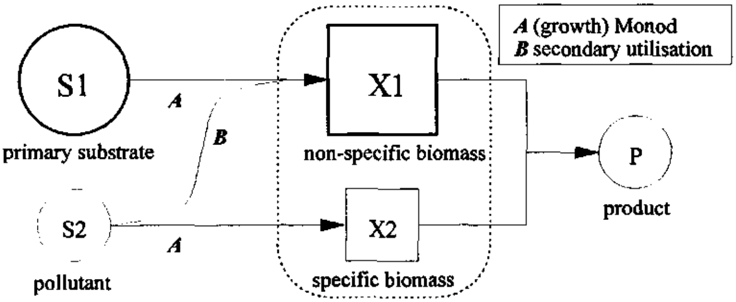

Fig. 2 *Simplified representation of the (bio)degradation of organic trace compounds by activated sludge.*

In contrast to LAS-C_{12} specific growth seems to be the dominant biodegradation mechanism for NTA, at least at higher influent concentrations (chapter 5). However, results presented by Bally *et al.* (1994) indicate that at low influent concentrations induction of the proper enzymes by the indigenous NTA degraders may be more important than enrichment of these degraders. In addition, Bally and Egli (1996) demonstrated that the presence of other easily biodegradable substrates can play an important role in the induction process.

The examples with LAS-C_{12} and NTA illustrate that a proper choice between secondary utilisation kinetics and Monod kinetics is not easy. It seems that this choice can only be made by careful experimentation in continuous systems, operated at various influent concentrations of the target pollutant. Microbial tools to count the number of micro-organisms involved in the degradation process and/or techniques to determine the specific enzyme concentrations would be extremely helpful. The examples with NTA and LAS-C_{12} also showed that more fundamental knowledge is required about microbial growth in the presence of multiple (easily biodegradable) substrates.

Variability of biodegradation parameters - Activated sludge fate models treat biodegradation parameters as compound inherent properties, i.e., they do not vary with the wastewater composition or with the operating conditions of a plant. This is in conflict with results reported by Sokol (1987) and others, who showed that these parameters are not unique for a given organism and substrate, but depend strongly on the growth history of the biomass. Also, the literature demonstrates a large variation in biodegradation parameters, even for pure cultures growing on single substrates. For example, E. *coli* growing on glucose can exhibit a maximum specific growth rate μ_{max} between 0.5 and 1.3 h^{-1} and a half-velocity constant K_C in a range of 20 $\mu g \cdot l^{-1}$ to 10000 $\mu g \cdot l^{-1}$ (Kovárová-Kovar and Egli, 1998). Similar, and probably even larger variations in biodegradation parameters can be expected for mixed cultures such as activated sludge. An example is the concentration $C_{1/0}$ at which the kinetics of LAS-C_{12} degradation changed from a first- to a zero-order regime (chapter 4). This parameter can be considered a measure of the affinity of the sludge to scavenge LAS-C_{12}. Depending on the influent concentration of LAS-C_{12}, $C_{1/0}$ varied between 600 $\mu g \cdot l^{-1}$ and 4.4 $mg \cdot l^{-1}$, with lower values at higher influent concentrations. If this effect of the influent concentration on $C_{1/0}$ is neglected, large errors can be expected in the model predictions. This is also illustrated in Figure 3. For the same activated sludge plant, fed with the same amount of LAS-C_{12} and operated under the same conditions, extremely different effluent and waste sludge concentrations of LAS-C_{12} were calculated when different values for $C_{1/0}$ were used in the calculations.

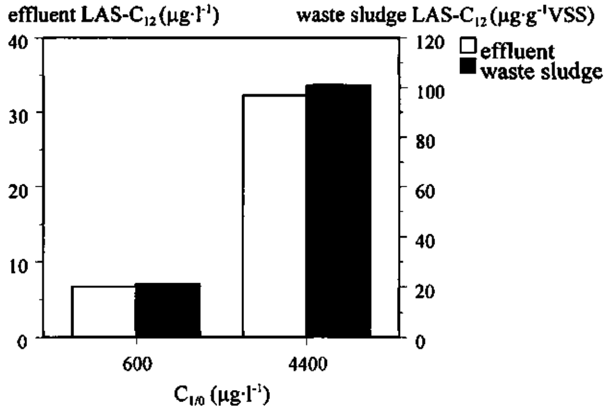

Fig. 3 Effect of the concentration where the kinetics change from first- to zero-order ($C_{1/0}$) on predicted effluent and waste sludge concentrations of LAS-C_{12}.

For NTA a similar phenomenon with lower values for $C_{1/0}$ at higher influent concentrations of NTA was observed (chapter 5). It was discussed that this effect may be explained by a combination of the metal chelating properties of NTA and differences in the stability and biodegradability of the different metal complexes. Although in principle these effects can be modelled, it would require a large number of additional equations to describe the necessary chemical equilibria.

Apart from the effect of the influent concentration on the affinity of activated sludge to scavenge the trace compounds LAS-C_{12} and NTA, information from the literature indicates that also the effect of other factors on the biodegradation parameters could be important, including the availability of alternative (easily biodegradable) substrates and the operating conditions, particularly the SRT. Although some *qualitative* (empirical) information about these effects is available (Kovárová-Kovar and Egli, 1998), the underlying microbial mechanisms are largely unknown, which makes (mechanistic) modelling an extremely difficult task.

Biodegradation tests - For modelling purposes the biodegradation parameters should reflect the kinetics as they prevail in the activated sludge plant of interest. Consequently, tests to assess these kinetics should mimic as closely as possible the conditions in this plant. As they all may have an effect on the kinetics, during the test care must be exercised not to change the microbial population or the physiological state of the existing population, and to maintain as much as possible the floc structure and the environmental conditions (pH, oxygen concentration, etc.). Unfortunately in most standardised biodegradation tests, for instance the tests prescribed by the Organisation for Economic Co-operation and Development (OECD, 1993), these requirements are not met. Therefore, a new biodegradation test was developed (chapter 3) which was referred to as a "by-pass" test. The test methodology was tested with the three test pollutants: NTA, LAS-C_{12} and toluene. It was found that more experience with the test certainly is required, in particular with respect to selection of the optimum test settings which are compound related. Still, the test can provide useful information, in particular in a research environment, for instance to investigate the effect of plant operational parameters and influent trace compound concentrations on biodegradation kinetics.

Biodegradation response in case of variable influent concentrations - In practice municipal activated sludge plants are subject to variable influent concentrations of trace pollutants and a true steady-state distribution for these compounds will never be achieved. This raises the question whether steady-state models are suitable tools for exposure assessment or that dynamic models should be used instead. Because the biodegradation response of activated sludge to varying influent concentrations is the key phenomenon in this regard, this response was investigated for a step increase of the influent concentration of the three test compounds LAS-C_{12}, NTA and toluene (chapter 7). After a certain adaptation period, for all three compounds this increase in influent concentration was compensated for by an increased biodegradation capacity of the sludge to give effluent concentrations similar to those observed before the step increase. The transient responses however were different, indicating that different adaptation mechanisms were involved. For LAS-C_{12} and toluene the biodegradation response was much faster (on the order of hours) than what could be expected from growth of specific degraders (on the order of days). In contrast, the biodegradation response for NTA was relatively slow, probably due to enrichment of specific degraders. These results suggest that, depending on their release pattern, for some compounds stationary models would be sufficiently accurate whereas for others dynamic models are required. The results also indicate that development of accurate dynamic models requires more fundamental knowledge about the underlying microbial adaptation processes.

