Combined Carbon and Nitrogen Removal in Integrated Anaerobic/Anoxic Sludge Bed Reactors for the Treatment of Domestic Sewage

Ghada Nassri Kassab

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Promotor
Prof. Dr. Ir. J. B. van Lier
Hoogleraar in de Anaerobie afvalwaterbehandeling voor hergebruik en irrigatie bij de sectie Milieutechnologie

Co-promotoren
Dr. Ir. B. Klapwijk
Universitair Docent bij de sectie Milieutechnologie

Prof. Dr. M. Fayyad
Professor of analytical chemistry
Chemistry department/ Faculty of Science
University of Jordan

Samenstelling promotiecommissie

Prof. Dr. F. El-Gohary,	National research center, Cairo, Egypt
Prof. Dr. Ir. A.J.M. Stams	Wageningen Universiteit
Prof. Dr. Ir. D. Brdjanovic,	UNESCO- IHE, Delft
Dr. Ir. R. Kleerebezem,	Technische Universiteit Delft

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Abstract

Kassab Gh. (2009). Combined carbon and nitrogen removal in integrated anaerobic/anoxic sludge bed reactors for the treatment of domestic sewage. PhD thesis, Wageningen University, The Netherlands.

The main objective of this research is to assess the applicability and effectiveness of integrating anaerobic digestion and denitrification processes in a single sludge system. The integrated concept is of particular interest for the treatment of high-strength domestic wastewater and is accomplished by means of a sequential anaerobic-aerobic system. The anaerobic pre-treatment can consist of a single anaerobic stage or two anaerobic stages, conditioned mainly by the wastewater characteristics, the prevailing ambient temperatures and the scale of application.

Effluent nitrogen level adjustments using treatment system consisting of a single anaerobic stage followed by an aerobic stage can be incorporated by circulating part of the nitrified aerobic effluent to the influent of the anaerobic stage, integrating denitrification and anaerobic digestion. In the sequential treatment system consisting of two anaerobic stages followed by an aerobic stage, nitrogen level adjustments can be incorporated by partially circulating the nitrified aerobic effluent to the secondary anaerobic stage (methanogenic stage).

The first part of this research is focused on studying the potentiality of integrating denitrification and methanogenesis processes in single stage UASB reactors operated with flocculent sludge, simulating a single anaerobic stage receiving circulated nitrified effluent.

Operating a Lab scale UASB reactor on synthetic wastewater, simulating high-strength domestic wastewater, under integrated conditions with a COD/NO_3-N ratio of 23 have resulted in 92% COD and 97% nitrate removal. Denitrification was the main nitrate reduction pathway. Yet, 12% of applied nitrate nitrogen was reduced via dissimilatory nitrate reduction to ammonium (DNRA), for the apparent reason that glucose was a constituent in the carbon substrate medium. Integrated operation lead to the consumption of 18% of applied COD in nitrate reduction processes, while the rest of removed COD was converted to methane. Compared to operation under strict anaerobic conditions, methanogenesis reduction upon launching the integrated process coincided with reduction of COD available for methane production. Thus, indicating that methane production in the UASB reactor was not distressed by the denitrification process inhibitory effects. This conclusion is compatible with results obtained from the operation of a semi-technical UASB reactor on raw domestic wastewater supplemented with synthetic nitrate. Compared to operation under strictly anaerobic conditions, integrated operation of the semi-technical UASB reactor at a COD/NO_3-N ratio ranging between 20 and 38 didn't result in significant reduction in observed methanogenesis. Interestingly, a higher degree of hydrolysis was observed under integrated conditions, increasing the available COD for either methanogenesis and/or denitrification. It is speculated that preservation of methanogenesis under integrated conditions, is most likely due to shielding of the methanogenic biomass in the interiors of the flocculent biofilm from the oxidized N-compounds. In addition, stratification inside the sludge bed may also have

contributed to protection of the methanogenic biomass. Regarding nitrogen removal, nitrate was completely removed. However, it wasn't completely denitrified, as 33% of applied nitrate was reduced via DNRA process. This obviously decreases the efficiency of the integrated system in terms of achieving a high degree of nitrogen removal, at the prevailing range of COD/NO_3-N ratio, i.e. 20-38. Most interestingly, integrated operation didn't lead to deterioration of the flocculent sludge settleability features. Thus, sludge detainment problems in consequence of long term integrated operation are not expected. Integrated operation also didn't lead into deterioration of the flocculent sludge dewaterability features.

The second part of this research is focused on studying the integration of methanogenesis and denitrification processes in EGSB reactors for the treatment of pre-settled domestic wastewater. The EGSB reactor simulates the secondary anaerobic stage receiving part of the nitrified effluent of a succeeding aerobic stage, within a treatment system consisting of two anaerobic stages followed by an aerobic stage. Two lab scale EGSB reactors were employed in the study; the first was operated under integrated denitrification and methanogenic conditions, whereas the second was operated as a control reactor under strict methanogenic conditions. Moreover, the study was carried in two successive periods, in which the applied upflow velocity in the first and second periods was 4.5 $m.h^{-1}$ and 8 $m.h^{-1}$, respectively. In the integrated reactor that was operated at a COD/NO_3-N ratio of 20, during the two experimental periods, nitrate was almost completely removed by means of the denitrification process.

In the integrated reactor, the COD consumed in methane production was only 67% and 69% of the COD available for its production during the first and second period, respectively. The observed low degree of methanogenesis is attributed to biomass loss from the reactor. This washout is attributed to the growth of denitrifiers in form of fluffy biofilm on the surface of methanogenic granules that deteriorated the granules' settleability. The liquid shear stress induced by the 4.5 $m.h^{-1}$ and 8 $m.h^{-1}$ upflow velocities was not enough to prevent accumulation of the fluffy biofilm.
Of practical interest is the preservation of specific methanogenic activity in the sludge grown under integrated conditions. Thus, if the encountered sludge detainment problems with integrated granules are resolved, EGSB reactors under integrated conditions can be operated at OLRs comparable to those under strict methanogenic conditions. Settleability of integrated granules can be substantially improved by periodic application of excessive detachment forces induced either by periodic increase in upflow velocity or by periodic circulation of the sludge bed from the bottom to the top of the reactor.

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Chapter 1

Introduction

1. Introduction

The control of nitrogen levels in wastewaters discharged into watercourses is vital in order to avoid the acceleration of eutrophic conditions and declination of oxygen levels in receiving water bodies. However, eutrophication is not the only problem caused by excessive nitrogen input. Ammonia, nitrite and nitrate are toxic to aquatic life at elevated concentrations. Nitrite, which has a greater affinity for hemoglobin than oxygen may act as substitute in the bloodstream, potentially causing blue baby disease in infants.

Adverse effects of excessive nitrogen input are not only related to wastewater discharge into watercourses and drinking water reservoirs, but also to the agricultural use of treated wastewater, despite the fact that nitrogen is a fertilizing agent. The extent into which agricultural use of treated wastewater affects the receiving environment, whereby the movement of nitrogen from wastewater irrigated soils to surface and ground water is of particular concern, depends on many factors such as chemical, physical and biological characteristics of the soil, plant uptake, effluent composition, volumes and rates of application and prevailing weather conditions (Sparling *et al*., 2006; Barton *et al*., 2005 and Boom *et al*., 2008). Wastewater applied nitrogen can be removed by plant uptake, denitrification, volatilization and immobilization into the soil organic matter. If any excess still remains it is likely to be leached considering the mobility of the nitrogen ions.

In countries of moderate daily per capita water consumption rates, such as the Middle East countries, the total nitrogen concentration in domestic wastewater range between 80-120mg.l^{-1} (Duqqah, 2002 and Mahmoud, 2002). Use of these wastewaters as sole irrigation water implies nitrogen application rates higher than required by most crops (Huibers and van Lier, 2005 and Boom *et al*, 2008). Accordingly, serious threats on the receiving environment will be imposed. Moreover, irrigation with wastewater implies inseparable application of irrigation water and nitrogen. Generally, the required water demand, determines the irrigation rate. Henceforth, when nitrogen needs are not synchronized with irrigation needs, receiving environments shall also be imperiled. In addition to the imposed threats on the environment, over-fertilization with nitrogen via wastewater irrigation causes excessive vegetative plant growth, reduction in crop yield and encouragement of weed growth (Asano and Pettygrove, 1987 and Bouwer and Idelovitch, 1987). This thesis presents research describing the amendment of nitrogen concentrations in the effluent of wastewater treatment plants prior to subsequent use in agricultural systems.

Nitrogen removal processes can be grouped into two main categories: biological and physical-chemical processes. Nitrogen in municipal wastewater is predominately present in the organic form and/or as ammonia, both analytically measured as N-Kjeldahl. Hydrolysis converts organic nitrogen to ammonia and biological removal of ammonia is then generally achieved by first oxidizing ammonia to nitrite/nitrate via the nitrification process which, in turn, is reduced to nitrogen gas via denitrification. Considering the relatively low nitrogen concentrations in domestic wastewater, biological processes are much more feasible than the physical-chemical processes. The current challenge is to incorporate biological nitrogen removal in efficient, sustainable and cost effective organic matter removal systems.

Sequential anaerobic-aerobic systems offer a potential effective and sustainable approach for domestic wastewater treatment. Wherein, anaerobic stage(s) ascertain at least 60-80% reduction in the organic load applied on the subsequent aerobic stage. Anaerobic systems are characterized by low construction and operational costs, low excess sludge production, production of energy in form of biogas and applicability in small and large scale (Lettinga *et al*., 2001; Foresti, 2002; Aiyuk *et al*., 2006 and van Lier *et al.*, 2008). Thus, compared to conventional aerobic systems energy consumption is reduced and production of excess poorly stabilized sludge is lowered.

Biological nitrogen removal can be incorporated in the sequential treatment systems by recycling part of the nitrified aerobic effluent to the anaerobic stage to achieve denitrification. In such approach, anaerobic digestion and denitrification takes place simultaneously in the anaerobic stage. Here, part of the organic carbon content in the wastewater serves as carbon source for the denitrification process and the rest is converted to methane. Removal of residual organics takes place in the aerobic stage in addition to the oxidation of ammonia to nitrate/nitrite (nitrification). This system eliminates the need for a separate denitrification unit (anoxic stage), and thus offers appreciable economic advantages.

In the course of this dissertation, a system will be referred to as the integrated system if denitrification and anaerobic digestion takes place in a single sludge process and the anaerobic reactor in which denitrification and methanogenesis takes place will be referred to as the integrated reactor.

The integrated system is of particular interest for the treatment of concentrated sewage, i.e. COD concentration exceeding 1 $g.l^{-1}$. This can be attributed to the fact that for treatment of more concentrated sewage, the volumetric design of the anaerobic system is not limited by the hydraulic loading rate, and thus the extra hydraulic load brought about by the recirculation of the nitrified aerobic effluent can be accommodated.

In view of the limited water resources and limited additions of (small-scale) industrial wastewaters, sewage concentrations in many arid climate countries range between 1-2 $gCOD.l^{-1}$ or even higher, e.g. the Middle Eastern and North African countries (Halalsheh, 2002; Mahmoud, 2002 and van Lier, 2008). Therefore, the foreseen application of the integrated system in such countries is viable option. However, the design of the anaerobic systems should take into consideration the diurnal and seasonal temperature fluctuations typical for arid climates.

Since domestic wastewater contains high fractions of suspended solids, at low to moderate temperatures, the application of single stage anaerobic pre-treatment will result in an accumulation of suspended solids in the sludge bed due to the slow hydrolysis of the entrapped solids. Therefore, to achieve adequate treatment, long HRTs should be applied (Zeeman and Lettinga, 1999). Otherwise, the suspended solids must be separated from the raw streams before the sewage enters the methanogenic reactor. Separation of suspended solids can be achieved by applying two anaerobic stages in series. In which the first anaerobic stage can be designed for either (van Lier *et al*., 2001): *(i)* physical entrapment of suspended solids, *(ii)* entrapment, hydrolysis and acidification of solids and *(iii)* pre-digestion of solids including methanogenesis.

Elmitwalli (2000) recommended the application of an anaerobic filter (AF) for physical removal of suspended solids prior to further anaerobic treatment. The AF was operated at a process temperature of 13°C and an HRT of 4 h, achieving total and suspended COD removal of 55% and 82%, respectively. Furthermore, treatment of domestic sewage in an AF followed by an anaerobic hybrid (AH) reactor, with a granular sludge bed in the bottom and a gas-solids separator in the middle and filter media in the top, was investigated by Elmitwalli *et al*. (2002). The system was investigated at 13°C, applying different HRTs. Best overall performance with 71% removal of total COD and 91% removal for suspended COD was achieved at HRTs of 4 and 8 h for the AF and the AH reactor, respectively.

Wang (1994) studied the combination of a hydrolysis up flow sludge bed (HUSB) reactor for the pretreatment of domestic sewage followed by an expanded granular sludge blanket (EGSB) reactor at ambient temperature (9-21°C). The HUSB reactor can be considered as a relatively highly loaded UASB reactor for the removal and hydrolysis of suspended COD. Operating the HUSB reactor at an HRT of 3 h (5 $KgCOD.m^{-3}.d^{-1}$) resulted in 58% removal of suspended COD, 0.9% increase in soluble COD and 23% removal of colloidal COD. The EGSB reactor was operated at an HRT of 2 h and 32%-58% removal of soluble COD was achieved. Overall process provided 71% and 51% total COD removal efficiency at T >15°C and T =12°C, respectively. However, according to calculations presented by Zeeman *et al*. (1997) only 0.7% hydrolysis was achieved in the HUSB reactor researched by Wang (1994). Thus, the HUSB reactor was providing mainly physical removal of suspended solids, so it was further referred to as an up flow anaerobic solids removal (UASR) reactor (Zeeman *et al*., 1997).

Successful application of the two stage concept, wherein the first stage serves for entrapment, hydrolysis and acidification of suspended solids and the second stage serves for the conversion of dissolved COD to methane, calls for minimum gas production in the first stage (Halalsheh, 2002). Nevertheless, a study carried out in completely stirred tank reactors (CSTRs) for digestion of primary sludge (Halalsheh *et al*., 2005/a) showed that methanogenesis started at a sludge retention time (SRT) ranging between 30-50 days at operational temperature of 15°C and between 5-15 days at operational temperature of 25°C. Thus, the achievement of phase separation at 25°C calls for maintaining a SRT in a range of (5-15) days. Controlling the SRT by sludge discharge at such narrow span is practically very difficult (Halalsheh, 2002). Thus, applying a two stage anaerobic system in which non-methanogenic and methanogenic phases are carried out in separate reactors appears not to be viable. This line of reasoning was further emphasized by operating a two stage UASB reactor under ambient conditions for treatment of concentrated sewage, i.e. sewage concentrations range between 1.5-2 $gCOD.l^{-1}$ (Halalsheh *et al*., 2005/b). The first stage was designed for entrapment, hydrolysis and acidification of suspended solids, while the second stage was designed for methane production. The first stage was operated at an HRT of 8-10 h (OLR= 3.6-5 $KgCOD.m^{-3}.d^{-1}$) and the second stage was operated at an HRT of 5-6 h (OLR=2.9-4.6 $KgCOD.m^{-3}.d^{-1}$). Results have shown that under summer conditions the HRT time applied in the first stage was sufficient to initiate methanogenic conditions. The percentage of applied total COD transferred into methane (methanogenesis %) was 46%, resulting in considerable gas production and consequently low suspended COD removal (57%).

Sayed and Fergala (1995) researched an alternating two-stage anaerobic system for the treatment of domestic sewage. The first stage consisted of two identical UASB reactors operated in parallel and intermittently with flocculent sludge while the second stage consisted of a UASB reactor operated with granular sludge. Either of the two first stages was operated as batch digester for entrapped solids digestion, meanwhile the other received continuous feeding. The experiments were carried out at ambient temperatures of 18-20 °C and HRTs of 4-8 h for the first stage and 2 h for the second stage. A total COD removal efficiency of 80% was achieved by the overall system. In which, most of the removal took place in the first stage with methanisation percentages of 21%, 21% and 28% during the feed-less periods at HRTs of 8, 6 and 4 h, respectively. While, during the feeding periods, the methanisation percentages were 14%, 11% and 2% at the HRTs of 8, 6 and 4 h, respectively. On the hand, up-scaling and full-scale operation of such system seems rather complex.

As was stated earlier, the achievement of adequate pre-treatment by means of a single anaerobic stage, calls for the application of long SRTs at low to moderate temperatures. For the UASB reactor, Zeeman and Lettinga (1999) proposed a model to calculate the required HRT (equation 1). In which, the HRT is related to the required SRT, which is in turn affects the hydrolysis percentage. Moreover, it is related to the concentration of sewage and the removal of suspended solids.

$$HRT = \frac{C \times R_{SS}}{X} \times R \times (1 - H) \times SRT \quad \text{..............................} \ (1)$$

Where:
C: Total COD concentration in the influent (gCOD.l^{-1}).
R_{SS}: The ratio of suspended COD to total COD in the influent (%).
X: Sludge concentration in the reactor (gCOD.l^{-1}); based on common sludge composition of 1g VSS=1.4 g COD
R: Fraction of the suspended COD removed.
H: Fraction of the removed COD that is hydrolyzed; no distinction had been made between the fraction of suspended COD that is removed but not hydrolyzed and the biomass yield.

A study carried out in a CSTR for the digestion of primary sludge by Halalsheh *et al*. (2005/a) showed that at an operational temperature of 15°C, the hydrolysis and methanogenesis percentages achieved at 75 days SRT were limited to 24% and 25%, respectively. Accordingly, at temperatures in the vicinity of 15°C, an SRT above 75 days is essential to achieve adequate methanogenic activity. Table 1 shows the calculated HRT according to the previously presented model for treatment of domestic wastewater with a concentration of 1.5 gCOD.l^{-1} of which 70% is suspended at SRT of 100, 125 and 150 days, assuming suspended COD removal of 50% and hydrolysis percentages of 50% and 75%. These sewage characteristics are typical for sewage produced in the Middle Eastern countries where the per capita share of fresh water is limited.

The results presented by Halalsheh *et al*. (2005/b) for studying the treatment of domestic sewage with an of average COD content of 1.5 g.l^{-1} by a single stage

Table 1: Calculated HRT according to the model proposed by Zeeman and Lettinga (1999) to achieve the required SRT*.

Hydrolysis%	HRT (days)		
	SRT=100 day	SRT=125 day	SRT=150 day
50	1.25	1.5	1.9
75	0.6	0.8	0.98

* Calculations are made for sludge concentration in the reactor amounting to 15 $gVS.l^{-1}$ (about 25 $gTS.l^{-1}$)

UASB reactor were in accordance with the model presented by Zeeman and Lettinga (1999). The study was carried out under ambient conditions with a prevailing average temperature of 25°C at summer time and 15°C at winter time. Application of 23-27 h HRT resulted in achieving an SRT of 137 days for winter and 186 days for summer, which in turn attained hydrolysis percentages of 46% and 76% for winter and summer, respectively. The total and suspended COD removal efficiencies achieved were respectively, 62% and 55% at summer and 51% and 50% at winter.

Obviously, if a single stage anaerobic reactor is to be designed under low to moderate temperature conditions, long HRTs are required. Whereas, applying a two stage system the total HRTs can be reduced. In addition to the prevailing ambient temperatures, the preference between single stage and two stages anaerobic pre-treatment depends on the characteristics of wastewater. Obviously, the rate of solids accumulation correlate with the concentration of domestic sewage, thus requiring increased HRTs. Another difference that might impact the decision is that with a two stage anaerobic pre-treatment the sludge produced in the first stage is not well stabilized and needs further digestion, whereas the sludge produced in the single anaerobic stage is well stabilized. This makes the scale of application a key factor in the preference between the single and two stages anaerobic pre-treatment. In case of centralized sanitation, in which sludge thickening and digestion can be feasible, two stage anaerobic pre-treatment can be preferential. In case of decentralized sanitation and treatment, in which sludge handling can be problematic, single stage is more favorable (Halalsheh, 2002).

The referred integrated concept can be employed in the treatment of domestic wastewater, accomplished by means of a sequential anaerobic-aerobic system. The anaerobic pre-treatment then consists of either a single anaerobic stage or two anaerobic stages, conditioned mainly by the wastewater characteristics, prevailing ambient temperatures and the scale of application. Effluent nitrogen level adjustments can be incorporated in the sequential system that consists of a single anaerobic stage followed by aerobic stage, by re-circulating part of the nitrified aerobic effluent to the anaerobic stage to achieve denitrification (Figure 1). In the sequential system consisting of two anaerobic stages followed by an aerobic stage, nitrogen level adjustment can be incorporated by partially circulating the nitrified aerobic effluent to the secondary anaerobic stage (Figure 2). The latter configuration is particularly appropriate for the treatment of concentrated sewage. This is due to the fact that with raw sewage of low COD content, the COD remaining in the first anaerobic stage effluent might not be sufficient for nitrate reduction.

The extent of total nitrogen (TN) removal is controlled by the recycle to feed (Q_r/Q) ratio, wherein raising the Q_r/Q ratio results in higher TN removal efficiency. This in turn results in lowering the carbon to nitrate ratio of the integrated reactor influent ($COD_{F\text{-}int}/NO_{3\ F\text{-}int}$). The $COD_{F\text{-}int}/NO_{3\ F\text{-}int}$ ratio determines the partitioning of COD consumption between denitrification and methane production processes (Lee *et al*., 2004).

For the single stage anaerobic-aerobic configuration, the $COD_{F\text{-}int}/NO_{3\ F\text{-}int}$ is given by;

$$\frac{COD_{F-int}}{NO_{3\,F-int}} = \frac{(Q\times COD_{inf} + Q_r \times COD_{ae})/(Q+Q_r)}{NO_{3\,ae}\times Q_r/(Q+Q_r)}$$

If COD_{ae} is neglected; $\dfrac{COD_{F-int}}{NO_{3\,F-int}} = \dfrac{COD_{inf}}{r\times NO_{3\,ae}}$

Where; $COD_{F\text{-}in\ t}$ = COD content of the integrated reactor influent ($mg.l^{-1}$).
Q = flow rate of raw wastewater stream, ($m^3.d^{-1}$).
COD_{inf} = COD content of raw wastewater ($mg.l^{-1}$).
Q_r = flow rate of recycle stream, ($m^3.d^{-1}$).
COD_{ae} = COD content of aerobic reactor effluent, ($mg.l^{-1}$).
$NO_{3\ F\text{-}int}$ = nitrate concentration in the integrated reactor influent, ($mgN.l^{-1}$).
$NO_{3\ ae}$ = nitrate concentration in the aerobic reactor effluent, ($mgN.l^{-1}$).
r = recycle to feed ratio = Q_r/Q.

For the two stage anaerobic-aerobic configuration; COD_{inf} should be replaced with COD_{an}. Where; COD_{an}= COD content of the first anaerobic stage effluent ($mg.l^{-1}$).

The amount of COD consumed in the denitrification process is given by:

$$COD_{den} = \frac{2.86\times(NO_{3\,F\text{-}int} - NO_{3\,E\text{-}int})}{(1-y_{anox.})}$$

Where; $y_{anox.}$ is the denitrifying biomass yield, $gCOD_{biomass}.gCOD_{removed}^{-1}$.

2. Limitations of the integrated system

The integrated scheme encounters three critical limitations that should be taken into account while assessing the biotechnological potentials of the system: ***(i)*** reduction of nitrate and nitrite is more energy yielding than methanogenesis, consequently carbon metabolism via denitrification will be expected to have a preference. ***(ii)*** inhibitory effects of nitrate and its denitrification intermediates on methane production. ***(iii)*** ammonium formation via dissimilatory nitrate reduction (DNRA), resulting in nitrogen conservation instead of removal by means of denitrification process.

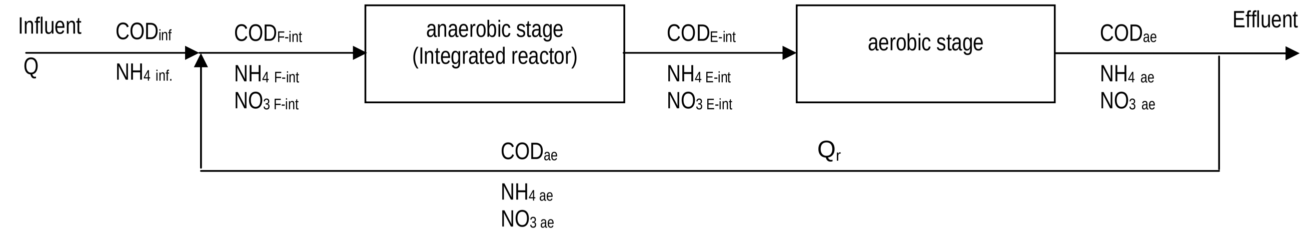


Figure 1: process configuration of a single stage anaerobic- aerobic integrated system

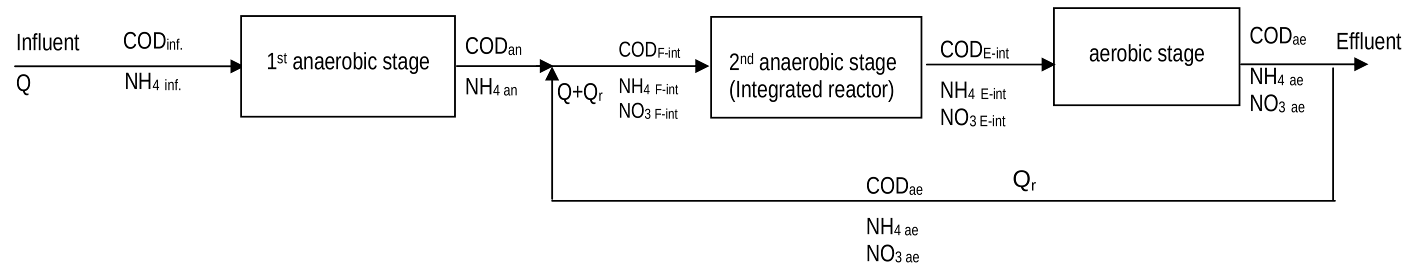


Figure 2: process configuration of two stage anaerobic-aerobic integrated system

Domination of carbon metabolism via denitrification

The coexistence of denitrifiers and methanogenesis in a single reactor implies competition between these two trophic groups of microorganisms over the available substrates. The theoretical energy yield of these competing processes (Table 2) indicates that the denitrification process provides more potential energy. Thus, if substrate is limited, the denitrification process would be more favorable. Moreover, the high potential energy provided by the denitrification process implies higher cell yields and hence a substantial part of the sludge bed will consist of denitrifiers.

Table 2: Theoretical energy yield of denitrification and methanogenesis processes.

Reaction	Energy yield (ΔG^{O}) (KJ.mol e d^{-1})*
Methanogenesis	
$4H_2+CO_2 \rightarrow CH_4+2H_2O$	-131
$CH_3COO^-+H_2O \rightarrow CH_4+HCO_3^-$	-31
$4CH_3CH_2COO^-+6H_2O \rightarrow 7CH_4+CO_2+4HCO_3^-$	-57
$C_6H_{12}O_6 \rightarrow 3CH_4+3CO_2$	-428
Denitrification	
$5H_2+2NO_3+2H^+ \rightarrow 6H_2O+N_2$	-224
$5CH_3COO^-+8NO_3^-+8H^+ \rightarrow 9H_2O+5CO_2+5HCO_3^-+4N_2$	-797
$5CH_3CH_2COO^-+14NO_3^-+14H^+ \rightarrow 7H_2O+10CO_2+5HCO_3^-+7N_2$	-1398
$5C_6H_{12}O_6+24NO_3^-+24H^+ \rightarrow 12N_2+30CO_2+42H_2O$	-2720

*The change in Gibbs free energy at standard condition in KJ mol.elector donor^{-1}

Inhibitory effects

Several studies have demonstrated that nitrate and its denitrification intermediates suppress methane production. Balderston and Payne (1976) demonstrated that introduction of nitrogen oxides into salt marsh sediments batches and its utilization by denitrifying bacteria suppressed methanogenesis. The duration of suppression as well as the rate of methane production after resumption was related to the type and concentration of nitrogen oxides added. Nitrite was the stronger of the two ionic inhibitors and stronger inhibitory effects were exerted by gaseous nitrogen oxides than ionic oxides with N_2O having the highest impact.

Chen and Lin (1993) have also shown that in a mixed culture system of denitrifying sludge and methanogenic sludge in an anaerobic bioreactor, methanogens were suppressed as long as nitrate and nitrite were made available in the mixed system. The degree of inhibition was increased with an increasing concentration of nitrogen oxides.

Akunna *et al*. (1994/a) stated that in batch cultures incubated with digested anaerobic sludge and nitrate at initial concentrations of 35, 70 and 143 $mgNO_3$-N.l^{-1}, methane production was resumed only in the culture incubated with 35 $mgNO_3$-N.l^{-1} and after complete elimination of all the nitrates and nitrites. In the cultures incubated with higher initial nitrate concentrations i.e. 70 and 143 $mgNO_3$-N.l^{-1}, methane production was completely suppressed. Also in mixed methanogenic cultures, Tugtas and Pavlostathis (2007/a) found that the level of methane production suppression, i.e. period of suppression and rate of methane production after the complete reduction of nitrogen oxides, was a function of the type and amount of nitrogen oxides. They have found that NO was the most toxic nitrogen oxide, whereas nitrate was the least inhibitory. Simultaneous methane production and denitrification was identified in cultures amended with less than 30 $mgNO_3$-N.l^{-1}.

It is worth mentioning that in addition to the type and concentration of the nitrogen oxides applied, inhibitory effects depend on the methanogenic species (Belay *et al*., 1990 and Kluber and Conrad, 1998/a). Generally, concentrations of nitrate>20mM; nitrite<100μM; N_2O<1mM and 1-2μM NO were found to be sufficient to completely inhibit hydrogen or acetate consuming methanogens (Chidthaisong and Conrad, 2000).
Three mechanisms for the suppression have been suggested: first hypothesis is that nitrate increases the redox potential, which prevents methanogens from being active. Bollag and Czlonkowski (1973) stated that, since the duration of the methane production lag phase in soil samples is correlated to the more reduced state of nitrogen oxides, the redox potential is the responsible factor for the suppression of methane production. Nevertheless, Balderston and Payne (1976) have shown that in salt marsh sediments the effect of nitrogen oxides can't be attributed to the increase in redox potential. This result was emphasized by Chen and Line (1993) in anaerobic sludge, as the inhibition of methane formation was still observed in the presence of nitrogen oxides, despite the fact that redox potentials were adjusted to values ranging between -340 to -530 mV. Akunna *et al*. (1998) stated that for anaerobic sludge, the inhibition of methane production by nitrogen oxides was still observed at a redox potential of -290mV. This was also confirmed by Roy *et al*. (1997) and Kluber and Conrad (1998/b) in anoxic rice soils. Kluber and Conrad (1998/a) has affirmed that in cultures of *Methanosarcins barkeri* and *Methanobacterium bryantii* the inhibitory effect of nitrogen oxides were not due to an increase in the redox potential that stayed well below -400 mV.

The second hypothesis is that methane suppression is the consequence of substrate (acetate, H_2) competition between denitrifiers and methanogens. However, Balderston and Payne (1976) showed that in salt marsh sediments substrate competition is not influential on methane production suppression by nitrate, since hydrogen and carbon dioxide were continually present in excess. Roy and Conrad (1999) have also shown that in anoxic rice field soil, competition for substrate between denitrifiers and methanogens is not the main mechanism for suppression of methanogenesis.

The third hypothesis is that denitrification leads to accumulation of intermediates, such as nitrite, N_2O, NO, that are toxic to methanogenic archae. This hypothesis was proposed by Balderstion and Payne (1976) in salt marsh sediments, by Chen and Lin (1993) in anaerobic sludge and by Roy and Conrad (1999) in anoxic rice fields. It is known that NO is especially toxic to bacterial cells by attacking Fe groups in enzymes, and since a number of enzymes involved in the acetoclastic or hyrogentrophic pathway of methanogenic archae contain Fe-S clusters or Fe hemes, these enzymes may be susceptible to inhibition by NO or nitrite which may form metal nitrosyl (Roy and Conrad, 1999). Enzymes that are crucial for methanogens may also be inhibited by N_2O which is known to inactivate cobalamin dependent enzymes (Fischer and Thauer, 1990). Apparently, the toxicity effects of denitrification intermediates explain the suppression of methane production by nitrogen oxides most appropriately. These toxicity effects lead to a reduction in bacterial populations and methanogenesis activity (Scheid *et al*., 2003).

In order to successfully apply the integrated systems and sidestep the inhibitory effects of nitrate and its denitrification intermediate on methanogenesis, researchers suggested systems in which spatial distinct of denitrification and methanogenic environments exist. Chen *et al*. (1997) studied the denitrification and methanol removal efficiency of a co-immobilized mixed culture system containing denitrifiers and methanogens in polyvinyl alcohol gel. It was observed that the heterotrophic denitrifiers tend to grow on the gel beads surface where nitrate is sufficient and anaerobic methanogens grow in the beads interior. Simultaneously, the influent's nitrate and methanol were effectively removed under long term operation in a CSTR. The previous habitat segregation concept was employed in systems operated with granular sludge as well (Hendriksen and Ahring, 1996 and Lee *et al*., 2004). Here the denitrifiers grow on the granules' surface where nitrate is abundantly available, whereas methanogens grow in the inner part where nitrate is deficient. Biofilm reactors where methanogens grow in the deep biofilm layers wherein nitrate is limited, has also been proposed (Hanaki and Polprasert, 1989; Akunna *et al*., 1994/b and Zellner *et al*., 1995).

Dissimilatory nitrate reduction to ammonium

The competition between denitrification and dissimilatory nitrate reduction to ammonium (DNRA) processes is of particular interest since both occur under similar conditions, i.e. low oxygen concentrations, but one result in a loss or removal of combined nitrogen (denitrification) and the other conserves nitrogen (DNRA) (Tiedje *et al*., 1982). The DNRA process has been recognized in anaerobic habitats such as in the rumen (Farra and Saatter, 1970), anaerobic sludge digesters (Akunna *et al*., 1994/a and Tugtas and Pavlostathis, 2007/b), paddy soils (Yin *et al*., 2002), and anoxic sediments (Kelso *et al*., 1997). Bacteria involved in DNRA process have fermentative rather than oxidative metabolism, which is the opposite of denitrification process (Tiedje, 1988). Bacteria capable of DNRA include mainly obligate anaerobes (e.g., *Clostridium, Veillonella and Desulfovibrio*) and facultative anaerobes (e.g., *Enterobacteriaceae, Klebsiella and Photobacterium*).

The DNRA process is carried out in two steps. First, membrane bound nitrate reductase catalyses reduction of nitrate to nitrite, while nitrite reductase completes the reduction to ammonium.

In most DNRA organisms the first step is coupled to energy production, while the second step lacks energy conservation (Tiedje, 1988). Although, the first step is not the distinctive step for the DNRA process, since it is not the rate limiting step and since it accumulates nitrite that can be converted to N_2. Hence, the conversion of nitrite to ammonium is the distinctive step for the DNRA process.

The theoretical energy yield of DNRA and the denitrification processes (Table 3) indicates that if evaluated on energy yield per electron donor, the denitrification provides more potential energy. However, if evaluated on energy yield per electron acceptor (nitrate) DNRA is more favorable, in a sense that DNRA has the capacity of accepting eight electrons versus five for the denitrification process. Thus, when the electron acceptor is limited relative to electron donor i.e. high COD/NO_3 ratio the DNRA process is favored since the highest need in metabolism is for maximum electron acceptor capacity. Whereas, when the electron donor is limited i.e. low COD/NO_3 ratio, denitrification is favored, since it gains higher energy per reduction of nitrate (Tiedje, 1982). Akunna *et al*. (1994/a) have found that in anaerobic digested sludge cultures fed with glucose, the activity of nitrate reduction to ammonium increases with the increase in COD/NO_3 ratio. Tugtas and Pavlostathis (2007/b) have found as well that in anaerobic digested sludge cultures fed with Dextrin/peptone and glucose, the DNRA was the dominant pathway of nitrate reduction at COD/NO_3-N ratios higher than 20 for the Dextrin/peptone culture and higher than 60 for glucose culture. Additionally, the prevailing COD/NO_3 ratio influences the population composition of nitrate reducers i.e. percentages of denitrifiers and DNRA bacteria. Consequently, the COD/NO_3 ratio determines the partitioning of nitrate reduction between denitrification and DNRA process.

Table 3: Theoretical energy yield and electron accepting capacity of denitrification and DNRA.

Reaction	Energy yield		Electron accepted per NO_3^-
	ΔG^O (KJ.mol^{-1}) electron donor	ΔG^O (KJ.mol^{-1}) electron acceptor	
Denitrification			
$5H_2+2NO_3+2H^+\rightarrow 6H_2O+N_2$	-224	-560	5
$5C_6H_{12}O_6+24NO_3^-+24H^+\rightarrow 12N_2+30CO_2+42H_2O$	-2720	-567	5
DNRA			
$4H_2+2NO_3^-+4H^+\rightarrow 6H_2O+2NH_4^+$	-150	-600	8
$8C_6H_{12}O_6+24NO_3^-+48H^+\rightarrow 12NH_4^++48CO_2+12H_2O$	-1835	-612	8

It has been pointed out in literature that in addition to the COD/NO_3 ratio, the nature of carbon source also influences the reduction pathway of nitrogen oxides. Akunna *et al*. (1993) showed that in anaerobic sludge cultures cultivated on acetic and lactic acid, DNRA was not detected. Conversely, in cultures cultivated on glucose and glycerol, DNRA was predominant even at a relatively low COD/NO_3-N ratio of 15. Thus, in case of wastewaters containing fermentable carbon, such as domestic wastewater, determination of the conditions favoring the denitrification pathway is needed. Moreover, it has been reported in literature that inorganic forms of sulfur, affects the reduction pathway of nitrogen oxides as well. Mazeas *et al*. (2008) have shown that with batches of municipal solid waste, incubated with landfill leachate and amended with nitrate and injected with H_2S, there is a relationship between the H_2S concentration and the relative amount of nitrate reduced via DNRA. Denitrification didn't occur and DNRA was the main nitrate reduction pathway when H_2S concentration in leachate was above 17 mg.l^{-1}. Tugtas and Pavlostathis (2007/c) showed that when mixed methanogenic cultures cultivated on enriched sulfide bearing media (67 mgS.L^{-1}) was amended with nitrate, the percentage of nitrate reduced via DNRA was 19%, 70% and 93% at COD/NO_3-N ratios of 10, 20 and 60, respectively. In contrast, when mixed methanogenic culture cultivated on sulfide free media was amended with nitrate at COD/NO_3-N ratio in range of 5-20 and sulfide in range of 10-100 mg.l^{-1}, denitrification was the predominant reduction pathway at all sulfide levels tested. Percheron *et al*. (1999) have shown that in sulfide rich anaerobic sludge cultures acclimated with molasses wastewater, denitrification was the main nitrate reduction pathway at COD/NO_3-N ratio as high as 66.

3. Applications of the integrated system

The potentiality of integrating denitrification and methanogenesis in a single stage reactor has been researched for high strength wastewaters such as industrial wastewaters (Mosquera-Corral *et al.,* 2001 and Lacalle *et al.,* 2001), slaughterhouse wastewaters (Munez and Martinez, 2001 and Del Pozo and Diez, 2003), landfill leachate (Jeong-Hoon *et al*., 2001) and piggery wastewater (Huang *et al*., 2005 and 2007). The employment of the integrated concept in the treatment of low strength wastewaters such as the domestic wastewater has been limitedly studied as shown in Tables 4 and 5. Jun *et al*. (2005) studied an integrated system consisting of upflow sludge blanket (USB) reactor followed by aerated bio-filter (ABF) for the treatment of domestic sewage with average COD and TN contents of 350 mgCOD.l^{-1} and 51 mgN.l^{-1}, respectively. The nitrified effluent from the ABF was circulated to the USB reactor at a recycle to feed ratios of 1 and 2, bringing about COD/NO_3-N ratios of 38 and 20 in the USB reactor influent. The overall system achieved a total COD removal efficiency of 91% and 96% and TN removal efficiency of 70% and 73% at recycle to feed ratios of 1 and 2, respectively. Based on the presented results, the percentages of applied COD transferred to methane in the USB reactor were calculated to be 64% and 51% at recycle to feed ratios of 1 and 2, respectively.

Several configurations have been suggested for the integrated reactor (Table 4) as well as for the whole integrated system (Table 5). Generally, the feasibility of the integrated concept was assessed in systems wherein spatial distinction between denitrifying and methanogenic environments exist, more specifically, in biofilm systems and sludge bed systems operated with granular sludge. Among the different sludge bed systems, the integrated concept was mostly studied in the UASB system, wherein denitrifiers and methanogenesis were in general successfully integrated. However, the denitrifying/methanogenic granules (integrated granules) grew to be buoyant and tend to washout of the reactor (Hendriksen and Ahring, 1996; Lee *et al*., 2003 and Karim and Gupta, 2003). This was attributed to the growth of denitrifiers in the form of fluffy biofilm on the granules' surface. However, the high upflow velocities applied in EGSB reactors can detach this fluffy biofilm and consequently enhance integrated granules formation. Another plus point for the EGSB reactor, which can be operated at up flow velocities reaching 8-10 $m.h^{-1}$, is that it can easily accommodate the extra hydraulic load brought about by the recycling flows from the aerobic stage.

Up to our knowledge the only research that investigated the integration of denitrification and methanogenesis in EGSB reactor was carried out by Zhang and Verstraete (2001) (Table 4). The study was focused on the biological performance of the reactor, whereas the physical and biological characteristics of developed integrated granules weren't stated. In view of the EGSB reactor potentialities as integrated reactor, further research is indeed needed.

It is worth mentioning that in domestic wastewater treatment, the EGSB reactor applicability as integrated reactor can only be achieved through the process configuration of two stage anaerobic-aerobic sequential treatment. This is due to the fact that the presence of suspended solids in the raw wastewater can be detrimental to the maintenance of the good characteristics of granular sludge in the reactor (Uemura and Harada, 2000 and von Sperling and Chrnicharo, 2005).

Using a CSTR inoculated with anaerobic sludge of flocculent nature and fed with synthetic wastewater containing glucose as the only source of carbon, Akunna *et al*. (1992) showed that at COD/NO_x-N ratios ranging between 8.86 and 53, denitrification and methane production occurred simultaneously. Their results clearly indicate that an integrated concept can be applied in sludge bed systems operated with flocculent sludge. Since the sludge aggregates developing in anaerobic sludge bed reactors operated on raw domestic sewage are expected to be of flocculent nature, the results presented by Akunna *et al*. (1992) highlight the potential viability of an integrated system consisting of a single anaerobic stage followed by an aerobic stage. The feasibility of the integrated concept in anaerobic sludge bed reactors operated with flocculent sludge has only been limitedly studied in previous investigations (Jun *et al*., 2005). The effects of integrated process conditions on biological activities and physical properties of the developed flocculent sludge are not well identified yet.

In view of the UASB reactor prospect on one hand, and the simplicity of single stage anaerobic-aerobic treatment on the other hand, research is needed to optimize the performance of UASB reactor operation with flocculent sludge under integrated conditions. Potentialities of the UASB reactor as integrated reactor can as well be employed in an integrated system consisting of two anaerobic stages followed by an aerobic stage. As long as the reduction in organic load achieved by the first anaerobic stage, would not extensively shift UASB reactor design limitations from organic to hydraulic loads.