Concluding remarks

Based on the experimental results, it can be anticipated that a "generic" assessment of the fate of organic trace pollutants in municipal activated sludge plants should not rely on model calculations alone, particularly not if biodegradation is expected to be an important removal mechanism. An important question in this regard obviously is how accurate the model output should be. A model can be used for a first screening of the distribution of trace pollutants and for existing priority pollutants this type of information can be used to design field monitoring sessions resulting in a more accurate assessment of this distribution.

A decision to admit new (suspected priority) compounds should rely on more accurate information. Based on a few compound properties (Henry's law constant, a sludge-water partition coefficient and the results of one or more biodegradability tests) an indication of the distribution in activated sludge plants can be obtained. However, this certainly needs to be supported by monitoring data which have to be collected in laboratory or pilot-scale activated sludge plants. It is very important to operate these plants with municipal wastewater rather than synthetic wastewater because the complex mixture of carbon sources in municipal wastewater may very well increase the opportunity for biodegradation and provides a more realistic representation of full-scale plants. Also, these "simulation" plants should be fed with various influent concentrations of the target pollutant and be operated at a wide range of SRTs as both parameters may have a significant effect on the removal efficiency.

The results of this study have demonstrated that biodegradation of trace compounds by activated sludge is a complex process involving many factors which are poorly understood. Microbiological and technological knowledge should be combined to gain more insight in the biodegradation of mixtures of organic compounds by mixed cultures such as activated sludge. The focus should be on the following aspects: (i) the kinetic regime that has to be selected and which is growth or non-growth related, (ii) the adaptation mechanisms that are involved (enrichment, enzyme induction, etc.), (iii) the mechanisms that cause variations in the biodegradation parameters, etc. This type of fundamental knowledge not only is required to improve existing activated sludge fate models, but may also be used to design treatment systems to accomplish an enhanced elimination of priority trace compounds.

To really make progress in the field of activated sludge fate modelling the problems associated with direct chemical analysis somehow should be circumvented. One possibility could be to use radiolabeled compounds. Also, microbial tools to count the number of micro-organisms and/or techniques to determine specific enzymes would be very useful for this purpose.

Finally, the selected test compounds in this thesis (LAS-C_{12}, NTA and toluene) each represented one of the possible removal pathways in activated sludge plants (effluent, sorption followed by discharge with the excess sludge and air stripping followed by removal with the off-gas), but at the same time they also were readily biodegraded by the sludge. In fact, biodegradation was the dominant process for all three test compounds and extremely low effluent and off-gas concentrations could be achieved. It would be very interesting to carry out similar validation experiments with less biodegradable trace compounds to be able to investigate in more detail the competition between biodegradation and the mass-transfer processes of sorption and air stripping.

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Summary

Introduction

The production, use and disposal of many compounds inevitably leads to their presence in the environment as organic trace pollutants. Although their concentrations may be low, these trace compounds (and synonyms thereof such as xenobiotic compounds, organic micropollutants, priority pollutants, etc.) can present an environmental hazard associated with their toxicity for human beings, their potential to accumulate in biota and ecosystems and in some cases their function as a catalyst in the destruction of the ozone in the stratosphere.

In many cases trace compounds enter the sewage system and will finally appear in municipal activated sludge plants. In contrast to industrial wastewater treatment plants, designed to remove specific pollutants, municipal plants have to eliminate the bulk of organic compounds, expressed by the sum parameter biochemical oxygen demand (BOD) or chemical oxygen demand (COD). Several processes determine the distribution of trace compounds in these plants. Hydrophobic compounds can partition to the sludge and in this manner may create a potential hazard associated with sludge disposal. Volatile organic compounds are amenable to air stripping and surface desorption and therefore are frequently found in the off-gas of activated sludge plants. Whereas sorption and volatilisation merely rearrange the distribution of compounds among the different environmental compartments (sludge, air and water), only a destructive process such as biodegradation can actually remove them from the environment. Municipal activated sludge plants are known to have a large biodegradation potential, both qualitatively and quantitatively. At the same time however, biodegradation is a complicated process involving numerous factors such as the molecular structure of trace compounds, biomass composition, environmental and operating conditions, the presence of other organic substances, etc. Besides, biodegradation has to compete with the aforementioned mass-transfer processes of sorption and volatilisation.

During the last two decades several activated sludge fate models have been developed. These models are used (i) to predict the environmental exposure to specific organic trace compounds expected to appear in municipal wastewater's, (ii) to optimise the design and control of treatment plants with respect to the removal of these pollutants and (iii) to establish limits on treatment plant influent loads based upon allowable effluent loads. The reliability of these models only has been demonstrated to a limited extent and therefore their applicability remains uncertain. A validation study may help to gain some confidence in these models, but also can reveal some of their shortcomings. Although it seems logic to focus such a validation study on one particular model, this would ignore that other models employ completely different mathematical equations. In particular for biodegradation numerous mathematical approaches are used.

The aim of this thesis was to analyse the shortcomings of existing activated sludge fate models and to develop scientific knowledge which may help further improvement of these models and their implementation in practice. For this purpose a literature review was carried out, a new test method was developed to study the biodegradation kinetics of organic trace compounds by activated sludge, and validation experiments were carried out to assess the behaviour of a number of selected trace compounds in a pilot-scale municipal activated sludge plant.

Literature review

A literature review was carried out (chapter 2) to provide information about (i) the relevant processes that determine the behaviour of organic trace compounds in activated sludge plants, (ii) mathematical equations that predict the rates at which these processes proceed, (iii) methods to assess the model parameters, (iv) activated sludge fate models proposed in the literature and (v) studies that have been undertaken to validate these models.

There appears to be a consensus with respect to the processes that have to be included in activated sludge fate models, being the mass-transfer processes of sorption and volatilisation (air stripping and surface volatilisation) and biodegradation. For some compounds other conversion processes such as photolysis and chemical oxidation/reduction also may take place. However, these abiotic processes generally are considered of minor importance as compared to biochemical oxidation by micro-organisms.

Sorption of trace compounds to activated sludge is modelled as an instantaneous equilibration process with linear sludge-water partitioning. If it is ascertained that sorption is only determined by hydrophobic interactions, the sludge-water partition coefficient can be estimated from an empirical relationship with the compound's octanol-water partition coefficient. Otherwise, the sludge-water partition coefficient should be assessed in separate sorption experiments.

Volatile trace compounds are amenable to air stripping and surface volatilisation. In the aerated bioreactors of activated sludge plants air stripping usually is the dominant process. Surface volatilisation only becomes important for compounds of low volatility and at extremely low air flow rates. In completely mixed bioreactors with diffused aeration systems air stripping usually is described by a first-order model with respect to the (dissolved) concentration of the target trace compound. The first-order stripping rate constant can be calculated from Henry's law constant H, which is a compound property, and from the overall mass-transfer coefficient $K_{ol}a$ which depends on the characteristics of the aeration equipment. The $K_{ol}a$ can be estimated from the mass-transfer coefficient for oxygen $K_{ol}a_{O2}$, which is often a known parameter for aeration systems.

The mathematical equations for sorption and volatilisation already have been verified under a wide range of conditions and seem to generate reliable predictions. The opposite applies to biodegradation, which is a very complicated process. This complexity is also reflected by the large number of mathematical equations that is used to predict the rate of biodegradation, including Monod kinetics, secondary utilisation kinetics and pragmatic first- and zero-kinetics. Which kinetic regime should be used is unclear and currently seems to be dictated by data availability rather than by knowledge about the underlying microbial mechanisms. Another complicating factor is the difficulty to obtain reliable biodegradation parameters. It is generally accepted that the most reliable parameters are obtained in those tests which closely mimic activated sludge plant conditions. However, such tests are extremely laborious and expensive and in practice simple (standardised) laboratory tests are used instead. Unfortunately, these test are not designed to yield kinetic information and extrapolation of their results to activated sludge plant conditions is extremely difficult, if not impossible. A third complicating factor is that biodegradation parameters can vary with the plant operational characteristics and the exposure history of the sludge. Consequently, these factors should be included in the biodegradation kinetics and in laboratory tests to assess these kinetics.

The last 10-15 years several activated sludge fate models have been developed, ranging from models that describe site-specific situations to generic models that can be used for an environmental exposure assessment. Many similarities can be found between these models, in particular regarding the equations they employ for sorption and air stripping. However, they also show large differences. A large variety in biodegradation kinetics certainly has to be mentioned because these kinetics can have an enormous impact on the model output that will be generated. Most of the models focus on the aerated bioreactor of activated sludge plants whereas other unit operations (pre-settlers, grit chambers, sludge digesters, etc.) also may have an impact on the distribution of organic trace compounds.

Validation of activated sludge fate models requires accurate monitoring data. Although the results of several monitoring studies have been reported, most of these were carried out in full-scale plants and (i) essential information about the plant characteristics and operational conditions is missing or (ii) information about the behaviour of the target trace compounds is incomplete or lacks the required level of detail. Besides, insufficient variation was applied to those parameters that activated sludge fate models are most sensitive to. In particular the sludge retention time (SRT) and (average) influent concentration of the trace compounds have to be mentioned in this regard.

A new biodegradation test method

One of the conclusions of the literature review of chapter 2 was that there is a lack of reliable tests which the biodegradation kinetics of organic trace compounds by activated sludge can be assessed with. As was mentioned earlier, it is evident that these tests most likely will provide useful kinetic data if the test conditions closely mimic the conditions in the activated sludge plant of interest. However, this usually is not the case and many differences in these conditions can be pointed out, including the operational mode (batch versus continuous), substrate to biomass ratio, presence of carbon sources other than the trace compound of interest, etc. It therefore was decided to develop a new kinetic test which was referred to as a "by-pass" test (chapter 3). The test methodology was tested with three trace compounds: nitrilotriacetic acid (NTA), the C_{12}-homologue of linear alkylbenzene sulfonate (LAS-C_{12}) and toluene. The results showed that more experience is required with this test, in particular with respect to selection of the optimum test settings which are compound related. The proposed test method is not suitable as a standardised biodegradation test but certainly could be applied in a research environment, for instance to investigate the effect of plant operation parameters on the biodegradation kinetics.

Set-up of the validation experiments

Apart from an activated sludge system (aerated bioreactor and a settler to retain the biomass in the system), most municipal wastewater treatment plants include several pre-treatment steps (primary settler, grit removal, screens, etc.) and facilities to treat excess sludge (thickeners, digesters, etc.).

Although it is recognised that all these process units may have an effect on the distribution of trace pollutants, the (aerated) bioreactor generally is considered the most important treatment unit in this regard. For that reason it was decided to focus the experiments entirely on the bioreactor.

Although in a way it is a model itself, it was also decided to use a pilot-scale activated sludge plant (aeration tank of 0.5 m^3) rather than a full-scale plant because (i) the conditions in a pilot-scale plant can be more conveniently manipulated, (ii) test compounds can be discharged without having to worry about detrimental effects on plant performance with respect to conventional wastewater parameters like COD, N and P and (iii) a pilot-scale plant can be covered which facilitates off-gas sampling. As they all may have an important effect on the actual behaviour of trace compounds, the *operational parameters* of the pilot-plant, i.e., the *sludge retention time* (SRT), *hydraulic retention* time (HRT), COD loading rate and aeration intensity, and the influent concentration of the trace compounds were varied in the experiments.

Because activated sludge fate models are extremely sensitive to the physical-chemical and biodegradation properties of trace compounds, it is necessary to investigate a large number of these compounds. Initially, a validation matrix was constructed with 17 test compounds of varying volatility, hydrophobicity and biodegradability. Unfortunately, a lack of reliable and accurate analytical techniques forced us to reduce this number to three compounds only: the C_{12}-homologue of linear alkylbenzene sulfonate (LAS-C_{12}) which is a highly sorptive compound, toluene which is a volatile compound and nitrilotriacetic acid (NTA) to represent the class of non-sorptive and non-volatile compounds.

Monitoring sessions to assess the behaviour of LAS-C_{12}, NTA and toluene in the pilot-plant are described in chapters 4, 5 and 6, respectively. In addition, the biodegradation kinetics of these trace compounds were determined. The results were used to check the model equations employed by existing activated sludge fate models, to obtain an impression of the reliability of these models and to identify model imperfections that need improvement and further research.

During the monitoring sessions the test compounds were discharged to the pilot-plant at a constant rate which means that their fate in this plant was determined under "steady-state" conditions. In reality this discharge rate will be time-varying and this may have a strong impact on the average removal efficiency that can be achieved. For that reason the dynamic behaviour of the test compounds was investigated in a number of step discharge experiments. The results of this part of the investigations are described in chapter 7.

Linear alkylbenzene sulfonate (LAS)

As part of this model validation study monitoring data were collected in a pilot-scale municipal activated sludge plant to establish the fate of the C_{12}-homologue of LAS (LAS-C_{12}) (chapter 4.1) The pilot-plant was operated at influent LAS-C_{12} concentrations between (background) concentrations in the pre-settled domestic wastewater of about 2000 $\mu g \cdot l^{-1}$ and an enhanced concentrations of 15000 $\mu g \cdot l^{-1}$. The plant was operated at SRTs of 10 and 27.3 days, HRTs of 7.3 and 12.4 hours and COD loading rates of 0.19 and 0.46 kg COD$\cdot kg^{-1}$ volatile suspended solids (VSS)$\cdot d^{-1}$. Irrespective of the height of the influent concentration of LAS-C_{12} and the operational characteristics of the plant, less than 0.5% of the influent load of LAS-C_{12} was discharged with the effluent and less than 0.4% was wasted with the excess sludge. Effluent and waste sludge concentrations were 5 to 10 $\mu g \cdot l^{-1}$ and 37 to 69 $\mu g \cdot g^{-1}$ VSS, respectively. In sludge samples, taken from the aeration tank of the plant, only a small fraction (2-8%), was present as dissolved LAS-C_{12} and the remaining 92-98% was found to be sorbed to the sludge. In spite of this high degree of sorption, more than 99% of LAS-C_{12} could be removed by biodegradation, showing that not only the soluble fraction but also the sorbed fraction of LAS-C_{12} is readily available for biodegradation.