4. Scope and thesis outline

This thesis describes the applicability and effectiveness of integrating anaerobic digestion and denitrification processes in a single sludge system. The integrated treatment concept would be employed in the treatment of domestic wastewater accomplished by means of a sequential anaerobic-aerobic system. The anaerobic pre-treatment can consist of single anaerobic stage or two anaerobic stages, conditioned mainly by the characteristics of wastewater, prevailing ambient temperatures and scale of application. In the single stage anaerobic-aerobic system, nitrogen level adjustments can be incorporated by re-circulating part of the nitrified aerobic effluent to the anaerobic stage to achieve denitrification in conjunction with anaerobic digestion. In the two stage anaerobic-aerobic system, nitrogen levels adjustments can be achieved by partly recirculating the nitrified aerobic effluent to the secondary anaerobic stage. This treatment approach shows interesting perspectives for off-site sewage treatment aiming for agricultural reuse of the treated effluent.

By means of literature review, an outline of researched sequential anaerobic-aerobic treatment configurations is given in **Chapter Two**. The reviewed systems are classified according to the mode of growth in the aerobic system i.e. suspended growth versus attached growth. The feasibility and effectiveness of these systems are highlighted.

Chapter Three presents the results of merging the methanogenic and the denitrification processes in a lab scale UASB reactor operated with flocculent sludge, treating soluble synthetic wastewater consisting of acetate, propionate, glucose and synthetic nitrate.

Chapter Four presents the results of operating a pilot scale UASB reactor under integrated conditions, treating raw domestic wastewater. The sewage was complemented with synthetic nitrate, simulating the recirculation of nitrified effluent to the anaerobic stage. The performance of the UASB reactor regarding carbon and nitrogen removal, along with modifications taking place within the sludge is evaluated.

Chapter Five describes the results of operating lab scale EGSB reactor under integrated conditions of methanogensis and denitrification, treating pre-settled domestic wastewater supplemented with synthetic nitrate. The EGSB reactor is regarded as the secondary anaerobic stage of two stage anaerobic-aerobic sequential treatment. The EGSB reactor performance, along with the effects of integrated process condition on sludge biological activities and physical properties are evaluated. The general discussion and conclusions of the thesis is presented in **Chapter Six**.

Table 4: Summary of researched integrated reactors and the resulting performance

Anaerobic reactor configuration	Sludge bed	Applied wastewater	Source of nitrate/nitrite	Operational conditions	Removal efficiencies	comments	Reference
Upflow filter	Seeded with digested municipal sludge	Synthetic wastewater (methanol) with COD concentration ranging between 113-900 mg $COD.l^{-1}$	Synthetic nitrate as KNO_3	• OLR: 0.7-2 $gCOD.l^{-1}.d^{-1}$ NLR: 0.3 g NO_3-$N.l^{-1}.d^{-1}$ COD/N:2.2-6.5 • OLR: 1.3-5.3 NLR: 0.3-1.3 COD/N: 4 Temp. 25-35°C	• COD : 95-98% NO_3-N: 100% at COD/N >3.5 • COD: 95-98% NO_3-N: 100%	• Methane production occurred with COD/N ratio>4. • Denitrification was the main nitrate reduction pathway.	Hanaki and Polprasert (1989)
UASB	Granular sludge	Synthetic wastewater (VFAs were the sole carbon source) with COD concentration of 3 $gCOD.l^{-1}$	Synthetic nitrate as $NaNO_3$	OLR: 6.6 $gCOD.l^{-1}.d^{-1}$ NLR: 0.34 g NO_3-$N.l^{-1}.d^{-1}$	COD: 100% NO_3-N: 100%	• Methanisation percent was 56% • Denitrification was the main nitrate reduction pathway.	Hendriksen and Ahring (1996)
Hyprid upflow sludge bed filter (USBF)	Granular and flocculent sludge (50% w/w)	Pretreated fish canning industry effluent with COD concentration ranging between 2.9-4 $gCOD.l^{-1}$	Synthetic nitrate as KNO_3	OLR: 1-1.25 $gCOD.l^{-1}.d^{-1}$ NLR: 0.1& 0.22 g NO_3-$N.l^{-1}.d^{-1}$ TOC/NO_x: 2-3 Temp 37°C	COD: 80% NO_3-N: 100%	• Methanisation percentage was 11% with TOC/NO_3-N ratio of 3. • Denitrification was the main nitrate reduction pathway.	Mosquera-Corral *et al.* (2001)
Expanded granular sludge bed (EGSB)	Granular sludge	Synthetic wastewater (glucose) with COD concentration of 0.17-2.2 $gCOD.l^{-1}$	Synthetic nitrite as NO_2-N	OLR: 0.38-6.56 $gCOD.l^{-1}.d^{-1}$ NLR: 0.03-0.9 NO_2-$N.l^{-1}.d^{-1}$ COD/NO_2-N: 7-11	COD: 92-95% NO_2-N: 92-100%	• Denitrification and methanogenesis synchronously took place with COD/NO_2-N ratio >5.3. • Denitrification was the main nitrite reduction pathway as no dissimilatory reduction of nitrite to ammonium took place.	Zhang and Verstraete (2001).
UASB	Granular sludge	Synthetic wastewater Acetate 2 Nitrophenol Acetate + 4- Nitrophenol Acetate + 2,4-Dinitrophenol Acetate (4.3 $gCOD.l^{-1}$)+ nitrophenols (30$mg.l^{-1}$)	Synthetic nitrate as $NaNO_3$	OLR: 2 $gCOD.l^{-1}.d^{-1}$ NLR: 0.1-0.6 g NO_3-$N.l^{-1}.d^{-1}$ COD/NO_3-N: 3.3-20.8 Temp: 27°C	COD: 84-95% NO_3-N: 100% at COD/NO_3-N $\geq$ 4	• Nitrophenols degradation increase with decrease in COD/NO_3-N ratio, with maximum removal at COD/NO_3-N ratio of 5. • Denitrification and methanogenesis synchronously took place with COD/NO_3-N ratio ranging from 5 to 21.	Karim and Gupta (2003)

Table 4 (continued)

Anaerobic reactor configuration	Sludge bed	Applied wastewater	Source of nitrate/nitrite	Operational conditions	Removal efficiencies	comments	Reference
Upflow sludge bed (USB)	Granular sludge	Synthetic wastewater (acetate as the carbon source) with COD concentration of 4g $COD.l^{-1}$	Synthetic nitrate, Mixture of $NaNO_3$ and KNO_3	OLR 3.3 $gCOD.l^{-1}.d^{-1}$ NLR: 0.06-1.3 g NO_3-$N.l^{-1}..d^{-1}$ C/N: 2.5-60 Temp: 35°C	COD: 75-95% NO_3-N: 100% at COD/N>2.5	• Nitrate was reduced via DNRA and denitrification processes. • Denitrification and methanogenesis synchronously took place with C/N ratio ranging from 5 to 60.	Lee *et al*. (2004)
Upflow sludge bed (USB)	Granular sludge	Synthetic wastewater (acetate as the carbon source) with COD concentration of 2 and 6 $gCOD.l^{-1}$	Synthetic nitrate as $NaNO_3$	OLR: 7.5 & 22 $gCOD.l^{-1}.d^{-1}$ NLR: 0.2-7.5 g NO_3-$N.l^{-1}..d^{-1}$ COD/N 1,5,10 and 100. Temp: 28-30°C	COD: Over 95% at COD/N ratio of 1, 5 and 100 and 50% at COD/N ratio of 10. NO_3-N: 100% at COD/N ratio > 5	• For COD/N ratio ≤10, denitrification represents the main route of COD consumption. For COD/N ratio of 100, methanization percentage over 97% was achieved. • Denitrification was the main nitrate reduction pathway	Ruiz *et al*. (2006)
Anaerobic inverse fluidized bed reactor	Granular sludge	Synthetic wastewater (glucose as the carbon source) with COD concentration of 3 $gCOD.l^{-1}$	Synthetic nitrate as $NaNO_3$	OLR: 15 $gCOD.l^{-1}.d^{-1}$ NLR: 0.67&1.67 gNO_3-$N.l^{-1}..d^{-1}$ COD/NO_3 15 & 30	COD: 65-70% NO_3-N: Over 90%	• At COD/N ratio of 30 nitrate reduction via the DNRA is favored, while at COD/N ratio of 15 denitrification was favored. • Simultaneous methanization and nitrate reduction was observed at COD/N ratios of 15 and 30.	Alvarado-Lassman *et al*. (2006)

Table 5: Summary of the researched integrated systems and the resulting performance

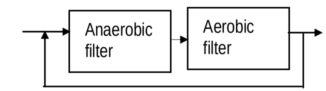
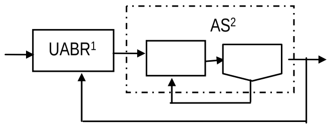
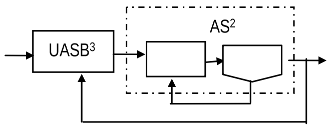
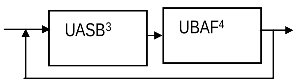
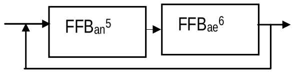
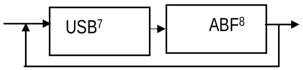
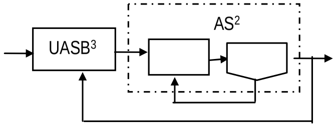
Process lay out	Applied wastewater	Operational conditions	Overall performance (removal efficincies)	Comments	Reference
	Synthetic wastewater (glucose) COD: 5.3 $gCOD.l^{-1}$ NH_4: 0.3 $gN.l^{-1}$	OLR_{ana}^*: 4.4 $gCOD.l^{-1}.d^{-1}$ NH_4-N load*: 0.25 $gN.l^{-1}.d^{-1}$ r♦= 1-5 Temp: 37°C	COD: over 97% TKN: 88-93%	• Methane gas production rate decreased from 1.04 under no recirculation conditions to 0.32 $m^3CH_4.m^{-3}.d^{-1}$ at recycle to influent ratio of 5.	Akunna *et al*. (1994/b)
	Leachate COD: (10-26) $gCOD.l^{-1}$ NH_4: (1-1.7) $gN.l^{-1}$ TKN: (1.7-1.9)$gN.l^{-1}$	OLR_{ana}^* :(2.6-19.2) $gCOD.l^{-1}.d^{-1}$ NH_4-N load*:(0.23-1.25) $gN.l^{-1}.d^{-1}$ r♦=3	COD: over 90%	• Denitrification and methanogenesis took place simultaneously in the UABR reactor, achieving denitrification efficiency of 99% • 80-90% of organics removed in the UABR was degraded by the methanogenesis.	Jeong-Hoon *et al*. (2001)
	Slaughter house wastewater COD: (0.6-3.7) $gCOD.l^{-1}$ TKN: (0.07-0.3) $gN.l^{-1}$	OLR_{ana}^*: (1.5-2.8) $gCOD.l^{-1}.d^{-1}$ TKN load* : (0.23-1.25) $g.N.l^{-1}.d^{-1}$ r♦: 1, 2 Temp: UASB at 35°C AS at 20°C	COD: 85% TN: 50% at r=1 70% at r=2	• Denitrification and methanogenesis took place simultaneously in the UASB reactor. • Dissimilatory reduction of NO_x to ammonium was insignificant even at high COD/NO_3 ratios.	Munez and Martinez (2001)
	Industrial wastewater COD: 10$gCOD.l^{-1}$ NH_4:0.2 $gN.l^{-1}$ TKN: 0.8 $gN.l^{-1}$	OLR_{ana}^*: (1.5-3) $gCOD.l^{-1}.d^{-1}$ TKN load*: (0.1-0.24) $g.N.l^{-1}.d^{-1}$ r♦: 2.4-6.7 Temp: 33°C	COD: over 95% TN: 50- 91%	• Methanogenic activity in the UASB reactor increased four times along the progress of the experiment. Methane percentage in the produced biogas was steady on 75%.	Lacalle *et al.* (2001)

Table 5 (Continued)

Process lay out	Applied wastewater	Operational conditions	Overall performance (removal efficincies)	Comments	Reference
	Slaughter house wastewater COD: 10 gCOD.l^{-1} TKN: 0.18 gN.l^{-1}	OLR♠: 0.4gCOD.l^{-1}.d^{-1} TKN load♠: 0.06gN.l^{-1}.d^{-1} r♦: 2-6 Temp: 20°C	COD:92% TKN: 95%	• Denitrification and methanogenesis took place simultaneously in the anaerobic reactor.	Del Pozo and Diez (2003)
	Domestic wastewater COD: 0..25-0.55 gCOD.l^{-1} NH_4: 0.02- 0.05 gN.l^{-1} TN: 0.04- 0.08 gN.l^{-1}	OLR♠: 0.6gCOD.l^{-1}.d^{-1} TN load♠: 0.1gN.l^{-1}.d^{-1} r♦: 1, 2 Temp: 20°C	COD: 91-96% TN: 70-73%	• Methanisation percentages at recycle to influent ratios of 1 and 2 were 64% and 51%, respectively. • Denitrification was the main nitrate reduction pathway.	Jun *et al*. (2005)
	Piggery wastewater COD: 2 gCOD.l^{-1} TKN: 0.4 gN.l^{-1}	OLR_{ana}*: 3 & 5 gCOD.l^{-1}.d^{-1} TKN load*: 0.6 &1 g.N.l^{-1}.d^{-1} r♦: 1,2 and 3 Temp: 30°C	COD:96-97% TN: 54-77%	• The UASB reactor was operated with granular sludge. • The abundance of denitrification process relative to the methanogenic process in the UASB reactor, increased with the increase of recycle to influent ratio.	Huang *et al.* (2007)

[1]Upflow anaerobic biofilm reactor (UABR).
[2]Activated sludge (AS).
[3]Upflow anaerobic sludge blanket reactor (UASB).
[4]Upflow biological anaerobic filter (UBAF).
[5]Anaerobic fixed film reactor (FFB_{an}).
[6]Aerobic fixed film reactor (FFB_{ae}).
[7] Upflow sludge blanket.
[8] Aerated bio-filter

* Loading rates based on the anaerobic reactor volume.
♦ Recycle to feed ratio.
♠ Loading rates based on the total volume i.e. volume of anaerobic+aerobic reactors

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Chapter 2

Sequential anaerobic-aerobic treatment for domestic wastewater- A review

Sequential anaerobic-aerobic treatment for domestic wastewater- A review

Kassab Gh., Halalsheh M., Klapwijk B., Fayyad M. and van Lier J.B.

Abstract
Introduction, consolidation and even standardization of expensive conventional aerobic systems for domestic wastewater treatment imposed significant financial constraints on the expansion of sanitary services including treatment in developing countries. A viable alternative is the sequential anaerobic-aerobic systems. If compared with the conventional aerobic technologies based on activated sludge processes, lower energy consumption and lower excess sludge production can be achieved with a high-rate anaerobic pretreatment step. Particularly with concentrated sewage, the energy benefit of applying anaerobic pre-treatment will become very significant. This study aims on putting the effectiveness of sequential systems for treatment of domestic wastewater on view, through displaying results presented in literature on the performance of these systems.

Keywords: anaerobic, aerobic, domestic wastewater, sewage, combined and post treatment.

1. Introduction

Conventional aerobic technologies based on activated sludge processes are dominantly applied for the treatment of domestic wastewater due to the high efficiency achieved, the possibility for nutrient removal and the high operational flexibility (Gavrilescu and Macoveanu, 1999). Nevertheless, the high capital and operational costs that coincide with the introduction of these technologies impose significant financial constraints on expanding the sewage treatment coverage, particularly in the low income countries. Therefore, to smooth the progress of sanitation services including conveyance and treatment, reliable, unsophisticated and cost effective treatment technologies should be adopted. Moreover, in countries of limited water resources like Jordan, treated wastewater is accounted for in the national water budget for mainly agriculture usage. Hence, extending sanitation services would result in the development of new urban wastewater ‘reuse’ schemes. Subsequently, agricultural use of treated sewage will stimulate the (peri)urban food production and will reduce the amounts of fresh water allocated to agriculture.

Anaerobic (pre-)treatment of domestic wastewater can serve a viable and cost-effective alternative (Lettinga, 1995) due to its relatively low construction and operational cost, operational simplicity, low production of excess sludge, production of energy in form of biogas and applicability in small and large scales. Moreover, owing to its compactness it can be located near or even inside the area of wastewater collection, stimulating (peri-)urban reuse. Since anaerobic treatment is a pre-treatment method, an adequate post treatment system is required to reach to local standards for discharge and/or agricultural reuse (Elmitwalli *et al*., 2003; Tawfik *et al*., 2005 and Chernicharo, 2006). Treatment of domestic wastewater in sequential anaerobic-aerobic processes exploits the advantages of the two systems in the most cost-effective set-up. In comparison with conventional aerobic technologies, the combined anaerobic-aerobic system consumes distinctly less energy, produces less excess sludge and is less complex in operation (van Haandel and Lettinga, 1994 and von Sperling and Chernicharo, 2005).

In the anaerobic system, solids are entrapped and organic matter is converted into biogas consisting mainly of methane and carbon dioxide. Organically bound nitrogen is converted to ammonium and sulfate is reduced to hydrogen sulfide. Sludge production in anaerobic systems is low and the excess sludge is already digested and can be directly dewatered, typically by drying beds. Regarding the microbiological indicators, coliform removal efficiency is low in anaerobic systems (Keller *et al*., 2004 and Pant and Mittal, 2007). However, helminth eggs are removed more effectively, particularly in the upflow anaerobic sludge blanket (UASB) reactor (Gerba, 2008). Anaerobic effluent's residual concentration of suspended solids and organic matter is polished in the aerobic system, along with ammonium oxidation to nitrite/nitrate via nitrification. Depending on the type of process and the operational conditions, aerobic treatment provides about 1-2 log pathogens removal (von Sperling and Cherincharo, 2005)

Nitrogen level adjustments can be incorporated in sequential anaerobic-aerobic system through partial recirculation of the nitrified aerobic effluent to the anaerobic reactor for denitrification to take place in conjunction with anaerobic digestion. In the integrated anaerobic reactor part of the organic carbon content in the raw wastewater serves as carbon source for denitrification and the rest is converted to methane. The proposed set-up is particularly of interest for concentrated wastewaters and/or lower ambient temperatures as under those conditions the volumetric design is not limited by the hydraulic loading rate (van Lier, 2008), i.e. there is already a volumetric spare capacity available to accommodate the recirculated flow.

To put the sequential anaerobic-aerobic treatment options on view and to state their feasibility and efficiency in domestic wastewater treatment, a desk review of the researched anaerobic-aerobic systems was performed with accentuation on high rate systems. The sequential systems were classified according to the mode of growth in the aerobic reactor i.e. suspended growth versus attached growth systems.

2. Sequential anaerobic- suspended growth aerobic systems

In the spectrum of suspended growth treatment processes, Activated Sludge (AS) is the most common configuration. By definition, the basic AS process consists of two basic units: *(1)* a reactor in which the microorganisms responsible for treatment are kept in suspension and aerated; *(2)* a liquid solids separation unit. An essential feature of the process is recirculation of part of solids removed from the liquid solids separation unit back to the aeration unit to maintain a high concentration of microorganism in the aeration tank.

A sequential system consisting of anaerobic baffled reactor (ABR) followed by an AS system was proposed by Garuti *et al.* (1992) for the treatment of domestic sewage. The proposed ABR comprises of: two anaerobic sludge blanket sections, an anoxic sludge blanket section and sludge trap section. Part of AS effluent is recycled to the anoxic section of the ABR to achieve denitrification. This configuration (Figure 1/a) with its ANaerobic, ANoxic and OXic sections (ANANOX) prevents biomass transfer, and thus it can be classified as a "separate biomass" system. Investigations on a pilot scale system resulted in achieving removal efficiencies of 90% for the total COD, 90% for the total suspended solids (TSS) and 81% for the total nitrogen (TN). Produced excess sludge was limited to 0.2 $KgTSS.KgCOD_{removed}^{-1}$. Once the successful operation of the ANANOX system was ascertained in pilot scale, its application on full scale took place (Garuti *et al*., 2001) with 30 m^3 ABR, 15 m^3 aeration tank and 32 m^3 secondary clarifier. At best operating conditions, total COD removal efficiency of 95% and TSS removal of 92% was achieved. The percentages of organic load removed by the anaerobic, anoxic and aerobic phases were 33%, 20% and 48%, respectively. It should be realized that this performance was obtained at a fairly low organic loading rate of 0.97 $KgCOD.m^{-3}.d^{-1}$ in the first anaerobic section. Apparently, the system is not optimized with regard to anaerobic phase efficiency. By means of nitrification, 80% conversion of ammonium was achieved in the aerobic stage and at a recycle to feed ratio of one, 58% of nitrate applied to the anoxic section was denitrified.

The feasibility of the ANANOX system for effective carbon and nitrogen removal from domestic wastewater has been evidently illustrated. However, ABRs encounter hydrodynamic limitations, which in turn impose constrains on achieving long sludge retention times (SRT) (van Lier *et al*., 2008). This makes the ANANOX system less favorable. However, its potentiality for simultaneous removal of carbon and nitrogen, owing to the fact that denitrifiers and methanogenesis can be cultivated separately, intensify its prospects. Nevertheless, under the condition that denitrifiers and methanogenesis are successfully integrated in a UASB reactor, which is capable of maintaining long SRT at relatively short HRT, an integrated system consisting of UASB reactor followed by an aerobic reactor, with partial recirculation of aerobic effluent to the UASB reactor to achieve denitrification, likely out competes the ANANOX system.