Sorption and biodegradation of LAS-C_{12} by municipal activated sludge were also separately investigated (chapter 4.2). Sorption equilibration was within 5 to 10 minutes, showing that sorption of LAS-C_{12} by activated sludge is an extremely fast process. The sorption equilibrium could be described by a linear isotherm with a sludge-water partition coefficient of 3.2 $l \cdot g^{-1}$ VSS. The results of the biodegradation kinetic tests showed that primary biodegradation (i.e., removal of the parent compound) of LAS-C_{12} cannot be described by a Monod model, but a secondary utilisation model should be used instead. Combined first- and zero-order kinetics, related to the total concentration of LAS-C_{12} (the sum of dissolved and sorbed LAS-C_{12}), gave the best model fit. Remarkably, the concentration where the kinetics change from first- to zero-order were found to decrease when the sludge was exposed to higher loading rates of LAS-C_{12}.

From the results of the biodegradation experiments it was concluded that existing activated sludge fate models cannot accurately predict the behaviour of LAS-C_{12} in municipal activated sludge plants. Therefore, using the results of the sorption and biodegradation tests a new simple mathematical model was developed and this model was validated against the LAS-C_{12} monitoring data (chapter 4.3). The model could accurately predict the fate of LAS-C_{12} in the plant. Only the effluent concentrations were slightly overestimated, probably because too low a sludge-water distribution coefficient was used in the model calculations. A sensitivity analysis demonstrated that the distribution of LAS-C_{12} in municipal activated sludge plants mainly is determined by sludge-water partitioning. From this, it can be anticipated that LAS removal efficiency is favourable in plants operated at high sludge concentrations and with excellent sludge-water separation facilities.

Nitrilotriacetic acid (NTA)

NTA was a second test compound in this study (chapter 5). The pilot-plant was operated according to the conditions already mentioned earlier for LAS-C_{12} and received various influent concentrations of NTA between a background concentration in the wastewater of about 300 $\mu g \cdot l^{-1}$ and enhanced concentrations as high as 35 $mg \cdot l^{-1}$. More than 99% NTA removal could be achieved, irrespective of the operational conditions. Effluent concentrations of NTA always were lower than the method detection limit of 30 $\mu g \cdot l^{-1}$. Sorption and subsequent discharge with the excess sludge was found to be an insignificant removal pathway for NTA and removal could be entirely attributed to biodegradation.

Biodegradation tests, in combination with information from the literature, showed that (no growth) secondary utilisation kinetics and (growth) Monod kinetics that usually are employed by activated sludge fate models cannot be used to accurately predict the biodegradation rate of NTA by activated sludge because (i) at low influent concentrations of NTA the Monod model seems to be invalid and may have to be replaced by a different, more complex model and (ii) the potential of the sludge to scavenge NTA is dependent on the influent concentration of NTA. Only little is known about the underlying mechanisms that cause these deviations from existing biodegradation models.

Toluene

Toluene was the third test compound that monitoring data were collected for (chapter 6). Biodegradation of toluene in the pilot-plant was absent when the sludge was exposed to an average background concentration of toluene in the wastewater of 4 $\mu g \cdot l^{-1}$. When the influent concentration was raised above 100 $\mu g \cdot l^{-1}$ biodegradation was established and could eliminate more than 97% of the influent load. Toluene concentrations in the effluent and off-gas were low: less than 2 $\mu g \cdot l^{-1}$ and 0.3 $\mu g \cdot l^{-1}$, respectively.

The general trend in the observations could be predicted with a Monod model, but not with secondary utilisation kinetics. More reliable model predictions are feasible, but this requires more accurate values for the Monod parameters and for the ratio between the liquid-gas mass-transfer coefficients of toluene and oxygen. It remains unclear whether the Monod model also is valid at influent concentrations lower than 100 $\mu g \cdot l^{-1}$ or should be replaced by a different model.

Dynamic fate modelling

Most fate models consider steady-state situations, that is, they assume that a trace compounds is supplied with the wastewater at a constant loading rate. However, in reality activated sludge plants have to deal with time-variable loading rates and because each time the sludge may need time to adapt to the new concentration this may have a significant impact on the (average) removal efficiency of a trace compound. The dynamic behaviour of LAS-C_{12}, NTA and toluene was investigated in the pilot-plant by following the response in concentrations to a step increase of their influent concentration (chapter 7). For all three compounds the sludge could adapt to this higher influent concentration by increasing their rate of biodegradation, resulting in concentrations which were also observed before the step increase. However, major differences were observed in the transient response of the test compounds, indicating that completely different adaptation mechanisms may have been involved. Because of this compound related adaptation response and because only little is known about the underlying adaptation mechanisms, it was concluded that existing activated sludge fate models cannot be used to accurately predict the effect of highly variable influent concentrations.

Conclusions

In chapter 8 the results of the literature review and the validation experiments are discussed. It was concluded that activated sludge fate models can be a useful element of a first screening of the distribution of organic trace compounds in activated sludge plants but that a generic assessment of this distribution should not rely on model calculations alone. For existing priority compounds the model output could be used to design field monitoring sessions in order to obtain a more accurate assessment of this distribution. For new (suspected) priority compounds a first screening also may yield useful information but this should be supported by monitoring data from laboratory of pilot-scale activated sludge plants. These plants have to be operated with municipal wastewater, at various influent concentrations of the target trace compound and at a wide range of SRTs. Finally, the experiments have demonstrated that biodegradation of trace compounds by activated sludge is a very complex process, involving many aspects which are still poorly understood. Therefore, more accurate activated sludge fate models can only be developed if more research is directed towards biodegradation of mixtures of organic substances by mixed microbial cultures such as activated sludge.

Samenvatting

Inleiding

De productie en het gebruik van organische stoffen resulteert vaak in hun (ongewenste) aanwezigheid in het milieu. Hoewel hun concentratie in het milieu meestal laag is, kunnen deze organische microverontreinigingen (en synoniemen hiervan zoals xenobiotica, prioriteitstoffen, chemicaliën, etc.) een gevaar opleveren die samenhangt met hun humane toxiciteit, accumulatie in ecosystemen en katalyserende werking bij de destructie van de ozonlaag.
Vaak komen organische microverontreinigingen op de een of andere manier in het riool terecht en worden ze uiteindelijk op een rioolwaterzuiveringsinrichting (rwzi) geloosd. In tegenstelling tot industriële zuiveringsinstallaties zijn deze rwzi's gericht op het verwijderen van de bulk aan organische stoffen, uitgedrukt als biochemisch zuurstofverbruik (BZV) of chemisch zuurstofverbruik (CZV). Meestal wordt hiervoor het zogenaamde actiefslibproces toegepast. Het gedrag van organische microverontreinigingen in deze actiefslibinstallaties wordt door verschillende deelprocessen bepaald. Hydrofobe stoffen kunnen door ad- en absorptie in het slib terechtkomen en een gevaar vormen bij de verdere verwerking van dit slib. Vluchtige stoffen zijn onderhevig aan strippen en oppervlaktevervluchtiging en worden regelmatig in de lucht boven (open) rwzi's of in het afgas van (overkapte) rwzi's aangetroffen. Vervluchtiging en sorptie bepalen louter en alleen de verdeling van organische microverontreinigingen over de milieucompartimenten water, lucht en slib. Een derde proces, te weten biodegradatie, zorgt ervoor dat een stof daadwerkelijk uit het milieu verwijderd kan worden. Actiefslibinstallaties hebben een grote biodegradatiecapaciteit , zowel kwalitatief als kwantitatief. Tegelijkertijd is biodegradatie een gecompliceerd proces waarbij een groot aantal factoren betrokken is zoals de moleculaire structuur van de te verwijderen stoffen, de samenstelling van de microbiële populatie waaruit het actiefslib is opgebouwd, de operationele condities van de actiefslibinstallatie, de aanwezigheid van andere organische stoffen, enz. Bovendien is er sprake van een competitie voor organische microverontreinigingen tussen biodegradatie en de eerder genoemde overdrachtsprocessen vervluchtiging en sorptie.
De afgelopen twee decennia zijn diverse actiefslibmodellen ontwikkeld waarmee het gedrag van organische microverontreinigingen in actiefslibinstallaties kan worden voorspeld. Deze modellen worden gebruikt (i) als hulpmiddel om de blootstelling van het milieu aan deze ongewenste stoffen in te schatten, (ii) bij het ontwerp en de bedrijfsvoering van actiefslibinstallaties om de verwijdering van deze stoffen te optimaliseren en (iii) om, in geval van industriële lozingen, beperkingen aangaande de belasting van actiefslibinstallaties met deze stoffen op te leggen. Echter, de betrouwbaarheid van deze modellen is slechts in zeer beperkte mate onderzocht zodat hun toepasbaarheid onzeker blijft. De resultaten van een validatiestudie kan tegelijkertijd het vertrouwen in deze modellen verhogen en hun tekortkomingen duidelijk kunnen maken.
Het doel van de studie die in dit proefschrift wordt beschreven was om de tekortkomingen aan te tonen van bestaande modellen die het gedrag van organische microverontreinigingen in actiefslibinstallaties voorspellen. Een tweede doel was om wetenschappelijke kennis te verzamelen waarmee deze modellen verbeterd zouden kunnen worden om zo een praktijktoepassing dichterbij te brengen. Hiertoe werd een literatuurstudie uitgevoerd, een nieuwe testmethode ontwikkeld

waarmee de biodegradatiekinetiek van organische microverontreinigingen door actiefslib bestudeerd kan worden en werden een aantal validatie-experimenten uitgevoerd waarin het gedrag van een aantal teststoffen in een proefinstallatie werd gemeten.

Literatuuronderzoek

Hoofdstuk 2 beschrijft de resultaten van een literatuurstudie uitgevoerd met als doel informatie te achterhalen over (i) relevante processen die het gedrag van organische microverontreinigingen in actiefslibinstallaties bepalen, (ii) wiskundige vergelijkingen waarmee de snelheid van deze processen kan worden voorspeld, (iii) methoden om de modelparameters te bepalen, (iv) bestaande actiefslibmodellen en (v) pogingen die zijn ondernomen om deze modellen te valideren.

Er bestaat een zekere concensus over de processen die moeten worden meegenomen in een model, te weten de overdrachtsprocessen sorptie en vervluchtiging (strippen en oppervlaktevervluchtiging) en biodegradatie. Voor sommige stoffen kunnen naast biodegradatie ook andere conversieprocessen optreden zoals fotolyse en chemische oxidatie/reductie. Echter, over het algemeen wordt aangenomen dat deze abiotische processen minder belangrijk zijn dan biochemische oxidatie door de micro-organismen in actiefslib.

Sorptie van organische microverontreinigingen aan actiefslib wordt gemodelleerd als een instantaan proces met een lineaire evenwichtsverdeling tussen het water en het slib. Indien is vastgesteld dat sorptie louter en alleen door hydrofobe interacties wordt bepaald dan kan de slib-water partitiecoëfficiënt van een stof berekend worden uit een empirische relatie met de octanol-water partitiecoëfficiënt van die stof. In alle andere gevallen zal eerstgenoemde parameter op experimentele wijze moeten worden vastgesteld.

Vluchtige microverontreinigingen zijn vatbaar voor strippen en oppervlaktevervluchtiging. In de biorector (aëratietank) van actiefslibinstallaties is strippen het dominante proces. Alleen in het geval van stoffen met een relatief lage vluchtigheid en bij zeer lage luchtdebieten kan ook oppervlaktevervluchtiging een significante bijdrage leveren aan de emissie naar de lucht. In volledig gemengde bioreactoren die zijn voorzien van bellenbeluchting wordt strippen meestal beschreven met een eerste-orde vergelijking in relatie tot de opgeloste concentratie van de betreffende microverontreiniging. De eerste-orde stripconstante kan worden berekend uit de Henry constante van die stof (H), en uit de overdrachtscoëfficiënt ($K_{ol}a$) die afhankelijk is van de eigenschappen van de beluchtingsapparatuur en geometrie van de bioreactor. De $K_{ol}a$ voor organische microverontreinigingen is moeilijk te meten maar kan wel worden geschat uit de $K_{ol}a$ voor zuurstof die wel relatief eenvoudig gemeten kan worden.

De literatuur laat zien dat de wiskundige vergelijkingen voor sorptie en vervluchtiging reeds uitgebreid en onder zeer uiteenlopende omstandigheden zijn gevalideerd zodat nauwkeurige voorspellingen verwacht mogen worden. Het tegenovergestelde geldt voor biodegradatie dat een zeer gecompliceerd proces is. Dit uit zich onder andere in het grote aantal vergelijkingen dat beschikbaar is voor de kinetiek van het biodegradatieproces, waaronder de Monod vergelijking, vergelijkingen voor de omzetting van de microverontreiniging als een secondair substraat en diverse pragmatische eerste- en tweede-orde vergelijkingen. Welke vergelijking onder welke omstandigheden gebruikt moet worden is onduidelijk en deze keuze lijkt vooralsnog gedicteerd te worden door de beschikbaarheid van de meetgegevens en niet door een gedegen kennis van de onderliggende microbiële processen. Een bijkomend probleem is de moeilijkheid om betrouwbare biodegradatieparameters te kunnen bepalen. Over het algemeen wordt aangenomen dat de meest betrouwbare parameters worden bepaald indien tijdens de meting dezelfde omstandigheden heersen als in de betreffende actiefslibinstallatie. Testen waarin dit het geval is zijn niet beschikbaar en meestal worden in plaats daarvan eenvoudige (gestandaardiseerde) laboratoriumtesten gebruikt. Echter, deze testen zijn niet ontwikkeld om kinetische informatie te genereren en bovendien is extrapolatie van de resultaten naar de omstandigheden in een actiefslibinstallatie uiterst twijfelachtig. Een derde complicerende factor is dat biodegradatieparameters kunnen variëren met de operationele omstandigheden in een actiefslibinstallatie en de blootstellingsgeschiedenis van het actiefslib aan een microverontreiniging. Dit betekent niet alleen dat deze factoren in de biodegradatiekinetiek moeten worden verwerkt, maar ook moeten worden betrokken bij de testen waarmee deze kinetiek wordt vastgesteld.