Combining an AS system with an UASB reactor was suggested by many researchers (e.g. von Sperling and Chernicharo, 1998). If compared with conventional AS system, less energy is consumed and much less excess sludge will be produced. For the sake of comparison von Sperling and Chernicharo (2005) presented designs of conventional AS system and combined UASB-AS system, using the same input data, i.e. low strength domestic wastewater with a BOD_5 amounting to 340$mg.l^{-1}$. Results have shown that preceding the AS system with a UASB reactor resulted in 60% reduction in sludge production and 40% reduction in aeration energy consumption. Furthermore, the UASB reactor acts as an influent equalization tank and it substitutes the primary clarifier. Excess aerobic sludge can be recycled to the UASB reactor for thickening and stabilization. Thus no special reactor for sludge stabilization is required (Souza and Foresti, 1996). However the extra load exerted on the UASB reactor by aerobic sludge recirculation should be taken into consideration in the UASB reactor design.

The configuration of UASB-AS system is also interesting because it highlights the possibility of upgrading existing AS plants by installing a UASB reactor before the aeration tank (Halalsheh and Wendland, 2008).

Von Sperling *et al*. (2001) reported results from 261 days of operation of a UASB reactor followed by an AS system under tropical conditions (Table 1). The UASB reactor had a volume of 416 l, feeding besides the AS system other post treatment lines, while the aeration tank had a volume of 23 l. The overall system (Figure 1/b) achieved at total HRT of 7.9 h, of which 4 h UASB, 2.8 h aeration tank and 1.1 h final clarifier, a total COD removal efficiency ranging between 85% and 93%. The percent of COD removed by the UASB reactor, relative to the COD removed by the overall system was in the range of 81% to 94%. An interesting point clarified in this study is the fact that in consequence of by-passing 20% of raw sewage to the AS, bulking problems systematically resulted while it was only occasional in case of applying UASB effluent only.

Under low to moderate temperatures ranging between 15-30°C, Motta *et al.* (2007) investigated the performance of UASB-AS system with recirculation of excess activated sludge to the UASB reactor for digestion (Table 1). UASB reactor of 396 l was operated at an HRT of 3.2 h. The influent had an average total COD of 341 $mg.l^{-1}$, bringing about an OLR of 2.6 $KgCOD.m^{-3}.d^{-1}$. The up flow velocity was maintained at 1 $m.h^{-1}$ through internal recirculation. The AS system was tested at an aeration chamber's HRTs of 2 and 3 h, which, in consequence of the UASB reactor performance, resulted in F/M ratios of 1.5 and 0.9 $KgCOD.KgVSS^{-1}.d^{-1}$, respectively. Keeping a constant flow rate, the aeration tank was operated at different HRT by volume adjustments. The tank volume used to achieve 2 h HRT was 240 l, and that used to achieve 3 h HRT was 360 l. The overall system (Figure 1/c) achieved 87% total COD removal efficiency and 92% TSS removal efficiency, regardless of the aeration chamber's HRT. Nevertheless, increasing the HRT from 2 to 3 h in the aeration chamber resulted in more stable operation including better particle flocculation and better sludge settling characteristics. The contribution of the UASB reactor to the total COD removed by the overall system was limited to 34%. Production of methane relative to removed COD was on average 0.1 $m^3.KgCOD_{removed}^{-1}$. The authors have stated that in spite of the UASB reactor low removal efficiencies, the performance of the overall system was satisfactory since the secondary effluent water quality requirements were met. Nevertheless, optimizing the operation of the UASB reactor through increasing the HRT and reducing the up flow velocity would reduce the load on the subsequent AS system resulting in less consumption of energy and less production of excess activated sludge.

Based on the outputs of the Brazilian Research Program on Basic Sanitation (PROSAB), von Sperling and Chernicharo (2005) presented the criteria and parameters for the design of an AS system acting as post treatment for UASB reactor effluents.

The applicability of the intermittent flow activated sludge system, i.e. sequencing batch reactor (SBR) system for post treatment of anaerobic effluents has also been investigated by many researchers (Table 1). The principal of an SBR consists of incorporation of all the units, processes and operations normally associated to the conventional activated sludge within a single tank. SBR systems have five steps carried out in sequence; (1) fill, (2) react/aeration, (3) settle, (4) draw and (5) idle. Since the flow of domestic sewage is continuous, at least two SBR tanks must be available. Sousa and Foresti (1996) have investigated the performance of combined system consisting of a UASB reactor followed by an SBR (Figure 1/d). In addition to pretreatment of applied wastewater, the UASB reactor was operated as digester for the SBRs' excess sludge. The researched setup was composed of 4 l UASB reactor followed by two identical 3.6 l SBRs. The system was operated at 30°C and fed with synthetic wastewater containing 422 $mgCOD.l^{-1}$. The UASB reactor was operated at an HRT of 4 h, resulting in an OLR of 2.5 $KgCOD.m^{-3}.d^{-1}$, while the SBRs were operated at 4 h cycles. The circulation of SBRs' excess sludge increased the OLR applied on the UASB reactor by only 0.7%.

Table 1: Sequential anaerobic-suspended growth aerobic systems.

System configuration	Scale and operational conditions							Overall performance Removal efficiency (%)		Anaerobic unit contribution* (%)		Reference
	Temp (°C)	Anaerobic unit			Aerobic unit							
		Volume (L)	HRT (h)	OLR (KgCOD.m^{-3}.d^{-1})	Volume (L)	HRT (h)		Total COD	TSS	Total COD	TSS	
UASB-AS	Tropical condition	416	4	2.3-4.4	23	2.8	F/M (KgCOD.Kg.VSS.d^{-1}) 0.6-0.9	85-93	85-95	81-94	84-94	von Sperling *et al.* (2001)
UASB-AS	15-30	396	3.2	2.6	240 and 360	2 and 3	F/M (KgCOD.Kg.VSS.d^{-1}) 1.5 and 0.9	87	92	34	36	Motta *et al.* (2007)
UASB-SBR	30	4	4	2.5	3.6	(Cycle time) 4	OLR (KgCOD.m^{-3}.d^{-1}) 0.4	95	96	91	89	Sousa and Foresti (1996)
UASB-SBR	21	150	6	2.1	90	(Cycle time) 24, 12, 6 and 4	-	91	84	72	79	Torres and Foresti (2001)
UASB-SBR	25	10	4	3.8	7	1 (aeration time) 0.5 (settling time)	Sludge ages (9, 11 and 15 days)	92	98	82	83	Guimaraes *et al.* (2003)
Anaerobic SBR - Aerobic SBR	28	14	(Cycle time) 12	0.9	14	(Cycle time) 12	F/M (KgCOD.Kg.VSS.d^{-1}) 0.08	94	-	75	-	Callado and Foresti (2001)
UASB-JLR		3	3,4,6 and 10	4.8, 3.6, 2.3 and 1.4	2.6	2.5, 3, 5 and 8	-	87-95	-	Over 90	-	Tai *et al.* (2004)

* Calculated as the percent of COD or TSS removed by the anaerobic unit relative to the COD or TSS removed by the overall system.

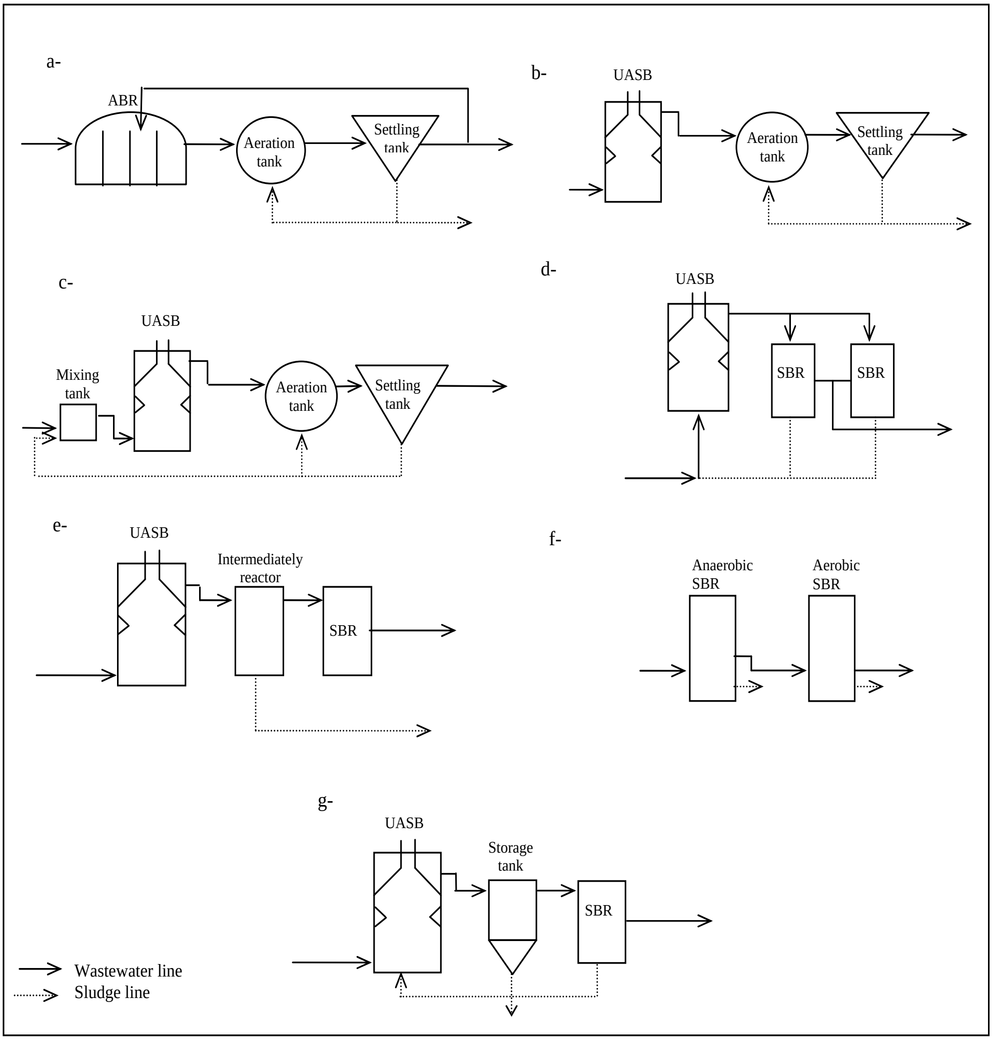

Figure 1: Schematic diagram of sequential anaerobic- suspended growth aerobic systems, researched by (a) Garuti *et al*. (1992 and 2001), *(b)* von Speriling *et al*. (2001), *(c)* Motta *et al.* (2007), *(d)* Sousa and Foresti (1996), *(e)* Torres and Foresti (2001), *(f*) Callado and Foresti *(2001)* and *(g)* Guimaraes *et al.* (2003).

The overall system achieved total COD removal efficiency up to 95%, of which 91% was mostly achieved by the UASB reactor. In relation to the removal of most of the organic matter by the UASB reactor, the authors have emphasized that the performance of the SBR is interrelated to the performance of the UASB reactor. Improved performance of the UASB reactor implies lower SBR performance since the fraction of persistent organic matter in UASB reactor effluent is expected to be higher. The system excess sludge fraction, which was discharged from the UASB reactor, corresponded to approximately 4% of the applied total COD. Taking into consideration that the applied wastewater was composed of meat extract (50%), sucrose and starch (32%), cellulose (8%) and vegetable oil (10%), in addition to the SBR excess sludge, the excess sludge fraction is lower than what is expected based on anaerobic yield calculations as suggested by Batstone *et al*. (2002), which then should be about 10%. The observed low value was not explained by the authors. Regarding the nitrification efficiency, the system converted 85% of the applied organic and ammonium nitrogen to the oxidized forms of nitrogen, mainly nitrate.

Torres and Foresti (2001) have also studied the UASB-SBR system for the treatment of domestic sewage with a median COD content of 570 $mg.l^{-1}$ and median TSS of 131 $mg.l^{-1}$ at an average temperature of 21°C. The experimental system (Figure 1/e) was composed of an UASB reactor of 150 l followed by an SBR reactor of 90 l with an intermediary tank to store the anaerobic effluent. The UASB reactor was operated at an HRT of 6 h, with a concomitant loading rate of 2.1 $KgCOD.m^{-3}.d^{-1}$, whereas the SBR was monitored for different cycle times of 24, 12, 6 and 4 h; corresponding to aeration times of 22, 10, 4 and 2 h. The overall system achieved total COD and TSS removal efficiencies up to 91% and 84%, respectively and showed to be independent from the applied aeration time in the SBR. The UASB reactor, which was inoculated with anaerobic granular sludge, was responsible for 72% and 79% of total organic matter and TSS removed in the overall system, respectively. Complete nitrification was achieved in the SBR reactor at aeration times $\geq$ 4 h. The overall system achieved total kjeldahl nitrogen (TKN) removal efficiencies ranging from 80% to 89%, at SBR aeration times ranging from 4 to 22 h. Phosphate removal, with efficiency amounting to 72%, was significant only at aeration time of 2 h. This condition was unfavorable to nitrification, wherein the ammonium removal was limited to 69%.

Two sequential SBRs forming a combined anaerobic-aerobic system were evaluated for treatment of domestic sewage (Callado and Foresti, 2001). The first reactor (anaerobic SBR) was meant to remove organic matter and convert organic nitrogen to ammonium. The second reactor (aerobic SBR) was operated under alternating aerobic and anoxic conditions to establish nitrification, denitrification and phosphate removal conditions. The researched setup (Figure 1/f) was composed of two bench reactors with useful volume of 14 l. The system was operated at 28°C and fed with synthetic wastewater containing 800 $mgCOD.l^{-1}$. The anaerobic SBR was inoculated with granular sludge whereas the aerobic SBR was not inoculated. Each of the two SBRs were operated at 12 h cycle time. The reaction time in the anaerobic SBR amounted to 9.5 h and that in the aerobic SBR was divided into three phases: aerobic (3 h), anoxic (3h) and aerobic (3.5 h) phase. The overall system achieved a total COD removal efficiency of 94% in which 75% was achieved by the anaerobic SBR. Complete nitrification was achieved in the aerobic SBR in addition to denitrification resulting in total nitrogen removal of 96%. Phosphate removal efficiency of 90% was achieved. However, denitrification and phosphate removal occurred only when sodium acetate was added at the beginning of the anoxic phase. This need of supplementary addition of an external carbon source is not convenient from the sustainability point of view. However, the external carbon source can be supplied from domestic solid waste (Chernicharo, 2006). Moreover, with concentrated sewage treatment, i.e. COD content ranges from 1500 to 2000 mg.l^{-1}; an external carbon source for denitrification is most likely not needed.

It should be taken into consideration that the anaerobic reactors employed in the former two studies were inoculated with granular sludge. Given that the presence of suspended solids in the raw wastewater can be detrimental to the maintenance of the good characteristics of granular sludge (Uemura and Harada, 2000). The significance of these results in the treatment of concentrated domestic wastewater, in which the TSS content can be as high as 500 mg.l^{-1} is low. Such concentrated sewage is produced in countries of limited per capita share of fresh water, like the Middle Eastern countries, or when septic tank and/or night soil additions are allowed to the centralized sewer grid such as often happens in African cities.

A UASB-SBR system with circulation of SBR excess sludge to the UASB reactor for digestion was also studied by Guimaraes *et al*. (2003) for the treatment of domestic sewage with an average COD of 587 mg.l^{-1}, at an average temperature of 25°C. The system (Figure 1/g) was composed of *(1)* 10 l UASB reactor, *(2)* 6 l storage/equalization/settling tank and *(3)* 7 l SBR reactor. The UASB reactor was operated at 4 h HRT, with a concomitant loading rate of 3.8 KgCOD.m^{-3}.d^{-1} and up flow velocity of 0.3 m.h^{-1}, whereas the SBR was operated at 1 h aeration time and 0.5 h settling time. The filling and decanting times were very short, i.e. < 1 min. At sludge ages of 9, 11 and 15 days, the overall system achieved total COD and TSS removal efficiencies of 92% and 98%, respectively, regardless of the SBR sludge age. The anaerobic pretreatment, i.e. the UASB reactor + storage tank, contributed to the total COD and TSS removal by 82% and 83%, respectively. For sludge ages of 11 and 15 days, the aeration period of 1 h was sufficient for the oxidation of both organic material and nitrification. However, at a sludge age of 9 days the aeration period of 1 h was insufficient for complete nitrification. Sludge formed in the SBR was characterized by a very good settleability with sludge volume index (SVI) values less than 80 ml.$gTSS^{-1}$. Thus, indicating that anaerobic pre-treatment has no negative effects on the settling properties of the aerobic sludge. This result is of particular importance, since good settleability of sludge in the aerobic post treatment system, means that the system can be operated at a high sludge concentration, and thus mitigating the reduction in the metabolic activity of both heterotrophic and autotrophic bacteria in aerobic post treatment systems compared to conventional aerobic systems (van Haandel and van der Lubbe, 2007).

A novel anaerobic-aerobic system consisting of a UASB reactor and a Jet Loop Reactor (JLR) was developed by Tai *et al*. (2004) for the treatment of domestic wastewater. The JLR is a sort of aeration tank that incorporates a recycle line with a venture and a draft tube, thus allowing the introduction of air drawn from the atmosphere and mixing of the reactor contents. The experimental setup was consisting of 3 l UASB reactor followed by 2.6 l JLR and conventional clarifier of 1.4 l. The system was operated on synthetic wastewater of 595 mgCOD.l^{-1}. The overall system achieved total COD removal efficiency ranging between 95 to 88% at (UASB, JLR) HRTs of (10, 8); (6, 5); (4, 3) and (3, 2.5). TKN removal efficiencies of 94% and 95% were achieved in the JLR at HRTs of 8 and 5 h equivalent to OLRs of 0.21 and 0.26 KgCOD.m^{-3}.d^{-1}. Further HRT reduction significantly deteriorated the nitrification efficiency.

3. Sequential anaerobic- attached growth aerobic systems

Attached growth systems can be classified into three general processes: (1) non-submerged attached growth processes, (2) submerged attached growth processes and (3) processes with suspended packing for attached growth.

Non-submerged attached growth processes

Among the non-submerged attached growth processes, trickling filter (TF) and rotating biological contactors (RBC) are the most widely used. The performance of combined systems comprising of a UASB reactor followed by either a TF or RBC have been studied in literature by many researchers (Table 2). Chernicharo and Nascimento (2001) studied the UASB-TF system (Figure 2/a) for treatment of domestic sewage of 520 $mgCOD.l^{-1}$ at average temperature of 26°C. Pilot scale UASB reactor of 416 l was operated at an average OLR of 3.8 $KgCOD.m^{-3}.d^{-1}$ and an HRT of 4 h with an upflow velocity of 1.4 $m.h^{-1}$. UASB reactor effluent was directed into a splitting box, in which flow with different rates, comprising different operational phases used to be pumped from it to the TF. The TF had a useful volume of 60 l and used to be operated as an intermediate rate filter at surface loading rates of 6.8 $m^3.m^{-2}.d^{-1}$ and as a high rate filter at surface loading rates of 10.3, 13.7, 17.1, 20.4 and 30.6 $m^3.m^{-2}.d^{-1}$. The highest removal efficiencies were obtained when operating the TF under surface loading rates varying from 6.8 to 17.1 $m^3.m^{-2}.d^{-1}$. Under these conditions the overall achieved COD removal efficiencies ranged between 80-88% with contributions from the UASB reactor ranging between 82-90%. This research was expanded by Pontes *et al.* (2003) who studied the incorporation of semi continuous recirculation of TF's excess sludge to the UASB reactor for digestion (Figure 2/b). UASB reactor of 416 l useful volume was operated at an HRT of 5.6 h corresponding to an OLR of 1.9 $KgCOD.m^{-3}.d^{-1}$. The TF which had a useful volume of 106 l was operated at a surface loading rate of 25 $m^3.m^{-2}.d^{-1}$. The flow of circulated sludge corresponded to 0.7% of the raw sewage flow introduced to the UASB reactor. Results have shown that circulating the excess aerobic sludge from the TF to the UASB reactor, did not affect the overall performance of the UASB reactor, nor did it contribute to a significant increase in COD and TSS concentration in the influent. The overall system achieved an average COD removal efficiency of 81%, with 93% contribution from the UASB reactor. Upon sludge circulation from the TF, the average specific sludge production in the UASB reactor insignificantly increased, i.e. 0.15 against 0.14 $KgTS.KgCOD_{removed}^{-1}$, whereas the average percentage of total volatile solids (TVS) slightly increased from 60% to 63%. Nevertheless, this percentage is lower than that found in the TF sludge which amounted 70%. The latter justifies the discharge of excess sludge only from the UASB reactor. Moreover, the sludge developed in the UASB reactor had excellent settling characteristics with an SVI < 50$ml.gTS^{-1}$.