De laatste 10-15 jaar zijn diverse modellen ontwikkeld waarmee het gedrag van organische microverontreinigingen in actiefslibinstallaties voorspeld kan worden. Het betreft zowel algemene modellen die gebruikt kunnen worden als hulpmiddel bij de voorspelling van de blootstelling van het milieu aan deze stoffen als modellen die meer specifieke situaties beschrijven. Tussen de verschillende modellen bestaan veel overeenkomsten, in het bijzonder wat betreft de vergelijkingen die worden gebruikt voor strippen en sorptie. Echter, de modellen vertonen ook grote verschillen. Een grote verscheidenheid in de biodegradatiekinetiek werd al genoemd en is zeer belangrijk omdat dit kan resulteren in extreem verschillende modelvoorspellingen. Ook de structuur van de actiefslibinstallaties is verschillend. De meeste modellen zijn gericht op de bioreactor maar soms worden ook andere processtappen (voorbezinker, zandvanger, slibvergister, etc.) beschouwd.

Validatie van bovenstaande modellen vereist een zeer uitgebreide set van nauwkeurige meetgegevens. Gegevens die in de literatuur worden gerapporteerd werden in de meeste gevallen verzameld in praktijkinstallaties. Echter, essentiële informatie over de configuratie van deze installaties en hun bedrijfsvoering ontbreekt in veel gevallen. Ook zijn meetgegevens van de microverontreinigingen vaak onvolledig en/of niet gedetailleerd genoeg. Daarnaast werd in de meeste studies onvoldoende variatie toegepast in die parameters waar de modellen juist het meest gevoelig voor zijn. Dit betreft vooral de slibleeftijd, de eigenschappen van de organische microverontreinigingen en hun (gemiddelde) influentconcentratie.

Een nieuwe testmethode voor biodegradatie

Een van de conclusies van het literatuuronderzoek van hoofdstuk 2 was dat er een gebrek is aan betrouwbare testen waarmee de biodegradatiekinetiek van organische microverontreinigingen door actiefslib kan worden bepaald. Zoals reeds eerder werd vermeld moeten in dergelijke testen de condities in een actiefslibinstallatie zo goed als mogelijk worden nagebootst. Echter, bij de meeste beschikbare (standaard)testen is dat niet het geval en bestaan er grote verschillen wat betreft de operationale omstandigheden (batch versus continue), verhouding tussen de concentraties substraat en biomassa, de aanwezigheid van extra organische stoffen naast de te testen microverontreiniging, etc. Om deze redenen werd besloten om een nieuwe test te ontwikkelen waarnaar gerefereerd zal worden als een "by-pass" test (hoofdstuk 3). De methode werd getest met drie stoffen: nitrilotriazijnzuur (NTA), de C_{12}-homoloog van lineair alkylbenzeen sulfonaat (LAS-C_{12}) en tolueen. De resultaten lieten zien dat meer ervaring met de testmethode moet worden opgedaan. Dit geldt vooral voor de selectie van de optimale testinstellingen die afhankelijk zijn van de te testen stof. De voorgestelde methode is dan ook niet geschikt als een standaardtest. Wel kan de test toegepast worden bij onderzoek, bijvoorbeeld onderzoek naar het effect van operationele parameters op de biodegradatiekinetiek van actiefslib.

Opzet van de validatie-experimenten

Naast een actiefslibsysteem (aëratietank en een nabezinker om de biomassa in het systeem vast te houden) bestaan de meeste huishoudelijke afvalwaterzuiveringsinstallaties uit een aantal extra voorbehandelingsstappen (rooster, voorbezinker, zandvangers, etc.) en diverse faciliteiten voor de behandeling van het surplusslib dat wordt geproduceerd (indikker, vergister, etc.). Hoewel al deze stappen een effect kunnen hebben op de distributie van organische microverontreinigingen, wordt de bioreactor over het algemeen als belangrijkste processtap beschouwd. Daarom werden de validatie-experimenten geheel op de bioreactor afgestemd.

Tevens werd besloten om de experimenten in een proefinstallatie (0.5 m^3) uit te voeren alhoewel dit in feite een model is van een praktijkinstallatie. De belangrijkste redenen hiervoor waren dat (i) de omstandigheden in een proefinstallatie eenvoudig kunnen worden gemanipuleerd, (ii) teststoffen in hoge concentraties op een proefinstallatie mogen worden geloosd en (iii) een proefinstallatie overkapt kan worden hetgeen bemonstering van het afgas mogelijk maakt. Omdat ze allen een effect op het gedrag van organische microverontreinigingen kunnen hebben werden tijdens de experimenten de slibleeftijd, de hydraulische verblijftijd, de CZV-belasting, de beluchtingsintensiteit en de influentconcentratie van de teststoffen gevarieerd.

Modellen die het gedrag van organische microverontreinigingen in actiefslibinstallaties voorspellen zijn vooral gevoelig voor de fysisch-chemische eigenschappen en biodegradeerbaarheid van deze stoffen. Daarom zou een groot aantal stoffen getest moeten worden en werd een validatiematrix van 17 verschillende stoffen geconstrueerd met uiteenlopende vluchtigheid, hydrofobiciteit en biodegradeerbaarheid. Echter, een gebrek aan betrouwbare en nauwkeurige analysetechnieken zorgde ervoor dat het aantal teststoffen teruggebracht moest worden tot drie: de C_{12}-homoloog van lineair alkylbenzeen sulfonaat (LAS-C_{12}) dat sterk aan slib kan adsorberen, tolueen dat een zeer vluchtige stof is en nitrilotriazijnzuur (NTA) dat noch vluchtig is noch aan slib adsorbeert.

De resultaten van verschillende meetsessies waarin de distributie van LAS-C_{12}, NTA en tolueen in een proefinstallatie werd bepaald worden achtereenvolgens beschreven in hoofdstukken 4, 5 en 6. De kinetiek waarmee deze stoffen door het actiefslib in de proefinstallatie werden omgezet werd gemeten met de eerder genoemde "by-pass" test (hoofdstuk 3). Op basis van de resultaten werden de vergelijkingen gecontroleerd waaruit bestaande modellen zijn opgebouwd.

Tijdens de meetsessies werden de teststoffen met een constante vracht op de proefinstallatie geloosd zodat hun uiteindelijke lot een evenwichtssituatie reflecteert. In werkelijkheid zal de belasting in de tijd variëren hetgeen een sterk effect kan hebben op het gemiddelde verwijderingsrendement. Daarom werd ook het dynamische gedrag van de teststoffen in de proefinstallatie onderzocht door in een aantal experimenten de belasting met deze stoffen opeens drastisch te verhogen (hoofdstuk 7).