Aiming at high loading rates, cost-efficiency and effective treatment of domestic sewage at low temperature, Elmittwalli *et al.* (2003) studied a system comprised of two anaerobic stages followed by an aerobic stage. The system consisted of an anaerobic filter (AF) of 60 l volume, anaerobic hybrid (AH) reactor of 65 l, followed by a TF (Figure 2/c). The AH reactor consisted of a granular sludge bed in the bottom, gas-solids separator in the middle and filter media in the top. The media of the TF were three vertical sheets of reticulated polyurethane foam with knobs. Each sheet had a height of

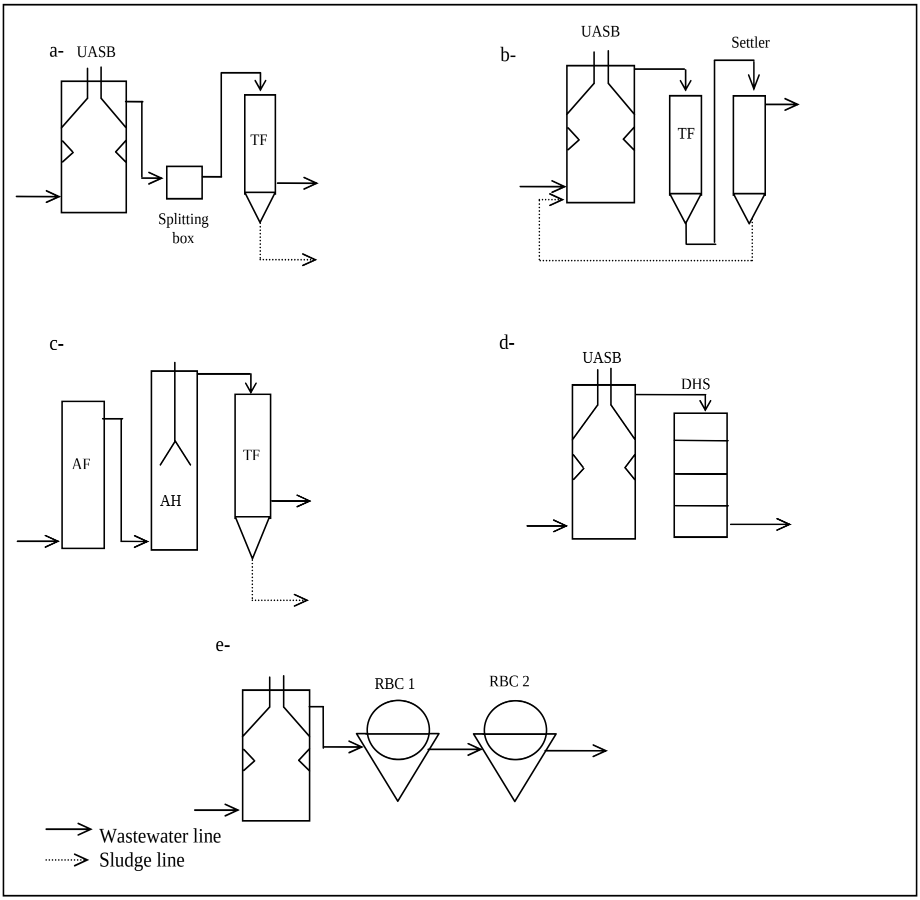

Figure 2: Schematic diagrams of sequential anaerobic- non-submerged attached growth aerobic systems, researched by; *(a)* Chernicharo and Nascimento (2001), (*b)* Pontes *et al*. (2003), *(c)* Elmitwalli *et al.* (2003), *(d)* Tawfik *et al*. (2006) and Tandukar *et al*. (2006) and *(e)* Castillo *et al.* (1997).

1.7 m and width of 0.06 m. At a controlled temperature of 13°C, the AF and the AH reactor were operated at HRTs of 3 and 6 h, respectively. The TF was operated at surface loading rates of 41, 15.4 and 2.6 $m^3.m^{-2}.d^{-1}$ at ambient temperatures ranging between 15 and 18 °C. At a surface loading rate of 41 $m^3.m^{-2}.d^{-1}$, the COD removal in the TF was limited; while at a surface loading rate of 15.4 and 2.6 $m^3.m^{-2}.d^{-1}$, high COD removal of 54%-57% was achieved without significant difference between the two applied surface loading rates. At the two lower surface loading rates, the overall system achieved 85% total COD removal efficiency in which the contribution of the AF+AH reactors amounted to approximately 80%. Moreover, the two step, AF+AH, system converted 46% of the influent COD to methane. The excess sludge produced in the AF+AH system, mainly originated from the AF reactor, amounted to 20-30% of the removed total COD in the AF+AH system. The amounts of excess sludge produced in the TF was indifferent for the two lower surface loading rates applied, reaching values of 0.61-0.69 $gVSS.gCOD_{removed}^{-1}$. The SVI of excess sludge produced in the AF was 39 $ml.gTSS^{-1}$ and that of excess sludge produced in the TF was 46 $ml.gTSS^{-1}$. The best nitrification efficiency was achieved at the surface loading rate of 2.6 $m^3.m^{-2}.d^{-1}$. However, only 60% of applied ammonium was removed and the amounts of produced nitrate were approximately equivalent to those of nitrite i.e. there was an incomplete nitrification. The removal of *E-coli* was limited in the anaerobic system, amounting to less than 1 log. The TF achieved 2 log removals without influence when the surface loading rate was decreased from 15.4 to 2.6 $m^3.m^{-2}.d^{-1}$.

Sousa and Chernicharo (2006) studied a compact on site treatment unit comprised of cylindrical tank, where half of the volume was used as modified septic tank and the other half is divided between AH reactor and TF. This AH reactor consisted of a UASB reactor in the bottom and an anaerobic filter in the top. The total volume of this unit corresponds to the volume of a septic tank, as determined according to the Brazilian directives. Raw sewage is fed through the bottom of the septic tank, flowing upwards to a lamella-sedimentation tank. Next, partly treated sewage is directed to the bottom of the AH reactor flowing upward to the top of the TF, where it is subjected to final polishing. In order to simulate flow patterns produced by a typical dwelling, the treatment unit was operated on an intermittent basis, with the flow rate peaking at different times of the day. The average HRT applied in the system was 24 h. COD removal efficiencies achieved by the system ranged between 77% and 80%. Low removal efficiencies were achieved by the modified septic tank and the TF, amounting to 40% and 30%, respectively. On the contrary, efficiencies achieved by the AH reactor ranged between 58% and 81%. The low removal efficiencies achieved by the TF can be attributed to the intermittent feeding pattern which resulted in biofilm desiccation and the fact that organic matter remained in the AH reactor effluent are more difficult to degrade. Sludge production rates for the septic tank and anaerobic hybrid reactor were approximately 0.24 and 0.13 $KgTSS.KgCOD^{-1}$, respectively.

The down flow hanging sponge (DHS) reactor is a novel biotower-TF system with polyurethane packing, developed for post treatment of UASB reactor effluents (Figure 2/d). The polyurethane packing material has a void space exceeding 90%, resulting in a significant increase in entrapped biomass and thus a longer SRT (Tandukar *et al*., 2006). The first generation of DHS reactor was the "cube type" DHS which exhibited good removal efficiency for carbonaceous and nitrogenous compounds (Agrawal *et al.*, 1997). Nevertheless, the impracticality of the cube-type resulted in the development of second generation DHS reactors: the "curtain-type" DHS. The UASB-curtain-type DHS achieved removal efficiencies of 84% and 61% for COD and ammonium nitrogen, respectively (Machdar *et al*., 2000). However, aiming on more feasible and more practical configurations the third (Tawfik *et al*., 2006/a) and fourth (Tandukar *et al*., 2006) generations were developed (Table 2). A combined UASB-third generation DHS system operated at average wastewater temperature of 15°C, achieved 90% removal for total COD and 86% removal for ammonium nitrogen. The contribution of the UASB reactor to the total COD removal was 67%. The UASB reactor had a volume of 155 l and was operated at an HRT of 8 h with an upflow velocity of 0.5 $m.h^{-1}$, corresponding to an OLR of 1.5 $KgCOD.m^{-3}.d^{-1}$. The DHS system had a capacity of 136 l and was operated at an HRT of 2.7 h, which in conjunction with UASB reactor performance, resulted in applied OLR of 1.6 $KgCOD.m^{-3}.d^{-1}$. The excess sludge produced in the UASB reactor, amounted to 0.2 $KgTSS.KgCOD_{removed}^{-1}$. The amounts of excess sludge produced in the DHS system was even lower reaching 0.09 $KgTSS.KgCOD_{removed}^{-1}$.

The combined UASB-fourth generation DHS system was studied under higher temperature conditions of 20-25°C, in which the UASB reactor had a volume of 1.15 m^3 and was operated at HRT of 6 h corresponding to OLR of 2.1 $KgCOD.m^{-3}.d^{-1}$. Based on average performance of the UASB reactor, the fourth generation DHS, which had a volume of 0.38 m^3, was operated at an OLR of 2.4 $KgCOD.m^{-3}.d^{-1}$ with 2 h HRT. The overall system achieved 91% removal of total COD applied, with 69% contribution from the UASB reactor. Nitrification efficiency was limited, achieving only 28% removal of ammonium nitrogen. The excess sludge produced from the UASB reactor was 0.11 $KgVSS.KgBOD_{removed}^{-1}$, while that from the DHS system was 0.12 $KgVSS.KgBOD_{removed}^{-1}$. Regarding the coliforms removal, 2.6 log and 3.45 log reductions were achieved in the third and fourth generation DHS reactors, respectively.

Tandukar *et al*. (2007) compared the performance of pilot scale UASB- second generation DHS system to that of an AS process for the treatment of the same municipal sewage. The study revealed that the performance of the UASB-DHS system was comparable to that of the AS system regarding organic removal efficiency. Regarding nitrogen removal efficiency, the UASB-DHS system performed inferiorly with total nitrogen removal efficiency of 56% compared to 72% for the AS system. Concerning pathogen removal, UASB-DHS system outperformed the AS system, with fecal coliform counts of 3.8×10^4 $MPN.100ml^{-1}$ in DHS effluent compared to 1.1×10^5 $MPN.100ml^{-1}$ in AS effluent. Interestingly, the volume of excess sludge produced in the UASB-DHS system was 15 times smaller than that from AS system.

TFs and DHS systems have high potentials in polishing effluents from anaerobic reactors particularly, UASB reactors. In addition, the operation of these systems is simpler and less expensive compared to AS systems. Therefore, sequential treatment systems consisting of a UASB reactor followed by TF or DHS system is highly recommended.

A combined system consisting of UASB-RBC reactors has also been proposed in literature for treatment of domestic wastewater. Castillo *et al*. (1997) have studied the potentials of UASB reactor followed by a two stage RBC (Figure 2/e) for domestic sewage treatment in coastal touristic areas under moderate climate conditions. Their argument on the suitability of anaerobic treatment lie in the fact that if the system is designed for local population under winter conditions, the increased tourists population in summer time is coped with by the increase in biodegradation rates taking place as a result of temperatures increase. UASB reactor of 750 l working volume, was operated at HRTs of 1.5, 3, 6 and 7.5 h. This scheme was followed in both summer (19°C) and winter (12°C) times. Part of the UASB effluent was introduced to the two stages RBC. The surface loading rate was set to be 0.17 $m^3.m^{-2}.d^{-1}$, whereas the applied OLRs ranged between 0.2-3.7 $gCOD.m^{-2}.d^{-1}$, depending on the wastewater characteristics and the HRT applied in the UASB reactor. The overall system achieved its best performance when the UASB reactor was operated at an HRT of 7.5 h in both summer and winter times. Wherein, the total COD removal efficiency achieved was approximately 95% and 91% under summer and winter conditions, respectively. The contribution of the UASB reactor amounted respectively to 74% and 60% under summer and winter conditions.

The similar removal efficiencies achieved in both seasons emphasized the concept of balancing the reduction in temperature by reduction in load. The applied surface loading rate of 0.17 $m^3.m^{-2}.d^{-1}$ didn't allow for nitrification to take place in both summer and winter times. Nevertheless, reducing the load to 0.06 $m^3.m^{-2}.d^{-1}$ and increasing the levels of dissolved oxygen in each stage to 4 $mg.l^{-1}$, resulted in achieving nitrification efficiency of 86%. The excess sludge produced from each stage of the RBC was ranging between 0.29 and 0.33 $KgVSS.KgBOD_{removed}^{-1}$, excess sludge produced in the UASB reactor was not measured.
The potentials of using RBC for post treatment of UASB reactor effluent have been extensively studied by Tawfik *et al*. (2002, 2005 and 2006/b). Single stage RBC of a working volume of 60 l and total effective surface area of 6.5 m^2 was employed in the study. The RBC was operated at a constant HRT of 2.5 h, with a surface loading rate of 0.09 $m^3.m^{-2}.d^{-1}$ at a temperature of 21°C. The RBC was operated at different OLRs due to variable performance of the UASB reactor, viz. OLRs of 20 and 15 $gCOD.m^{-2}.d^{-1}$, which resulted from operation of the UASB reactor at 15°C and 30°C, respectively. Although the removal efficiency of total COD at the two applied OLRs was not significantly different, i.e. 72% at an OLR of 15 $gCOD.m^{-2}.d^{-1}$ versus 63% at an OLR of 20 $gCOD.m^{-2}.d^{-1}$. The removal efficiency of ammonium nitrogen was significantly different, i.e. 64% at the lower applied load in which 86% occurred through nitrification versus 18% at the higher load. Regarding the *E-coli* removal, 1.2 log and 0.9 log reduction was achieved at the lower and higher applied loads, respectively. The performance of single stage versus two stages RBC system for post treatment of UASB reactor effluent at 12°C was evaluated as well. The single stage and two stages RBC system were operated at the same OLR of 26 $gCOD.m^{-2}.d^{-1}$ and at total HRT of 2.5 h, with a surface loading rate of 0.09 $m^3.m^{-2}.d^{-1}$, but at different flow rates. Results have shown that the total COD removal efficiency of the single stage and two stages system was limited to 65% and 77%, respectively. Moreover, nitrification was inadequate in both systems, most probably due to the fact that at the applied load the nitrifiers couldn't compete with the heterotrophs for space and oxygen. The *E.coli* reduction in the single and two stages RBC system was 0.9 log and 1.3 log, respectively.
The performance of the UASB-RBC system for the treatment of strong domestic sewage with a COD content of about 1500 $mg.l^{-1}$ was investigated within the scope of this dissertation (non published results). The treatment system was operated under ambient summer conditions (26°C) and consisted of a 1.4 m^3 UASB reactor followed by two stages RBC of 113 m^2 total effective surface area. In order to eliminate suspended bio film that is produced in the RBC reactor its effluent was introduced to a 0.1 m^3 settler operated at an HRT of 1.6 h. The UASB reactor was operated with an average OLR of 1.6 $KgCOD.m^{-3}.d^{-1}$ and an HRT of 23 h with an upflow velocity of 0.22 $m.h^{-1}$. The RBC was operated at HRT of 14 h, corresponding to a surface loading rate of 0.01 $m^3.m^{-2}.d^{-1}$ and based on the performance of the UASB reactor at an average OLR of 10 $gCOD.m^{-2}.d^{-1}$. The overall system achieved total COD removal efficiency of 95%, with contribution from the UASB reactor amounting to 56%. Complete ammonium removal was achieved in the RBC, of which 68% was found as nitrate and no accumulation of nitrite took place. The discrepancy between removed ammonium and produced nitrate might be attributed to ammonium assimilation and denitrification in the inner biofilm. The feasibility of the UASB-RBC system is clearly illustrated. The main advantages of

RBCs over TFs (Gray, 2005) are complete wetting of the medium, unlimited oxygen, no distribution problems, no recirculation required and excellent process control. Disadvantages include loss of treatment during power loss, frequent motor and bearing maintenance and problem of excessive film build up on discs after power failure resulting in damage and possible failure of the motor when restarted.

Submerged attached growth processes

Aerobic submerged attached growth processes include down flow packed bed reactors, up flow packed bed reactors and up flow fluidized bed reactors. The type and size of packing material affects the performance and operating characteristics of submerged attached growth processes. Designs differ by their packing configuration and inlet and outlet flow distribution and collection. As the biomass accumulates on the media the head loss across the reactor increases and may eventually become clocked. Thus, regular backwashing is required to remove the excessive film.

Goncalves *et al*. (1998) studied the association of a UASB reactor with submerged aerated up flow packed bed reactor termed as "submerged aerated biofilter- BF" (Table 2). UASB reactor of 46 l and BF of 6.3 l were employed in the study (Figure 3/a). The BF floating packing material consisted of S5 type polystyrene spheres with 3 mm diameter and 1200 $m^2.m^{-3}$ specific surface area. The UASB reactor was fed with raw wastewater of average COD content amounting to 460 $mg.l^{-1}$. The routine operation included sludge removal from the UASB reactor and BF backwash for excess biofilm removal. The backwashes were frequently preceded, at least once every 72 hours. Once steady state conditions were achieved the overall system was operated under two conditions. Firstly, The UASB reactor was operated at an HRT of 8 h corresponding to an OLR of 1.4 $KgCOD.m^{-3}.d^{-1}$, whereas the BF was operated at a surface loading rate of 0.73 $m^3.m^{-2}.h^{-1}$ and, in consequence of UASB reactor performance, on OLR of 4 $KgCOD.m^{-3}.d^{-1}$. Secondly, The UASB reactor was operated at an HRT of 6 h corresponding to OLR of 1.8 $KgCOD.m^{-3}.d^{-1}$, whereas the BF was operated at a surface loading rate of 0.97 $m^3.m^{-2}.h^{-1}$ and, in consequence of UASB performance, at an OLR of 4.9 $KgCOD.m^{-3}.d^{-1}$. The overall system achieved total COD removal efficiencies of 88% and 92% for the first and second operational conditions, respectively. The contribution from the UASB reactor was 83% irrespective of the operational conditions. Nitrification activities in the BF were satisfactory only during the start up phase when the applied hydraulic load was 0.36 $m^3.m^{-2}.h^{-1}$. Further increase in the loading limited the nitrification process. This is expected since at higher OLR the heterotrophic bacterium, which has a higher biomass yield, dominate the surface of the filter over nitrifying bacteria (Fdz-Polanco *et al*., 2000). Specific sludge production in the UASB reactor varied between 0.14 and 0.16 $KgTS.KgCOD_{removed}^{-1}$ while that of the BF amounted to 0.37 $KgTS.KgCOD_{removed}^{-1}$, measured during the second operational conditions. In conclusion, the authors have emphasized the viability of having an excellent quality final effluent through compact, efficient and low energy consumptions system.

Goncalves *et al*. (2002) investigated the performance of the UASB-BF configuration for the treatment of domestic sewage with continuous recirculation of BF excess sludge to the UASB reactor for thickening and digestion (Figure 3/b). Pilot scale plant composed of UASB reactor of 35 m^3 followed by BF of 12 m^3 volume was used for treatment of domestic sewage with average COD content of 523 $mg.l^{-1}$. The UASB reactor was operated at an HRT of 8 h corresponding to an average OLR of 1.6 $KgCOD.m^{-3}.d^{-1}$. The overall system achieved total COD removal efficiency of 85%. The contribution of the UASB reactor amounted to 76%. In consequence of aerobic sludge circulation, the organic load on the UASB reactor was increased by 3.7% and the system excess sludge fraction (discharged from the UASB reactor) corresponded to 6% of the applied total COD. Although the stabilization of aerobic sludge in the UASB reactor was very limited, obviously justified by the limited degree of hydrolysis, the thickening performance was rather satisfactory with UASB bottom sludge total solids concentration of 5%. Moreover, the VS/TS ratio of UASB reactor discharged sludge was 65%.

Upflow aerobic packed bed reactor, termed as aerated bio-filter (ABF) was investigated by Jun *et al*. (2005) for the treatment of domestic wastewater in association with a UASB reactor (Figure 3/c). The two reactors had a volume of 19 l and were operated at 20°C. The UASB reactor was operated at an HRT of 12 h corresponding to an OLR of 0.7 $KgCOD.m^{-3}.d^{-1}$ and in consequence of its performance the ABF was operated at an OLR of 0.13 $KgCOD.m^{-3}.d^{-1}$. Results have shown that the overall system achieved total COD removal efficiency of 93%. In which 88% was achieved by the UASB reactor. About 68% of ammonium applied to the aerobic bed reactor was converted and recovered as nitrate in the effluent.

A treatment system consisting of a UASB reactor followed by submerged aerated bio-filters is clearly capable of producing high quality effluents with respect to organic matter and suspended solids concentration. Nitrification is possible at low OLRs, although, due to the regular backwashing required for carbonaceous systems, generally on daily bases, it is normal to have separate reactors for nitrification. This in turn represents a serious drawback of the UASB-BF system, when nitrogen removal is required.

A combined system consisting of an upflow multi layer bioreactor (UMBR) and aerobic biofilm reactor was studied by Kwon *et al.* (2005). Pilot scale plant consisting of UMBR of 7.5 m^3 capacity and aerobic biofilm reactor of 9 m^3 capacity was operated for treatment of sewage with an average COD content of 483 $mg.l^{-1}$ and average TSS of 500 $mg.l^{-1}$, at a temperature varying between 12°C and 28°C. The UBMR is used as anaerobic, primary settling tank and thickener. The influent is fed uniformly by distributors at the bottom and overflowed supernatant at the top flows into the aerobic biofilm reactor. The distributor at the bottom of the UBMR is attached to a rotating shaft at the low revolution rate of 4-5 $m.min^{-1}$ tip speed. The UMBR is divided into sludge blanket layer, supernatant layer and thickening layer, the thickening layer below the distributor is used as sludge thickener. When the sludge blanket level detected by optical or ultrasonic sensor in the UMBR is higher than a set point, excess sludge in the thickening layer is automatically drained by a discharge pump. The aerobic biofilm reactor comprises a reaction zone packed with rope like fiber (5.5 m^3) and a clarification zone equipped with lamella separator (3.5 m^3). Nitrified effluent was recycled to the UMBR with internal recycle to feed ratio of 2. Detached biomass from the aerobic biofilm reactor was recycled to the UMBR as well. The HRT of the sludge blanket layer and supernatant layer were 3.5 h and 2.3 h, respectively. The aerobic biofilm reactor was operated at HRT of 6.4 h. Operation for 200 days, revealed total COD and TN removal efficiencies of 93% and 75%, respectively. The systems excess sludge was discharged only from the UMBR with a rate of 0.7 $KgTSS.KgCOD_{removed}^{-1}$, with VSS/TSS ratio of 0.55. SRT in the UMBR reactor amounted to 8.3 days. Such low SRT can be explained by the fact that the UMBR doesn't encompass phase separator which in turn adversely affect sludge retention and result in short SRT. However, the high stability of accumulated sludge, indicated by the low VSS/TSS ratio, reflects longer SRT, especially with temperatures as low as 12°C. Such discrepancy in results is not explained by the authors.

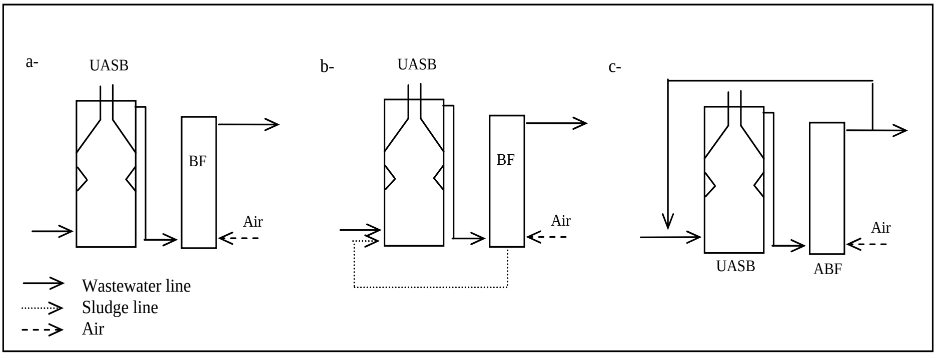

Figure 3: Schematic diagrams of sequential anaerobic- submerged attached growth aerobic systems, researched by *(a)* Goncalves *et al.* (1998), *(b)* Goncalves *et al*. (2002) and *(c)* Jun *et al*. (2005).

Mendonça *et al.* (2006) have studied the behavior of upflow expanded packed bed reactor with overlaid anaerobic and aerobic zones. The reactor that consist of 84 m^3 reaction zone and 75 m^3 sedimentation zone was employed for treatment of domestic wastewater with a COD content ranging between 171-1118 $mg.l^{-1}$ and TSS ranging between 99-544 $mg.l^{-1}$. The reaction zone of the reactor was packed with 0.4-2 mm granular activated carbon particles. Non-aerated and aerated volumes were formed through oxygen injection above the non-aerated area. The reactor was firstly operated under anaerobic conditions after inoculation with anaerobic granular sludge. After confirming operational stability under anaerobic conditions the anaerobic-aerobic system was initiated. Operating the reactor with an HRT of 5.4 h and an OLR of 4 $KgCOD.m^{-3}.d^{-1}$ resulted in COD and TSS removal efficiencies of 79% and 76%, respectively. Granulometry, nitrification kinetic and aerobic biofilm formation were presented in details in Mendonca (2004).

Processes with internal suspended packing for attached growth

In order to increase the biomass concentration in the aeration basin and thus to potentially reduce its volume, packing material of various types is placed to support biofilm growth. Bodik *et al*. (2002 and 2003) have investigated the feasibility of baffled reactors composed of anaerobic and aerobic compartments. The system consisted of a compartmentalized reactor (baffled reactor) in which the first compartment with 0.25 m^3 volume and the last compartment with 0.08 m^3 volume were operated as primary and secondary settling units. In between, four compartments filled with plastic material were operated anaerobically and a fifth one, filled with hanging polypropylene fibers, was operated aerobically. Each of the in between compartments had a volume of 0.1 m^3. The HRT in the anaerobic and aerobic parts were 15 and 4 h, respectively. The

temperature during the experimental period varied between 4.5 and 23°C. Total COD and SS removal efficiencies of 79-82% and 81-91% were, respectively, achieved. Nitrification process was observed during the whole year, even at the very low temperature periods. Nevertheless, it was not complete as shown by different ammonium concentrations in the effluent. As a result of circulating sludge produced in the secondary settling unit to the anaerobic part, partial denitrification was observed.
The feasibility of moving bed biofilm reactors (MBBR) for onsite treatment of two phase UASB-septic tanks effluent was investigated by Luostarinen *et al*. (2005 and 2006). The two phase UASB-septic tank treated a mixture of kitchen waste and black water (BWKW) at 20°C. The basic idea behind the MBBR is to develop a non-clogging biofilm reactor with high specific biofilm surface. A non-clogging biofilm is achieved by having the biofilm growing on small carrier elements that move along with the water in the reactor (Odegaard *et al*., 1994), i.e. kept in fluidized state. The movement is achieved by aeration during aerobic periods and by mechanical mixing during periods of no aeration. There is no need for backwash or recycling of biomass and the head loss though the reactor is insignificant. The average concentration of applied BWKW in the above cited studies was 1715 $mg.l^{-1}$. whereas by operating the first phase of the UASB-tank at an HRT of 3.5 days and the second phase at an HRT of 1.6 days, an OLR of 0.5 and 0.25 $KgCOD.m^{-3}.d^{-1}$ was imposed to the system. Two MBBRs with a volume of 2 l each were operated with intermittent aeration at an HRT of 2 days. One was continuously fed and the second was operated in a sequenced batch mode with 8 h cycles. Regardless of the MBBRs modes of operation the overall system removed over 96% of applied total COD, mostly achieved by the anaerobic pretreatment (95%) as it was meant for in the design. Complete nitrification was achieved in the MBBR as long as the DO concentration was at least 2-3.5 $mg.l^{-1}$ and an aeration phase of 1 h with continuous feeding or an aeration phase of 2-2.5 h with sequenced batch operation. Dentrification removed 50-60% of oxidized nitrogen despite being restricted by lack of carbon source. Thus, the authors emphasized on the fact that anaerobic pretreatment should be designed to retain sufficient carbon to ensure high nitrogen removal in the MBBR. Although, efficient removal of nitrogen and COD was achieved in the MBBR reactor regardless of the operation mode, continuous feeding was recommended given that it's simpler in operation.

4. Other combined processes

Other, and likely more expensive, systems such as the aerobic membrane biological reactors (Sheng-bing *et al*., 2003) and the dissolved air flotation (Reali *et al*., 2001; Pinto Filho *et al*., 2001; Odegaard, 2001 and Tessele *et al*., 2004) have been proposed in literature for treatment of domestic wastewater in conjunction with anaerobic reactors.

5. Treatment of concentrated sewage

The superiority of sequential anaerobic-aerobic systems over conventional aerobic systems is more profound with treatment of concentrated sewage. In countries of limited per capita share of water, like many of the Middle Eastern countries, the COD content of the produced sewage range between 1500 to 2000 $mg.l^{-1}$ (Halalsheh, 2002 and Mahmoud, 2002). Treatment of such concentrated sewage via conventional aerobic systems is highly expensive, especially with respect to operational costs. Pilot trials in

Jordan showed that employing single stage UASB reactor, operated with HRT of 24 h, for pre-treatment of domestic sewage with average COD content of 1500 $mg.l^{-1}$, results in 50-64% removal efficiency for the total COD (Halalsheh *et al*., 2005). These results consolidate the feasibility of UASB reactor for efficient and low cost pre-treatment of concentrated sewage, in temperate climate characterized by hot summer and cold winter. The UASB reactor was operated at such high HRT in order to achieve long SRT that would in turn provide sufficient hydrolytic and methanogenic activities under the low temperature conditions prevailing in winter.
In order to illustrate the advantages of sequential anaerobic-aerobic system over conventional aerobic system for the treatment of concentrated sewage, the same input data (Table 3) were used for the design of two treatment configurations; conventional AS system and AS system preceded by UASB reactor. This illustration is being made based on AS system owing to the fact that it is the most widely applied technology in the Middle East region. Each of the treatment configurations were designed for BOD removal only as well as for BOD removal with nitrification. To set a reference for comparison, the aeration tank within the two treatment scenarios was designed based on equivalent F/M ratios. For BOD removal with nitrification the F/M ratios were about 0.16 $KgBOD.KgVSS^{-1}.d^{-1}$, while for BOD removal only F/M ratios were about 0.3 $KgBOD.KgVSS^{-1}.d^{-1}$. The main parameters resulting from the two designs are listed in Table 4. The advantage of introducing UASB reactor ahead of AS system is obvious, mainly in terms of sludge production and energy consumption. Corresponding to UASB reactor performance under summer and winter conditions, the quantities of poorly digested sludge were respectively reduced by 66% and 56%, regardless of whether nitrification is taken into account or not. Power required for aeration was reduced by 40% for BOD removal and nitrification under summer condition and by 33% under winter condition. Reduction on required power for aeration is even more significant for BOD removal only, wherein 72% reduction can be attained under summer conditions and 60% under winter conditions. Moreover, if electric power is generated from the combustion of methane in an appropriate generator, the demand for aeration in the AS system can be covered. With respect to the total HRTs needed for treatment, i.e. total volumes needed, the outputs of the two scenarios were comparable. Aiming at BOD removal from concentrated sewage, Halalsheh and Wendland (2008) showed based on design calculation that at least 45% reduction in sludge production and 58% reduction in aeration energy consumption can be achieved by introducing anaerobic pre-treatment consisting of a UASB reactor followed by anaerobic filter ahead of an AS system. The UASB reactor was succeeded by the anaerobic filter so as to entrap the solids washed out with the UASB reactor effluent, since previous studies (Halalsheh *et al*., 2005) showed that 80% of effluent COD is in suspended form.
In view of the fact that aeration costs increase linearly with increasing organic loads, adopting the AS system for polishing of anaerobic effluent may not be the most "sustainable" option for concentrated sewage. Sequential anaerobic-aerobic systems reviewed in this paper, although mostly employed for treatment of low to moderate strength domestic wastewater, are promising options for concentrated sewage management. The assessment of different sewage treatment systems should be made based on their sustainability. Seghezzo *et al.* (2003) presented rationale, simple and comprehensive method to assess the sustainability of different sewage treatment

technologies. The method is based on four basic criteria; technical, environmental, social and economic aspects.

Generally, the UASB-DHS system and the UASB-TF are considered systems of high potentials for concentrated sewage treatment, owing to effectiveness and reliability in treatment, operational simplicity and relatively low investment and operational costs.

The potentials for nitrogen removal in sequential system consisting of UASB reactor followed by aerobic system, by partially circulating the nitrified aerobic effluent to the UASB reactor for denitrification to take place is considerable with concentrated sewage. Since in this case the UASB reactor is limited by the maximum OLR rather than the hydraulic load (van Lier, 2008), and thus the extra hydraulic load induced by the recirculation is endurable.

6. Conclusions

Literature results obtained so far clearly show the effectiveness of the sequential anaerobic-aerobic systems in domestic wastewater treatment and consolidate their advantage over the conventional aerobic systems. The significant contributions of anaerobic treatment in the sequential systems' overall performance, emphasize the discernment of reduction in energy consumption and excess sludge production upon replacing conventional aerobic systems by sequential anaerobic-aerobic systems. Further research and further developments on the application of combined systems under low to moderate temperature conditions are deemed necessary to overcome challenges on extensive applications.

Table 2: Sequential anaerobic-attached growth aerobic system

System configuration	Scale and operational conditions							Overall performance		Anaerobic unit contribution[1]		Reference
	Temp (°C)	Anaerobic unit			Aerobic unit			Removal efficiency (%)		(%)		
		Volume (L)	HRT (h)[2]	OLR ($KgCOD.m^{-3}.d^{-1}$)	Volume[2] (L)	HRT (h)[2]	SuLR[3] ($m^3.m^{-2}.d^{-1}$) and/or OLR ($KgCOD.m^{-3}.d^{-1}$)	Total COD	TSS	Total COD	TSS	
Sequential anaerobic- non submerged attached growth aerobic processes												
UASB-TF	26	416	4	3.8	60	12 - 2	SuLR, 6.8 - 17.1	80-88	80-89	82-90	76-91	Chernicharo and Nascimento (2001)
UASB-TF	26	416	5.6	1.9	106	1.5	SuLR, 25	81	89	93	99	Pontes *et al.* (2003)
AF-AH-TF	13, (AF+AH) 15-18, (TF)	60+65 (AF+AH)	(3+6) (AF +AH)	(4+1) (AF +AH)	-	2.5 and 15	SuLR, 15.4 and 2.6	85	-	80	-	Elmittwalli *et al.* (2003)
UASB-DHS	15	155	8	1.5	136	2.7	OLR, 1.6	90	94	67	87	Tawfik *et al.* (2006)
UASB-DHS	20-25	1150	6	2.1	380	2	OLR, 2.4	91	93	69	81	Tandukar *et al* (2006)
UASB- RBC	Summer, 19 Winter, 12	750	7.5 7.5	1.6 2.1	55	1.5 1.5	SuLR, 0.17 0.17	95 91	-	74 60	-	Castillo *et al.* (1997)
UASB-RBC	26	1400	23	1.6	$113m^2$ surface area	14	SuLR, 0.01	95	-	56	-	This dissertation

Table 2: Continued

System configuration	Scale and operational conditions							Overall performance		Anaerobic unit contribution[1]		Reference
	Temp (°C)	Anaerobic unit			Aerobic unit			Removal efficiency (%)		(%)		
		Volume (L)	HRT (h)[2]	OLR (KgCOD.m^{-3}.d^{-1})	Volume[2] (L)	HRT (h)[2]	SuLR[3] (m^3.m^{-2}.d^{-1}) and/or OLR (KgCOD.m^{-3}.d^{-1})	Total COD	TSS	Total COD	TSS	
Sequential anaerobic- submerged attached growth aerobic processes												
UASB-BF	-	46	8 and 6	1.4 and 1.8	6.3	0.23 and 0.17	SuLR, 0.73 and 0.97 OLR, 4 and 4.9	88 and 92	92 and 94	83	79 and 83	Concalves *et al.* (1998)
UASB-BF	-	35000	8	1.6	12000	0.3	SuLR, 2.2	85	88	76	72	Concalves *et al.* (2002)
UASB-ABF	20	19	12	0.7	19	12	OLR, 0.13	93	-	88	-	June *et al.* (2005)
UMBR-aerobic biofilm	12-28	7500	12.3	-	9000	6.4	-	93	96	95	96	Kown *et al.* (2002)
Processes with internal suspended packing for attached growth												
Baffled reactor	Summer (12-23) Winter (4.5-12.5)	400	15	-	100	4	-	79-82	81-91	-	-	Bodik *et al.* (2002 and 2003)
Two phase UASB/ septic tank-MBBR	20		Phase I, 3.5 days Phase II, 1.6 days	Phase I, 0.5 Phase II, 0.15	2	2 days	OLR, 0.03, 0.04 and 0.09	92	-	95	-	Luostarinen *et al.* (2005 and 2006)

[1] Calculated as the percent of COD or TSS removed by the anaerobic unit to the COD or TSS removed by the overall system.
[2] Unless stated otherwise.
[3] Surface loading rate.

Table 3: calculation bases for comparison between conventional AS system and sequential UASB- AS system.

Influent flow rate ($m^3.d^{-1}$)		1000
Characteristics of raw wastewater		
• Total COD ($mg.l^{-1}$)		1420
• BOD_5 ($mg.l^{-1}$)		900
• TSS ($mg.l^{-1}$)		352
• VSS($mg.l^{-1}$)		238
• TKN ($mg.l^{-1}$)		108
Primary clarifier performance		
• BOD_5 removal efficiency (%)		30
• TSS removal efficiency (%)		60
UASB reactor performance		
	Summer	*Winter*
• Total COD removal efficiency (%)	64	50
• BOD_5 removal efficiency (%)	80	72
• TSS removal efficiency (%)	62	55
• VSS removal efficiency (%)	65	62

Table 4: comparison between conventional AS system and sequential UASB- AS

	SRT (d)	HRT (h)		Power ($KW.h.d^{-1}$)		Sludge production ($KgTSS.d^{-1}$)	
Conventional AS system	aeration tank	Primary clarifier	Aeration tank		Aeration tank	Primary clarifier	Aeration tank
BOD_5 removal with nitrification	18	2	40		-1145	211	279
BOD_5 removal without nitrification	8	2	23		-633	211	352
UASB - AS system	Aeration tank	UASB	Aeration tank	UASB	Aeration tank	UASB	Aeration tank
AS designed for BOD removal with nitrification	10	24	14	+(1287** to 1322*)	-(690* to 765**)	133* to 158**	168* to 216**
AS designed for BOD removal without nitrification	5	24	8	+(1287** to 1332*)	-(180* to 254**)	133* to 158**	184* to 240**

* Corresponding to UASB reactor performance under summer conditions.

** Corresponding to UASB reactor performance under winter conditions.

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Chapter 3

Integration of methanogenesis and denitrification in UASB reactors operated with flocculent sludge

Integration of methanogenesis and denitrification in UASB reactors operated with flocculent sludge

Kassab Gh., Alnaimat H., Mateo-Sagast J., Halalsheh M., Klapwijk B., Fayyad M. and van Lier J.B.

Abstract Combined methanogenesis and denitrification in a single stage UASB reactor was researched by using an 18.7 l bench-scale reactor fed with synthetic wastewater, simulating the COD strength of domestic sewage. The reactor was inoculated with flocculent methanogenic sludge and initially operated under anaerobic conditions at an average organic loading rate (OLR) of 3.2 $kgCOD.m^{-3}.d^{-1}$, achieving COD removal efficiency of 93%. Hereafter, nitrate was added to the feed and the reactor was operated under integrated anaerobic and denitrifying conditions under approximately the same OLR and nitrate loading rate (NLR) of 0.15 $KgNO_3\text{-}N.m^{-3}.d^{-1}$. Removal efficiencies were 92% and 97%, for COD and nitrate-nitrogen, respectively. Denitrification was the main nitrate reduction pathway and no accumulation of nitrite took place. During the integrated operation, the methane content of the produced biogas only slightly decreased, i.e., from 80% to 75%, attributable to denitrifying activities. Using mass balances, an increase in the biomass production from 0.07 to 0.1 $gVS.gCOD_{removed}^{-1}$ was estimated, when shifting from anaerobic to integrated anaerobic-denitrifying conditions. In order to estimate the inhibitory effects of nitrate and its denitrification intermediates on methanogenesis, batch assays were inoculated with either methanogenic sludge or integrated sludge and were dosed with different nitrate concentrations, ranging between 10-300 $mgNO_3\text{-}N.l^{-1}$. Residual methanogenic activities of integrated sludge were in the range of 95-28%, while those of the strict methanogenic sludge were in the range of 74-17%.

Keywords Integrated, methanogenesis, denitrification, DNRA and UASB.

1. Introduction

Agricultural use of treated domestic wastewater contributes to mitigating water shortage problems in (semi)arid climate countries. Additionally, domestic wastewater contains significant amounts of micro- and macro-nutrients which farmers can beneficially use. However, non-controlled additions of too high levels of nutrients via ferti-irrigation induce vegetative growth and reduce crop production (Asano, 1996). This is particularly true for nitrogen, which is present in relatively high concentrations and which is a mobile compound that may affect the quality of underlying groundwater as well (Feigin *et al.*, 1991). Moreover, plants require varying amounts of nitrogen at different stages in the growth cycle. Therefore, the need for nitrogen on one hand and the limitations on its application on the other hand, calls for a flexible wastewater treatment system that is able to easily adjust the N-levels in the treated effluents.

Treatment of domestic wastewater in sequential anaerobic-aerobic processes has proven to be a sustainable technology, especially if effluent of high qualites is required. Moreover, nitrogen removal can be integrated by recycling part of nitrified aerobic effluent to the anaerobic stage for denitrification to take place. The process configuration of such a system- denominated as the integrated system- is shown in Figure 1. Denitrification and anaerobic digestion takes place simultaneously in the

anaerobic stage. In which part of the organic carbon content in the raw wastewater serves as carbon source for denitrification and the rest is converted to methane. In the aerobic stage, oxidation of ammonium (nitrification) takes place, in addition to degradation of the organics that weren't removed in the anaerobic stage.
However, the toxicity of nitrate and its denitrification intermediates on methanogenesis and especially on the main methanogenic metabolic pathway, i.e. acetate transformation to methane has been accentuated by many researchers (Akunna *et al*., 1994; Clarens *et al*., 1998 and Roy and Conrad, 1999). In addition, reduction of nitrate and nitrite is more energy yielding than methanogenesis, consequently carbon metabolism via denitrifications will be expected to dominate. Moreover, dissimilatory nitrate reduction to ammonium (DNRA) which is a step back in the nitrogen removal process is possible in anaerobic sludge (Akunna *et al*.,1993 and 1994). In order to sidestep the toxicity effects of nitrate and its denitrification intermediates on methane production, many researchers have studied the potentiality of simultaneous carbon and nitrogen removal in single stage reactor, in which spatial distinction of denitrification and methanogenic environments exists. More specifically, biofilm reactors and sludge bed reactors operated with granular sludge. However, it has been limitedly reported that denitrification and methanogenic processes can take place simultaneously in flocculent sludge based reactors (Akunna *et al*., 1992 and Jun *et al.*, 2005).

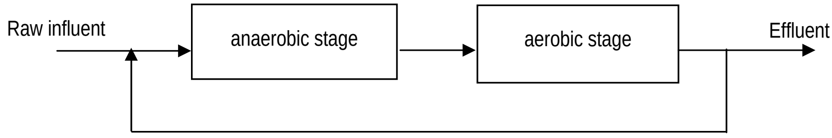

Figure 1: process configuration of the integrated system

The potentiality of the integrated system was mostly studied for wastewaters of high organic content such as industrial wastewater, slaughterhouse wastewater and landfill leachate (Lacalle *et al*. 2001; Mosquera-Corral *et al*. 2001 and Del Pozo and Diez, 2003). Simultaneous removal of carbon and nitrogen was achieved with COD and nitrogen removal efficiencies of 80-99% and 65-100%, respectively. Integrating denitrification and methanogenesis processes for municipal sewage treatment has not been recommended because carbon levels are usually too low to even support denitrification sufficiently (Hendriksen and Ahring, 1996). However, the process configuration of the integrated system implies that the organic content in the raw influent will be primarily used for nitrate reduction, whereas the excess carbon will go for methanogenesis. Moreover, a COD/TKN ratio of 5:1 in the influent wastewater is usually sufficient for effective nitrate reduction (Metcalf and Eddy, 2003). Therefore, for municipal wastewaters produced in countries of limited per capita share of freshwater such as the Middle East countries, which has a COD/TKN ratio in a range of 10-15, the integrated system is an adequate system for nitrogen and carbon removal.
Since the UASB reactor is the most widely and successfully used anaerobic technology for domestic wastewater treatment (von Sperling and Chernicharo, 2005), it is of interest to study the potentiality of integrating methanogenic and denitrification processes in a single stage UASB reactor operated with flocculent

sludge. The study was carried out with synthetic wastewater that simulates high strength domestic wastewater. The startup of the denitrification processes in the UASB reactor and the long term performance regarding carbon and nitrogen removal has been evaluated.
The effect of nitrate and its denitrification intermediates on methanogenesis have been extensively studied, but mostly with salt marsh sediments (Balderston and Payne, 1976), with pure cultures (Clarens *et al*., 1998 and Kluber and Conrad, 1998), with anoxic rice fields' soil (Roy and Conrad, 1999 and Chidthaisong and Conrad, 2000) and with fresh water sediments (Scholten and Stams, 1995). Studies with mixed anaerobic sludge (Akunna *et al*., 1992) are limited. Hence, in the present research we investigated concentration dependent effects of nitrate on methane production in flocculent methanogenic sludge using batch assays.