Lineair alkylbenzeen sulfonaat (LAS)

De resultaten van het onderzoek met LAS-C_{12} worden in hoofdstuk 4.1 beschreven. De proefinstallatie werd belast met huishoudelijk afvalwater dat concentraties LAS-C_{12} bevatte tussen 2 en 15 $mg \cdot l^{-1}$. De installatie werd bedreven bij slibleeftijden van 10 en 27.3 dagen, hydraulische verblijftijden van 7.2 en 12.4 uur en CZV-belastingen van 0.19 en 0.46 kg CZV per kg organische stof per dag. Onafhankelijk van de influentconcentratie LAS-C_{12} en de operationele omstandigheden werd minder dan 0.5 % van de influentvracht LAS-C_{12} in het effluent aangetroffen en verliet minder dan 0.4% de installatie via het spuislib. De concentratie LAS-C_{12} in het effluent en spuislib waren respectievelijk 5-10 $\mu g \cdot l^{-1}$ en 37-69 $\mu g \cdot g^{-1}$ organische stof. Slechts 2-8% van de LAS-C_{12} in de slibmonsters uit de aëratietank was aanwezig in opgeloste vorm en de overige 92-98% in (aan het slib) geadsorbeerde vorm. Ondanks dit hoge percentage geadsorbeerde LAS-C_{12} kon in totaal meer dan 99% LAS-C_{12} door middel van biodegradatie worden verwijderd. Dit toont duidelijk aan dat niet alleen de opgeloste fractie LAS-C_{12} beschikbaar is voor biodegradatie maar ook de geadsorbeerde fractie.

Sorptie en biodegradatie van LAS-C_{12} door actiefslib werden ook apart onderzocht (hoofdstuk 4.2). Tijdens de sorptie-experimenten werd binnen 10 minuten een evenwicht bereikt waaruit blijkt dat sorptie een zeer snel proces is. Het sorptie-evenwicht kon met een lineaire isotherm worden beschreven met een slib-water partitiecoëfficiënt van 3.2 $l \cdot g^{-1}$ organische stof. De resultaten van de biodegradatietesten lieten zien dat primaire biodegradatie (dat wil zeggen de verwijdering van de uitgangsstof) van LAS-C_{12} niet met een Monod model kan worden beschreven en in plaats daarvan een secondair verbruiksmodel zou moeten worden toegepast. De resultaten konden het beste worden beschreven met een gecombineerd eerste- en nulde-orde model dat gebaseerd is op de totale (som van opgeloste en geadsorbeerde LAS-C_{12}) concentratie LAS-C_{12}. Opvallend was dat de concentratie waar de kinetiek omschakelde van eerste- naar nulde-orde kleiner werd naarmate het slib was blootgesteld aan hogere LAS-C_{12} vrachten.

Op basis van de resultaten van de biodegradatietesten werd geconcludeerd dat bestaande modellen de uiteindelijke distributie van LAS-C_{12} in actiefslibinstallatie niet nauwkeurig kunnen voorspellen. Daarom werd een nieuw (wiskundig) model ontwikkeld dat in hoofdstuk 4.3 werd gevalideerd met behulp van de metingen in de proefinstallatie. Het model kon nauwkeurig het gedrag van LAS-C_{12} voorspellen. Alleen de berekende effluentconcentraties waren enigszins te hoog, waarschijnlijk omdat in de berekeningen een te lage slib-water partitiecoëfficiënt werd toegepast. Een gevoeligheidsanalyse liet zien dat de distributie van LAS-C_{12} voornamelijk wordt bepaald door de verdeling van LAS-C_{12} tussen het slib en het (afval)water. Hieruit werd geconcludeerd dat installaties met hoge slibconcentraties en een goede slib-water scheiding bevorderlijk zijn voor de eliminatie van LAS-C_{12}.

Nitrilotriazijnzuur (NTA)

NTA was een tweede teststof in dit onderzoek (hoofdstuk 5). De proefinstallatie werd op dezelfde wijze bedreven als bij LAS-C_{12} het geval was en werd belast met influentconcentraties NTA variërend tussen 300 $\mu g \cdot l^{-1}$ en 35 $mg \cdot l^{-1}$. Meer dan 99% van de NTA in het afvalwater werd in de installatie verwijderd, onafhankelijk van de influentconcentratie en de operationele omstandigheden. De effluentconcentratie NTA was altijd lager dan de detectielimiet van 30 $\mu g \cdot l^{-1}$. Sorptie en daarmee samenhangende distributie van NTA naar het spuislib was geen aantoonbare eliminatieroute zodat de verwijdering van NTA volledig kon worden toegeschreven aan biodegradatie.

Biodegradatietesten, gecombineerd met literatuurinformatie, toonde aan dat noch het op specifieke groei gebaseerde Monod model, noch secondaire verbruiksmodellen de biodegradatie van NTA door actiefslib kunnen voorspellen. Bij lage influentconcentraties moet het Monod model waarschijnlijk door een ander, meer complex, model vervangen worden. Daarnaast bleek de affiniteit van het slib voor NTA afhankelijk te zijn van de influentconcentratie NTA. Helaas is vrijwel niets bekend over de mechanismen die aan deze afwijkingen ten opzichte van bestaande modellen ten grondslag liggen.

Tolueen

De derde teststof in dit onderzoek was tolueen (hoofdstuk 7). Biodegradatie van tolueen trad niet op bij influentconcentraties lager dan 4 $\mu g \cdot l^{-1}$. Bij influentconcentraties hoger dan 100 $\mu g \cdot l^{-1}$ was dit wel het geval en werd meer dan 97% van de totale influentvracht in de proefinstallatie door biodegradatie verwijderd. Tolueenconcentraties in het effluent en in het afgas waren zeer laag: respectievelijk minder dan 2 en 0.3 $\mu g \cdot l^{-1}$.

De algemene trend in de waarnemingen kon goed met een Monod model worden voorpeld maar niet met een secondair verbruiksmodel. Betrouwbaardere voorspellingen lijken mogelijk maar dit vereist nauwkeuriger waarden voor de Monod parameters en voor de verhouding tussen de (water-lucht) massoverdrachtscoëfficiënten voor tolueen en zuurstof. Het blijft onduidelijk of het Monod model ook valide is bij influentconcentraties lager dan 100 $\mu g \cdot l^{-1}$ of in dat geval vervangen zou moeten worden door een ander model.

Modelleren van dynamisch gedrag

De meeste modellen gaan uit van een evenwichtssituatie waarbij een organische microverontreiniging met een constante vracht op een actiefslibinstallatie wordt geloosd. Echter, in werkelijkheid moeten actiefslibinstallaties tijd-variabele vrachten behandelen. Om het actiefslib zich telkens opnieuw moet aanpassen aan de veranderende vracht kan dit een behoorlijk effect hebben op het uiteindelijke (gemiddelde) verwijderingsrendement. Om dit te onderzoeken werd het dynamische gedrag van LAS-C_{12}, NTA en tolueen gevolgd in respons op een stapvormige verhoging van hun influentconcentratie (hoofdstuk 7). Voor alledrie de stoffen paste het slib zich zeer snel aan door de biodegradatiesnelheid te verhogen. Uiteindelijk resulteerde dit in effluentconcentraties die ook vóór de verhoging van de influentconcentratie werden waargenomen. Echter, er werden aanzienlijke verschillen in de overgangsrespons van de verschillende stoffen waargenomen hetgeen erop duidt dat er sprake was van verschillende adaptatiemechanismen. Er werd werd geconcludeerd dat met bestaande modellen het dynamische gedrag van organische microverontreinigingen in actiefslibinstallaties niet nauwkeurig kan worden voorspeld.

Conclusies

In hoofdstuk 8 werden de resultaten van het literatuuronderzoek en de validatie-experimenten bediscussieerd. Met bestaande modellen kan een eerste 'screening' van het lot van organische microverontreinigingen in actiefslibinstallaties worden uitgevoerd. Echter, een inschatting van dit lot mag niet alleen op modelvoorspellingen gebaseerd zijn. Voor bestaande microverontreinigingen zou het resultaat van de modelvoorspellingen gebruikt kunnen worden om meetsessies te ontwerpen. Voor nieuwe (verdachte) stoffen zou een eerste "screening" zinvolle informatie kunnen opleveren als ondersteuning voor meetgegevens die verzameld moeten worden in actiefslibinstallaties op laboratorium- of proefinstallatieschaal. Deze installaties moeten worden gevoed met huishoudelijk afvalwater dat verschillende influentconcentraties van de microverontreinigingen bevat en moeten worden bedreven bij verschillende slibleeftijden. Tenslotte hebben de experimenten aangetoond dat biodegradatie van organische microverontreinigingen door actiefslib een uiterst gecompliceerd proces is waarbij verschillende deelprocessen zijn betrokken die slechts ten dele worden begrepen. Daarom is de ontwikkeling van nauwkeuriger modellen alleen maar mogelijk indien meer onderzoek wordt gedaan naar de biodegradatie van mengsels van organische stoffen door mengcultures zoals actiefslib.

Dankwoord

Tot drie jaar geleden heb ik me altijd afgevraagd waarom mensen zo graag willen promoveren. Natuurlijk zijn daar wel redenen voor aan te dragen, maar in mijn ogen waren die niet erg belangrijk. Maar goed, de resultaten van een project lagen er nu eenmaal en waren reeds vastgelegd in een eindrapport. Volgens Bram Klapwijk was het een koud kunstje om dat rapport om te zetten in een proefschrift. Echter, zoals gewoonlijk moest ik het mezelf weer onnodig (?) moeilijk maken en heb ik aardig wat weekenden en avonden achter de PC doorgebracht om dit boekje te produceren. Toch heb ik daar geen moment spijt van omdat ik nu tenminste net als alle andere promovendi ook heb moeten lijden onder het schrijverschap, een (AIO-)dip heb ervaren, moest herstellen van het gebrek aan motivatie, etc. Echter, de grootste voldoening komt voort uit het idee dat ik mezelf de tijd heb gegund om eens rustig over één specifiek onderwerp na te denken. Vreemd genoeg komt dat er in de meeste projecten door tijd- (en dus geld-)gebrek te weinig van terwijl je toch zou mogen verwachten dat iemand die aan een universiteit werkt daar wel tijd voor heeft.

Ik moet een groot aantal mensen bedanken en reeds vooraf excuses als ik iemand vergeet. Bram, vooral bedankt voor jouw relativeringsvermogen en het vertrouwen dat je altijd in mij hebt gehad. Ik heb altijd een grote vrijheid gehad bij het opzetten en uitvoeren dit en andere projecten en dat stel ik zeer op prijs. Zeker ook een bijdrage heeft Wim Rulkens geleverd als promotor. Wim, alhoewel ik vind dat het wel heel erg lang heeft geduurd voordat je begonnen met het lezen moet ik toegeven dat je dat uiteindelijk wel heel grondig en in record tempo hebt gedaan.

Veel dank ben ik Vinnie de Wilde verschuldigd. Terwijl ik tijdens een groot deel van het project in Denemarken biologisch aan het defosfateren was, moest hij zich samen met Bram verweren tegen een begeleidingscommissie met nederlandse, duitse en engelse chemische bobo's. Iedereen, die Vinnie een beetje kent, weet dat hem dit geen enkele moeite heeft gekost, ondanks alle tegenslagen die we hebben gehad met de proefinstallatie en bij het uitvoeren van de analyses. Zijn inzet (ook buiten de reguliere werktijden werden grote hoeveelheden monsters genomen en geanalyseerd) wordt bijzonder gewaardeerd en ik vind het echt onvoorstelbaar dat hij pas sinds kort een vaste aanstelling heeft binnen de sectie Milieutechnologie.

Alhoewel ik de afgelopen 10 jaar ongeveer 30 studenten voorbij heb zien komen, hebben slechts enkele aan de inhoud van dit boekje bijgedragen. De tomeloze inzet van Lars Toräng was onmisbaar bij het opzetten van de LAS(tige) analyses en Stefan Mol, die als ex-student wat onbetaalde werkervaring wilde opdoen, heeft waarschijnlijk nog steeds een deuk in zijn vingers van het injecteren van de tolueen monsters.

De mensen in de proefhal in Bennekom (ik ga geen namen noemen, dat geeft alleen maar problemen) kennen mij waarschijnlijk het beste als de klagende en koekjes etende workaholic. Ik heb mezelf de laatste paar jaar diverse malen voorgenomen te ontsnappen aan die rare mix van stafleden, OBP'ers, binnen- en buitenlandse onderzoekers en studenten, maar dat is me nog steeds niet gelukt. Blijkbaar zijn jullie daar met z'n allen veel te leuk en aardig voor. Wanneer komt er trouwens weer eens een barbecue?

Iemand die ik natuurlijk niet vergeet is dr. ir. van Eekert, mijn grote steun en toeverlaat. Miriam, ik begrijp soms niet waar je de energie vandaan haalt om met mij samen te leven, zeker niet op

zaterdagochtenden als ik weer eens een "addi-haddi" aanval heb. Alhoewel we het eigenlijk nooit inhoudelijk over het onderzoek in dit boekje hebben gehad, ben je het allerbeste "klankbord" dat ik ken, en dat zal door diverse AIO's bij milieutechnologie worden onderstreept. Dat ik hoop dat we samen nog heel veel cashew-noten mogen verorberen zegt genoeg denk ik.
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Hardy Temmink
Wageningen, 2 mei 2001

Curriculum Vitae

Bernard Gerhard (Hardy) Temmink werd geboren op 26 september 1963 te Rheden. Na het behalen van het VWO-diploma op het Rhedens Lyceum te Rozendaal begon hij in 1981 met de studie Milieuhygiene aan de (toen nog) Landbouwhogeschool in Wageningen. Afstudeervakken werden uitgevoerd bij de vakgroepen waterzuivering, proceskunde en informatica. Stages vonden plaats op het gebied van de afvalwaterzuivering, achtereenvolgens bij de VAV in Örebro, Zweden en bij Thames Water in Londen, Engeland. De studie werd afgerond in 1988 en na enige tijd bij een software-bureau te hebben gewerkt kwam hij in dienst bij de vakgroep (nu zo genoemde) sectie milieutechnologie van Wageningen Universiteit. De eerste 4 jaar werd in samenwerking met Manotherm, een producent van een respiratiemeter die op de vakgroep was ontwikkeld, onderzoek gedaan naar de toepassing van dit meetinstrument. Nadat gedurende 1½ jaar bij de vakgroep chemische technologie van de Technische Universiteit in Kopenhagen onderzoek was gedaan naar biologische defosfatering, werd de draad op de vakgroep milieutechnologie in Wageningen weer opgepikt. Er werden diverse derde-geldstroom projecten uitgevoerd, waaronder het project dat in dit proefschrift is beschreven en diverse andere projecten op het gebied van de afvalwaterzuivering.

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ONTWERP OMSLAG
Willemien Schouten