2. Materials and Methods

2.1 Continuous experiment

Experimental setup

The continuous experiment was carried out using a lab scale UASB reactor of 18.7 l working volume, 0.1 m in internal diameter and 2.3 m in height. Five sampling ports were placed along the height of the reactor. The reactor was operated on 25±2°C by circulating water into a jacket that surrounds the reactor through an external thermostat (Julabo 25). The reactor was inoculated with 10 l of flocculent anaerobic sludge, originating from a pilot-research UASB reactor operated on raw domestic wastewater collected from Abu-Nussier residential complex, located 7 Km to the north of Amman; the capital of Jordan. Inoculum sludge had total and volatile solids content of 80 $gTS.l^{-1}$ and 60 $gVS.l^{-1}$, respectively.
The reactor was fed with a synthetic medium of approximately 1.5 $gCOD.l^{-1}$. Each one liter contained 1.62 g tri-hydrous sodium acetate, 0.31 g sodium propionate and 0.47 g glucose. Each one liter contained as well buffering agents; K_2HPO_4 (2.8 g) and NaH_2PO_4 (1.2 g), yeast extract (0.05 g) and macro nutrients; NH_4Cl (170 mg), KH_2PO_4 (38 mg), $CaCl_2$ (6 mg) and $MgSO_4.7H_2O$ (12 mg). Trace elements consisting of $FeCl_3.4H_2O$, $CoCl_2.6H_2O$, $MnCl_2.4H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, HBO_3 $Na_2SeO_3.5H_2O$, $NiCl_2$, $(NH_4)_6Mo_7O_2.4H_2O$ and EDTA were added. Buffering agents, macro nutrients and trace elements were added according to van Lier (1995). Forty five liter of feed used to be prepared on daily basis and preserved at 4°C.

Operational conditions

The experimental work was carried out over two successive periods. In the first period, the UASB reactor was operated under strict methanogenic conditions for 61 days, wherein within the first 35 days the applied organic loading rate (OLR) was gradually increased to 3.2 $KgCOD.m^{-3}.d^{-1}$. In the second period, the UASB reactor was operated for 134 days under integrated denitrification and methanogenic conditions by incorporating synthetic nitrate in form of KNO_3 in the feed. The average concentration of nitrate in the feed was 68 $mg.l^{-1}$. The applied nitrate loading rate (NLR) ranged between 0.14-0.16 $KgNO_3\text{-}N.m^{-3}.d^{-1}$ and the OLR ranged between 3.2-3.8 $KgCOD.m^{-3}.d^{-1}$. Thus, the resulted $COD/NO_3\text{-}N$ ratio applied is approximately 23. This $COD/NO_3\text{-}N$ ratio has been chosen based on the fact that in countries of limited water resources like many of the Middle East countries the total COD content of generated domestic wastewater ranges between 1 and 2 $g.l^{-1}$ and the total nitrogen content ranges between 80 and 120 $mg.l^{-1}$. Employing the integrated system for

adjusting the total nitrogen content in such domestic sewage into values acceptable for agricultural reuse, i.e < 30 $mg.l^{-1}$ (Westcot and Ayers, 1985), implies operating the system at a recycle to influent ratio around 2. Accordingly, the COD/NO_3-N ratio at the anaerobic reactor input is expected to range between 20 and 28.
During these two periods the reactor was operated at an HRT of 11.2 h and an up flow velocity of 0.2 $m.h^{-1}$.

Analytical methods
Samples of the reactor influent and 24 h composite samples collected from the effluent were taken twice a week. The samples were analyzed for total COD (COD_t), paper filtered COD (COD_{pf}), soluble COD (COD_{sol}), volatile fatty acids (VFA) and ammonium, nitrate and nitrite concentration. Effluent samples were additionally analyzed for total suspended solids (TSS) and volatile suspended solids (VSS). Solid fractionation for COD_{pf} was performed using Whatmann 40 paper filters (pore size of 4.4 μm). COD_{sol} was determined using Orange Scientific membrane filter (0.45 μm). The different COD fractions, TSS and VSS were measured according the standard methods (APHA, 1995). VFA were measured by Varian 3300 gas chromatograph. Ammonium was tested by distillation method according to the standard methods (APHA, 1995). Nitrate concentrations were measured by ion chromatography with chemical suppression of eluent conductivity method (Dionex DX 3000) as described in the standard methods (APHA, 1998). Nitrite concentrations were measured with colorimetric method as described in the standard methods (APHA, 1998).
The biogas production was monitored daily using a wet gas meter. Gas composition including CH_4, N_2 and O_2 was tested by Philips Pye unicam pu 4500 gas chromatograph equipped with thermal conductivity detector. Carrier gas was helium and the temperatures of the injector, the column and the detector were 100, 70 and 200°C, respectively.
Sludge samples withdrawn from the bottom of the reactor were tested for total solids (TS) content and volatile solids (VS) content, according to the standard methods (APHA, 1995). Specific methanogenic activity (SMA) and specific denitrification activity (SDA) were measured for sludge samples withdrawn from the bottom of the reactor at the end of each operational period, i.e, strict methanogenic and integrated operational period. SMA was measured in 250 ml capacity serum bottles in duplicate. The bottles were inoculated with sludge in a concentration corresponding to 0.8 $gVS.l^{-1}$. Sodium acetate in a concentration corresponding to 1.5 $gCOD.l^{-1}$ was added in addition to macro and micro nutrients, phosphate buffer and yeast extract added according to van Lier (1995). Anaerobic conditions were established by flushing with nitrogen for 3-5 minutes. During the test the bottles were incubated at 35°C under static conditions. The amount of produced methane was measured with time using the liquid displacement test.
SDA was measured in 250 ml capacity serum bottles in duplicate. The bottles were inoculated with sludge in a concentration corresponding to 3 $gVS.l^{-1}$. Sodium acetate in a concentration corresponding to 150 $mgCOD.l^{-1}$ and potassium nitrate in a concentration corresponding to 30 $mgNO_3$-$N.l^{-1}$ were added. Additionally macro and micro nutrients, phosphate buffer and yeast extract were added according to van Lier (1995). After flushing with nitrogen for 3-5 minutes, the bottles were placed on a shaker at room temperature (27 °C) and nitrate concentration was monitored with time.

2.2 Batch experiments

The effect of denitrification process on methanogenic activity has been studied with sludge withdrawn from the bottom of the continuously operated UASB reactor at the end of the strict methanogenic and integrated periods. Residual methanogenic activities are corrected for the blank, i.e. the nitrate free control assay, based on the steepest part of the methane production curve. Sludge corresponding to a concentration of 0.5 $gVS.l^{-1}$ was added to twelve 250 ml capacity serum bottles. Sodium acetate corresponding to an initial concentration of 1.5 $gCOD.l^{-1}$ was added as substrate. Macro and micro nutrients, phosphate buffer and yeast extract were added according to van Lier (1995). Different amounts of a 1 $gNO_3\text{-}N.l^{-1}$ potassium nitrate stock solution were added to ten of these bottles to achieve COD/NO_3-N ratios of 150, 40, 20, 10 and 5 in duplicates. The remaining two bottles didn't receive any potassium nitrate and served as blanks. The total volume of 250 ml was achieved by adding distilled water. Anaerobic conditions were established by flushing each bottle with pure helium for five minutes. Incubation was done at 32°C with agitation. Total pressure of cumulative biogas inside each bottle was measured by digital manometer (SPER Scientific). Cumulative biogas composition was measured by Philips Pye Unicam pu 4500 gas chromatograph operated under the previously mentioned conditions.

3. Calculations

Nitrate recovery percentage as nitrogen gas was calculated based on equation 1. Where, $NO_3\text{-}N_{reduced}$ refers to the amounts of nitrate removed in the UASB reactor. Percentage of ammonium produced in the UASB reactor was calculated according to equation 2. COD used for nitrate reduction processes ($COD_{\text{oxidized (nitrate reduction)}}$) was calculated using equation 3. Methanisation percentage was calculated based on equation 4 where; COD_{CH_4} is the COD equivalent to the amounts of collected methane in the biogas, in addition to the amounts of soluble methane. COD mass balance was made based on one week average performance of the reactor according to equations 5 and 6.

1. $$\text{Nitrogen recovery\%} = \frac{N_{2\,\text{gas phase}}}{NO_3-N_{reduced}} \times 100$$

2. $$\text{Ammonium production \%} = \frac{(NH_4-N_{effluent}) - (NH_4-N_{influent})}{NH_4-N_{influent}}$$

3. $$COD_{\text{oxidized(nitrate reduction)}} = 2.86 \times (NO_3-N_{\text{reduced, denitrification}}) + 4.57 \times (NO_3-N_{\text{reduced, DNRA}})$$

Where; $NO_3\text{-}N_{\text{reduced, denitrification}}$ is the amount of nitrate reduced via the dentirification process and the 2.86 is the oxygen equivalent value of denitrified nitrate.

$NO_3\text{-}N_{\text{reduced, DNRA}}$ is the amount of nitrate reduced via the DNRA process and the 4.57 is the oxygen equivalent value of the nitrate reduced via the DNRA.

4. $$Methanogenesis\% = \frac{COD_{CH_4}}{COD_{t,\,influent}} \times 100$$

Mass balance

Strict methanogenic conditions

5. $$COD_{influent} = COD_{effluent} + COD_{CH_4} + COD_{biomass}$$

Integrated conditions

6. $$COD_{influent} = COD_{effluent} + COD_{CH_4} + COD_{oxidized\left(nitrate,\,reduction\right)} + COD_{biomass}$$

4. Results

4.1 performance of the reactor

The UASB reactor was firstly operated under strictly methanogenic conditions to set a reference for assessment of the effects of imposing the integrated process conditions on the performance of the UASB reactor. Achieved efficiencies of soluble COD and VFA removal under strict methanogenic conditions were 93±3% and 95±2%, respectively. Total COD removal efficiency was limited to 86±6% as a consequence of biomass washout. The average TSS and VSS of the effluent were 0.042 mg.l^{-1} and 0.017 mg.l^{-1}, respectively. Methane content of the produced biogas was 80% corresponding to a methane production of 0.34 $m^3CH_4.KgCOD_{t,removed}^{-1}$. Sludge withdrawn from the bottom of the reactor had a VS/TS ratio of 0.54 and showed SMA of 1.4 gCH_4-COD.$gVS^{-1}.d^{-1}$ and SDA of 2.1$mgNO_3$-N.$gVS^{-1}.h^{-1}$.

Two days upon launching the integrated process the effluent nitrate concentration was below the detection limit of 0.045 $mgNO_3$-N.l^{-1}. During the next 132 days of integrated operation, average nitrate removal efficiency was 97±0.8% and level of nitrite in effluent didn't exceed 0.003 $mgNO_2$-N.l^{-1}. Removal efficiency of soluble COD and VFAs dropped respectively to 76% and 70% on the first day of integrated operation. Nevertheless, in the second day of integrated operation, COD_{sol} and VFA removal efficiencies were recovered to 92%, each. Average values of 92±2% and 97±3% were respectively maintained for COD_{sol} and VFA removal efficiencies during the next 132 days of operation. The TSS and VSS of the effluent were 0.043 and 0.023 mg.l^{-1}, respectively. Nitrogen content in produced biogas increased from 2.6% (average content in produced biogas under strict methanogenic conditions) to 16% on the first day of integrated operation but dropped immediately on second day to 9%, and demonstrated an average of 11±1% during the next 132 days of integrated operation. Methane content on first day of integrated operation was reduced to 69%, recovered to 74% on second day and demonstrated an average value of 75±2% afterwards. This methane content corresponded to a production of 0.30 m^3CH_4.Kg $COD_{t,\,removed}^{-1}$.

Average ammonium production of 50% was detected during the integrated operation, i.e. increase in ammonium concentration from 10 $mgNH_4$-N.l^{-1} in the influent to 15 $mgNH_4$-N.l^{-1} in the effluent. Such increase in ammonium concentration indicates that nitrate was partly reduced to ammonium via DNRA process, expectedly, due to the presence of glucose in the influent (Akunna *et al*. 1993 and

Quevedo *et al*., 1996). To check if DNRA was stimulated by glucose as carbon source, glucose was eliminated from the influent for a week and its COD contribution was compensated by acetate and propionate. As a result ammonium concentration dropped from 9.8 $mgNH_4$-N.l^{-1} in the influent to 6.4 $mgNH_4$-N.l^{-1} in effluent, emphasizing the fact that nitrate was partly reduced via DNRA. The percentage of nitrate reduced via the DNRA process was estimated to be 12%, based on attributing the increment in effluent's ammonium concentration induced by glucose, i.e. from 6.4 to 15 $mgNH_4$-N.l^{-1}, to the DNRA process.
Sludge withdrawn from the bottom of the reactor had a VS/TS ratio of 0.51 and compared to strict methanogenic sludge it showed elevated methanogenic and denitrification activities. The SMA and SDA were 1.8 gCH_4-COD.$gVS^{-1}.d^{-1}$ and 4.2 $mgNO_3$-N.$gVS^{-1}.h^{-1}$, respectively.

4.2 *Mass balance over the reactor*
Table 1 shows a five days average distribution of nitrogen and total COD in influent and effluent streams under integrated and strict methanogenic conditions. Methanogenesis percentage decreased from 83% prevailing under strict methanogenic conditions to 67% under integrated conditions. According to the mass balance, 9% and 14% of the total COD fed has been employed in biomass growth under operation of strict methanogenic and integrated conditions, respectively. These values correspond to biomass yields of 0.07 and 0.10 $gVS.gCOD_{removed}^{-1}$ based on biomass composition of $C_5H_7NO_2$. Assuming that the ratio of biomass production/ methane gas production under anaerobic condition would still be prevailing under anoxic conditions; the yield for the nitrate reduction process would be 0.35 $gCOD_{biomass}$.$gCOD_{removed}^{-1}$. This yield is calculated based on the estimated nitrate reduction of 88% via denitrification pathway and 12% via DNRA pathway. In view of the fact that 12% of nitrate was reduced via the DNRA pathway, the achieved nitrogen recovery percentage of 96% is higher than expected. This is most probably due to air intrusion during sampling and/or analysis.

4.3 Recovery of methanogens
For a sustainable agricultural use of treated effluents a flexible wastewater treatment system is required, with the ability to periodically remove the excess nitrogen from the wastewater. For this reason, the application of nitrate was stopped by the end of the experiment to investigate the performance of the reactor immediately after switching to complete anaerobic conditions. Within a day after the switch, the soluble COD removal increased from 93.6% (average of integrated operation week before) to 96%, maintaining an average removal of 94% within the following week. Gas production on the first day increased from 31 $l.d^{-1}$, with a methane content of 76%, to 37 $l.d^{-1}$, with methane content of 81%. Hereafter, the average gas production was 36 $l.d^{-1}$, with an average methane content of 80%.

4.4 Batch experiments
Inhibitory effects of nitrate and its denitrification intermediates on methane production were studied for five different concentrations using strict methanogenic and integrated flocculent sludge as inoculum (Figure 2). Residual methanogenic activities for the five applied nitrate concentration are summarized in Figure 3, showing that residual activities decrease significantly with increasing nitrate concentration for both types of incubated sludge ($p<0.05$). Moreover, residual activities of integrated sludge are also significantly higher than strict methanogenic

Table 1: COD and nitrogen mass balances for the strict methanogenic and integrated operational periods

	Strict methanogenic conditions	*Integrated conditions*
COD mass balance		
$COD_{influent}$ ($g.d^{-1}$)	61.8	71.5
$COD_{effluent}$ ($g.d^{-1}$)	4.9	5.7
$COD_{methanised}$ ($g.d^{-1}$)	51.2	48.0
Methanogenesis%	83	67
$COD_{nitrate\ reduction}$ ($g.d^{-1}$)		8.3
$COD_{biomass}$ ($g.d^{-1}$)	5.6	9.6
Anaerobic ($g.d^{-1}$)	5.6	5.2 (=48*5.6/51.2)
Nitrate reduction ($g.d^{-1}$)	0.0	4.4 (=9.6-5.2)
yield $_{nitrate\ reduction}$ ($gCOD.gCOD^{-1}$)		0.35 (=4.4/ (8.3+4.4)
Nitrogen mass balance		
NO_3-$N_{influent}$ ($g.d^{-1}$)	-	2.8
NO_3-$N_{effluent}$ ($g.d^{-1}$)	-	0.08
NO_2-$N_{influent}$ ($g.d^{-1}$)	-	2.1×10^{-4}
NO_2-$N_{effluent}$ ($g.d^{-1}$)	-	5.2×10^{-4}
NH_4-$N_{influent}$ ($g.d^{-1}$)	0.4	0.4
NH_4-$N_{effluent}$ ($g.d^{-1}$)	0.26	0.6
N_2 ($g.d^{-1}$)	0.7	2.6
Recovery%	-	96%

sludge ($p<0.05$), especially for nitrate concentrations less than 75 $mg.l^{-1}$. In the batches incubated with strict methanogenic sludge, about 88%-99% of added nitrate-nitrogen was recovered as nitrogen gas. Unfortunately, in the batches incubated with integrated sludge, the calculated recovery of nitrate-nitrogen as nitrogen gas was overestimated, most probably due to air intrusion.

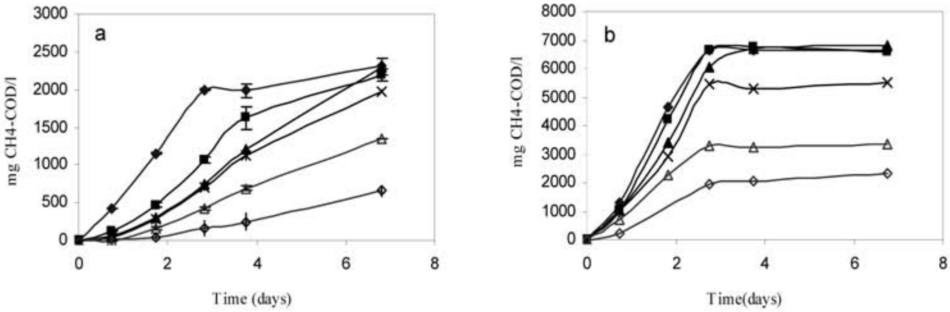

Figure 2: Methane production in batches inoculated with strict methanogenic sludge (a) and integrated sludge (b) after addition of different concentrations of nitrate: ♦ blank, ■ 10, ▲ 37.5, × 75, Δ 150 and ◊ 300 mg NO_3-$N.l^{-1}$.

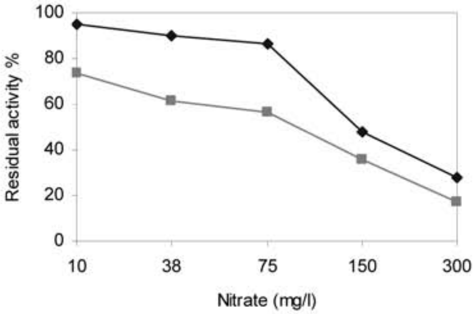

Figure 3: Residual activities with different nitrate concentrations for strict methanogenic sludge (■) and integrated sludge (♦).

5. Discussions

Results of our present research clearly demonstrate the potentials of combing anaerobic pre-treatment of domestic wastewaters with denitrification processes in a single stage reactor system. Furthermore, on-off switching of the integrated mode versus strict methanogenic conditions illustrates the flexibility of the system for actual field conditions. Our results show that treatment of low strength wastewater simulating the strength of Middle East domestic wastewater in a UASB reactor operated with flocculent sludge at average COD/NO_3-N ratio of 23, resulted in usage of 18% of the applied COD in nitrate reduction processes. COD removed and not consumed in nitrate reduction processes was completely converted to methane. Relative to the operation under strictly methanogenic conditions, methanogenesis of applied COD was reduced by only 20% as a consequence of imposing denitrification processes to the system. The latter result is in accordance with the results presented by Jun *et al.* (2005) who studied the removal of carbon and nitrogen from domestic sewage in integrated system composed of upflow sludge blanket (USB) reactor and aerate bio-filter (ABF). Based on their results, the reduction in methanogenesis relative to operation without recirculation amounted to 13% and 30% at COD/NO_3-N ratios of 38 and 20 resulting from recycle to raw influent ratios of 1 and 2, respectively.

Denitrification was the main nitrate reduction pathway. However, the presence of glucose in the feed resulted in the reduction of 12% of applied nitrate-nitrogen via DNRA. Akunna *et al.* (1994) found that 18% of reduced nitrate were found as ammonium in batches inoculated with anaerobic sludge using glucose as substrate at COD/NO_3-N ratio of 16.

Although the reactor from which the seed was originally taken received no nitrate, the strictly methanogenic sludge possessed denitrification activity that enabled the immediate initiation of the denitrification process. Indicating that if adjustment of nitrogen level in treated effluents is not needed and the partial recirculation of nitrified effluent to the anaerobic reactor was ceased, anaerobic sludge can still preserve its denitrification activity for the times when adjustments are needed again. The presence of denitrifying activity in methanogenic sludge was reported by many authors (e.g. Hendriksen and Ahring *et al.*, 1996; Mosquera-Corral *et al.,* 2001 and Akunna *et al.*, 1993).

As a consequence of denitrification processes, the total biomass yield under integrated conditions was increased by 43% in comparison with strict methanogenic conditions. Nevertheless, growth of denitrifiers within the flocculent anaerobic sludge didn’t result in increased biomass washout as shown by steadiness of effluent TSS content. This illustrates that integrating denitrification in flocculent anaerobic sludge doesn’t encounter sludge detainment problems as it is observed with integrating denitrification processes in granular anaerobic sludge (Hendriksen and Ahring, 1996; Ruiz *et al*., 2006 and Lee *et al*., 2004)
After incubating methanogenic and integrated sludges with nitrate in batch cultures, integrated sludge preserved higher methanogenic activities, illustrating the possibility of methanogenic sludge adaptation to the toxic effects of nitrate and its denitrification intermediates. Likely, a substantial fraction of the methanogenic biomass is shielded from the oxidized N-compounds in the interiors of the flocculent biofilms. Preservation of methanogenic activity in the integrated sludge was also observed in the continuous flow UASB experiments. Interestingly, the 20% reduction in applied COD methanogenesis coincided with the 18% employment of applied COD in the nitrate reduction processes. Apparently, the reduction in methanogenesis can be ascribed to reduction of available COD for methanogenesis rather than to toxic effects of nitrate and its denitrification intermediates.

6. Conclusions

- The present results illustrate the high potentiality of integrating denitrification and methanogenesis in mixed anaerobic sludge systems inoculated with flocculent sludge developed in UASB reactor.
- Operation of a UASB reactor under integrated conditions of denitrification and methanogenic processes at COD/NO_3-N ratio of 23 resulted in using 18% of applied COD in nitrate reduction processes and excess carbon, which was not used in nitrate reduction, was converted to methane.
- Methane production in the UASB reactor was not distressed by the denitrification process inhibitory effects, as the reduction in methanogenesis after launching the integrated process coincides with the reduction of COD available for methane production.
- Denitrification was the main nitrate reduction pathway. Yet, 12% of applied nitrate nitrogen was reduced via DNRA, likely for the reason that glucose was a constituent in the carbon substrate medium.
- Methane content in the biogas was 75%, enabling its use as energy source.
- The high residual methanogenic activities of integrated sludge compared to those of strict methanogenic sludge after incubation with nitrate in batch cultures, display the possibility of methanogenic sludge adaptation to inhibitory effects of nitrate and its denitrification intermediates whenever such effects are prevailing in the integrated reactor system.

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Chapter 4

Evaluation of UASB reactor performance for simultaneous removal of carbon and nitrogen from domestic wastewater

Evaluation of UASB reactor performance for simultaneous removal of carbon and nitrogen from domestic wastewater

Kassab Gh., Gonzalez P., Halalsheh M., Klapwijk B., Fayyad M. and van Lier J.B.

Abstract the integration of denitrification and methanogenic processes was studied in a single stage UASB reactor for the purpose of simultaneous removal of carbon and nitrogen from domestic wastewater. In order to set a reference for assessing the impacts of integrated process operation, the UASB reactor was operated firstly under strictly methanogenic conditions. The applied organic loading rate (OLR) ranged between 1.1- 2.2 $KgCOD_t.m^{-3}.d^{-1}$, achieving average total COD removal efficiency of about 71%. Secondly, synthetic nitrate in form of KNO_3 was included in the feed resulting in nitrate loading rate of 0.05 $KgNO_3$-$N.m^{-3}.d^{-1}$. The applied OLR in the second phase ranged between 1- 1.9 $KgCOD_t.m^{-3}.d^{-1}$. Nitrate was completely removed and average total COD removal efficiency of 68% was achieved. However, nitrate wasn't totally reduced via denitrification, as 33% of applied nitrate was reduced via dissimilatory nitrate reduction to ammonium. Compared to operation under strictly methanogenic conditions, integrated process operation didn't affect methanogenesis. This can be attributed to the higher degree of hydrolysis prevailing under integrated process conditions and the preservation of sludge SMA. Additionally, integrated process operation didn't affect the settleability or dewaterability features of the retained sludge.

Keywords: Denitrification, methanogenic, DNRA, domestic wastewater, flocculent sludge, integrated, UASB.

1. Introduction

Treatment of domestic wastewaters in consecutive anaerobic-aerobic processes shows interesting perspectives as a sustainable treatment approach (von Sperling and Chernicharo, 2005). Recent research indicates that this is not only the case under tropical conditions whereas temperatures are high year around, but also under cold and moderate climate conditions, characterized by large daily and/or seasonal temperature fluctuations (Elmitwalli *et al.,* 1999, 2000 and 2002 (a+b); Mahmoud *et al*., 2004 and Halalsheh *et al*., 2005). Nitrogen removal can be incorporated by recycling part of the nitrified aerobic effluent to the anaerobic unit to achieve denitrification (Figure 1). In this way, denitrification and anaerobic digestion takes place simultaneously in the anaerobic stage, wherein part of the organic carbon content in the raw wastewater serves as carbon source for the denitrification process and the rest is converted to methane. Removal of residual organics takes place in the aerobic stage in addition to the oxidation of ammonium to nitrate/nitrite (nitrification).

The above approach has been widely researched for strong wastewaters such as fish canning industry effluent, landfill leachate, slaughterhouse wastewater and industrial wastewater (Mosquera- Corral *et al*., 2001; Jeong- Hoon *et al*., 2001; Múñez and Martinez, 2001 and Lacalle *et al*., 2001). Simultaneous removal of carbon and nitrogen was achieved with COD and N-removal efficiencies of 80-99% and 65-100%, respectively. Nevertheless, up to our knowledge the integration of denitrification and methanogenesis in anaerobic reactors has been limitedly investigated for low strength wastewaters, such as domestic wastewater. Jun *et al*. (2005) investigated the feasibility of integrating carbon and nitrogen removal in upflow sludge blanket (USB) reactor by recycling aerated bio-filter's (ABF) nitrified effluent to the USB reactor for the treatment of domestic sewage, with an average COD content of 350 $mg.l^{-1}$ and total nitrogen (TN) concentration of 51 $mg.l^{-1}$. The USB-ABF system achieved total COD and TN removal efficiencies of 98% and over 70%, respectively.

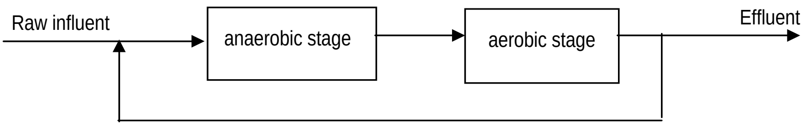

Figure 1: process configuration of sequential anaerobic- aerobic treatment with simultaneous removal of carbon and nitrogen in the anaerobic stage.

The main concerns associated with this strategy are the inhibitory effects of nitrate and its denitrification intermediates on methane production (Akunna *et al*., 1994/a and Chen and Lin, 1993) and the occurrence of dissimilatory nitrate reduction to ammonium (DNRA), which represents a step backwards in the nitrogen removal process (Akunna *et al*., 1993). Additionally, reduction of nitrate and nitrite is more energy yielding than methanogenesis, consequently carbon metabolism via denitrification will be expected to dominate eventually. However, the last concern is not noteworthy in domestic wastewater since the COD/N ratio in raw domestic wastewater is usually higher than the optimum COD/N ratio needed for effective nitrate reduction.

In order to sidestep the inhibitory affects of nitrate and its denitrification intermediates on methanogenesis, integrated concept was mostly studied in systems wherein spatial distinct of denitrification and methanogenic environments exist. More specifically, biofilm systems (Hanaki and Polprasert, 1989 and Akunna *et al*., 1994/b) and systems operated with granular sludge (Hendriksen and Ahring, 1996 and Lee *et al*., 2004). However, using a CSTR inoculated with anaerobic sludge of flocculent nature and fed with synthetic wastewater containing glucose as the only source of carbon, Akunna *et al*. (1992) showed that at COD/NO_x-N ratios ranging between 8.86 and 53, denitrification and methane production occurred simultaneously. Thus, pointing out that integrated concept can be applied in sludge bed systems operated with flocculent sludge. Accordingly, treatment configuration presented in figure 1 is applicable for domestic wastewater treatment, since the sludge aggregates developing in anaerobic sludge bed reactors operated on raw domestic sewage are expected to be of flocculent nature.

The purpose of the present research was to investigate the feasibility of integrating methanogenesis and denitrification processes in a single stage UASB reactor employed for the treatment of raw domestic wastewater. This is of particular interest for domestic wastewaters characterized by high COD content, viz. 1-2 $g.l^{-1}$, and high TN concentration, viz. 80-120 $mg.l^{-1}$, since in this case the anaerobic reactor is limited by the maximum applicable organic loading rate (OLR) instead of the hydraulic flow regime (van Lier, 2008). Such wastewaters are often found in

countries characterized by a moderate per capita water consumption rate, such as in the Middle East.
The semi-technical scale UASB reactor was firstly operated under strictly methanogenic conditions to set a reference for assessing the effects of integrated process conditions on the performance of the UASB reactor regarding carbon and nitrogen removal. Hydrolysis, acidification and methanogenesis percentages of applied COD were assessed as well. Additionally, the effects of integrated process conditions on the sludge biological activities and physical properties were evaluated.

2. Materials and Methods

The experimental work was carried out in a UASB reactor assembled from a 57 l effective volume reactor body, which is 2 m in height and 0.19 m in internal diameter, and 71 l head that contains the gas solid separator, being 1 m in height and 0.3 m in internal diameter. Six sampling taps were distributed over the first meter of the reactor height and two sampling taps were distributed over the second meter. The reactor was operated at 27±2°C by re-circulating water in tubes wrapping the reactor through an external thermostat (Julabo 25). In order to minimize heat losses, the reactor was further wrapped with thermal insulation sheets.
Due to the unavailability of flocculent anaerobic sludge, granular sludge was grinded and sieved over 200 μm sieve and used as seed material. The granular sludge was obtained from a full scale UASB reactor, treating paper mill wastewater (Eerbeek, The Netherlands). The grinded granular sludge inoculum had a total solids content of 84 mgTS.g wet sludge^{-1} and a volatile solids content of 65 mgVS.g wet sludge^{-1} respectively. The specific methanogenic activity of inoculum sludge amounted to 0.18 gCH$_4$-COD.gVS^{-1}.d^{-1}.

2.1 Operational conditions

The experimental work was carried out over three successive periods. In the first period (period I), the UASB reactor was operated under strict methanogenic conditions for 62 days at an HRT of 8.6 h, bringing about an upflow velocity of 0.23 m.h^{-1}. The first 35 days of this period were considered a start-up phase. In the second period (period II), the UASB reactor was operated for 60 days under integrated denitrifying and methanogenic conditions by incorporating synthetic nitrate in the form of KNO_3 in the feed. The HRT applied over period II was also 8.6 h, bringing about upflow velocity of 0.23 m.h^{-1}. As for the last period (period III) the reactor was operated for 30 days under integrated conditions with an HRT of 8.6 h, but with an upflow velocity of 0.46 m.h^{-1}, brought about by an internal circulation of the UASB reactor effluent. This increase in upflow velocity was carried out to simulate the increase expected to take place in consequence of recirculation of nitrified effluent to the anaerobic reactor (Figure 1). The height of the sludge bed during period I was kept below tap 7 (150 cm from the reactor bottom). During period II the sludge bed interface fluctuated between taps 7 and 8 and it was kept below tap 8 by discharging sludge that has accumulated above.

2.2 Wastewater

The UASB reactor was operated on raw domestic wastewater originating from the village of Bennekom, The Netherlands. The wastewater is collected in a combined sewer system, so it is diluted according to the intensity of rainfall. The concentration of this sewage was increased by continuously adding diluted primary sludge, in an attempt to simulate high strength Middle East sewage (Halalsheh, 2002). The primary sludge was obtained from a 26 m^3 primary clarifier after 2 h settling time. The settler received sewage from the wastewater treatment plant of Bennekom, The Netherlands, after passing screens and grit chamber. After sieving on a 1 mm sieve, the sieved primary sludge was diluted with wastewater with a dilution factor of 3 and stored in 80 l container with continuous mixing. Potassium nitrate was incorporated in the feed though peristaltic pump, resulting in average nitrate concentration of 17±3 $mgNO_3$-$N.l^{-1}$. Influent characteristics (Table 1) were measured by taking 24 h composite samples after mixing the raw sewage with the diluted primary sludge.

2.3 Analysis

Gas

The biogas production was monitored daily using a wet test gas meter (Schlumberger, Dordrecht, The Netherlands). The biogas composition of CH_4, CO_2, N_2 and O_2 was analyzed using a Fison 8000 gas chromatograph equipped with Teflon column (2*25m×0.53mm) connected in parallel to a steel column (30m×0.53mm). The carrier gas was helium and the oven and TCD detector temperatures were 40 and 100°C, respectively. The injected sample was 100 μl taken from a biogas sample collected in a gas bag.

Table 1: Characteristics of wastewater applied to the UASB reactor.

Parameter ($mg.l^{-1}$)	Total COD	Soluble COD	Suspended COD	Colloidal COD	VFA-COD	NH_4-N	Total Kjeldahl nitrogen
Period I	574±164 (380-794)*	188±50 (102-243)	276±163 (85-547)	110±23 (61-140)	49±22 (21-92)	38±13 (16-50)	n.m
Period II	517±90 (342-650)	163±39 (115-264)	237±67 (121-357)	117±19 (71-143)	50±28 (21-114)	48±4 (43-55)	67±1 (66-68)

* Values between brackets are the minimum and maximum vales obtained; n.m: not measured.

Wastewater

Twenty four hours composite samples of the reactor influent and effluent were collected twice per week. The samples were analyzed for total COD (COD_t), paper filtered COD (COD_{pf}), soluble COD (COD_{sol}), volatile fatty acids (VFA) and ammonium, nitrate and nitrite concentration. Solids fractionation for COD_{pf} test was performed using Schleicher & Schuell filter paper (595 1/2, pore size 4.4μm). Suspended COD (COD_{ss}) was considered as the difference between COD_t and COD_{pf}. The COD_{sol} was determined using Schleicher & Schuell membrane filter (ME 25, pore size 0.45μm). Colloidal COD (COD_{col}) was considered as the difference between COD_{pf} and COD_{sol}. The different COD fractions were measured using Dr. Lange cuvette test (LCK 514 & LCK 314), where organic material in the sample is oxidized by potassium dichromate in acid conditions and presence of catalyst (Ag^+). The reduced quantity of chromate is determined photometrically and related to the COD of the sample. VFAs were determined by HP 5890A gas chromatograph equipped with Supelco port (11-20 mesh) coated with 10% Fluorrad FC 431. The temperature of the injector, the column and the FID detector were 200, 130 and 280 °C. Ammonium, nitrate and nitrite concentration was determined using Segment Flow Analyzer (Skalar, the Netherlands). Total Kjeldahl nitrogen was determined according to the Standard Methods (APHA, 1998).

Sludge
Sludge samples obtained from the taps allocated along the height of the reactor were analyzed for total solids (TS), volatile solids (VS), sludge volume index (SVI), capillary suction time (CST), specific denitrification activity (SDA) and specific methanogenic activity (SMA). TS, VS, SVI and CST were determined according to the Standard Methods (APHA, 1998).
The SDA test was carried out under batch mode in vessels of 2 l working volume, equipped with side ports for sampling. Incubation was carried out in 1.8 l medium. Macro and micro nutrients, phosphate buffer and yeast extract were added according to van Lier (1995). Acetate corresponding to 250 $mg.l^{-1}$ and sludge corresponding to 5 $gVS.l^{-1}$ were added. Directly before the start of the experiment nitrate corresponding to a concentration of 25 $mgNO_3\text{-}N.l^{-1}$ in the form of KNO_3 was added. Batches were flushed with nitrogen for two minutes before taking the first sample. Afterwards, batches were incubated at 30°C with shaking (200 rpm). Liquid samples were taken every 15 minutes for the first hour and every 30 minutes thereafter. Samples were immediately paper filtered (4.4μm Schleicher& Schuell). The duration of the test was approximately 4 to 5 h. The concentration of nitrate and nitrite was determined in a Segment Flow Analyzer (Skalar, the Netherlands) immediately after the end of the test.
The SMA of sludge retained in the reactor at the end of each period was tested. The test was performed in 1 liter bottles, equipped with side port for gas sampling and closed with oxi-top® measuring heads that contains built in pressure sensor (WTW OxiTop® Control, OC 110) which register the increase in pressure relative to the initial pressure every 20 minutes. Incubation was carried out in 300 ml medium. Macro and micro nutrients, phosphate buffer and yeast extract were added according to van Lier (1995). Acetate corresponding to 1 $gCOD.l^{-1}$ and sludge corresponding to 2 $gVS.l^{-1}$ were added. To achieve anaerobic conditions, bottles were flushed with nitrogen gas for two minutes. Afterwards, the bottles were incubated with shaking (120 rpm) at 30°C. The biogas composition CH_4, CO_2, N_2 and O_2 was analyzed using Fison 8000 gas chromatograph operated on the conditions stated earlier. Methane production was computed by multiplying methane percent in the produced biogas by the prevailing pressure inside the bottles at the time of carrying out the composition analysis.

3. Calculations
Recovery percentage of nitrate as nitrogen gas was calculated using equation 1 where nitrate reduced refers to nitrate removed in the UASB reactor. Stripped nitrogen ($N_{2\ stripped}$) is the calculated amount of nitrogen released in the biogas due to stripping of dissolved nitrogen in the influent. It was calculated based on the difference between the saturation concentration of nitrogen gas ($C_{s,N2}$) in the influent stream and that inside the reactor. $C_{s,N2}$ in the influent stream was computed using Henry's law at ambient temperature and nitrogen gas partial pressure of 0.79 atm. $C_{s,N2}$ inside the reactor was computed at 27°C and nitrogen gas partial pressure calculated from the percentage of nitrogen gas in the produced biogas and total pressure of 1 atm. Ammonium production (Amm_{Pr}) percentage, which refers to ammonium production in the UASB reactor relative to the ammonium concentration in the influent, was calculated according to equation 2. The COD used in nitrate reduction processes was calculated using equation 3. Methanisation percentage was calculated based on equation 5. The amount of soluble methane was calculated according to Henry's law. Percentages of hydrolysis and acidification prevailing

during operation under strict methanogenic and integrated conditions were calculated according to equations 6 to 9. COD mass balances were made based on the reactor average performances over the two experimental periods, according to equations 10 to 12. The sludge residence time (*SRT*) was calculated according to equation 13. Specific sludge production in reference to total COD removed during period II operation was calculated based on equation 14. The filterability constant (χ) was calculated according to equation 15.

1. $$Nitrogen\ recovery\ \% = \frac{N_{2\ gas\ phase} - N_{2\ stripped}}{\left(NO_3 - N\right)_{reduced}} \times 100$$

2. $$Amm_{Pr}\ \% = \frac{\left(NH_4 - N_{effluent}\right) - \left(NH_4 - N_{influent}\right)}{NH_4 - N_{influent}}$$

3. $$COD_{Oxidized(nitrate\ reduction)} = 2.86 \times NO_3 - N_{reduced\ (denitrification)} + 4.57 \times NO_3 - N_{reduced(DNRA)}$$

4. $$COD_{CH_4} = COD_{CH_4\ gas\ phase} + COD_{CH_4\ soluble}$$

5. $$Methanogenesis\% = \frac{COD_{CH_4}}{COD_{t,\ influent}} \times 100$$

6. $$Hydrolysis\ (methanogenic)\ \% = \frac{\frac{COD_{CH_4}}{1 - Y_{anaer}} + COD_{sol,\ effluent} - COD_{sol,\ influent}}{\left(COD_{ss} + COD_{col}\right)_{influent}}$$

7. $$Hydrolysis(integrated)\ \% = \frac{\frac{COD_{CH_4}}{1 - Y_{anaer}} + \frac{COD_{oxidized(nitrate\ reduction)}}{1 - Y_{anox}} + COD_{sol,\ effluent} - COD_{sol,\ influent}}{\left(COD_{ss} + COD_{col}\right)_{influent}}$$

Where; Y_{anaer} and Y_{anox} are the anaerobic and anoxic yields amounting to 0.1 and 0.35 $gCOD_{biomass}$. $gCOD_{removed}^{-1}$, respectively. These yields are computed based on COD mass balance made over UASB reactor operated under strict methanogenic and integrated conditions (chapter three).

8. $$Acidification\ (methanogenic)\ \% = \frac{\frac{COD_{CH_4}}{1 - Y_{anaer}} + VFA\ as\ COD_{effluent} - VFA\ as\ COD_{influent}}{COD_{t,\ influent} - VFA\ as\ COD_{influent}}$$

9. $$Acidification\ (integrated)\% = \frac{\frac{COD_{CH_4}}{1-Y_{anaer}} + \frac{COD_{oxidized(nitrate\ reduction)}}{1-Y_{anox}} + VFA\ as\ COD_{effluent} - VFA\ as\ COD_{influent}}{COD_{t,\ influent} - VFA\ as\ COD_{influent}}$$

Mass balance

Methanogenic reactor:

10. $$COD_{influent} = COD_{effluent} + COD_{CH_4} + COD_{solids}$$

Integrated reactor:

11. $$COD_{influent} = COD_{effluent} + COD_{CH_4} + COD_{oxidized(nitrate\ reduction)} + COD_{solids}$$

COD_{solids} encompass the accumulated COD ($COD_{accumulated}$) and the COD corresponding to biomass growth

The accumulated COD is estimated as:

$$COD_{accumulated} = (COD_{SS} + COD_{col})_{removed} - (COD_{SS} + COD_{col})_{hydrolyzed}$$

and $$(COD_{SS} + COD_{col})_{hydrolyzed} = Hydrolysis\ \% \times (COD_{SS} + COD_{col})_{influent}$$

12. $$COD_{available} = (COD_{sol})_{biodegradable} + (COD_{SS} + COD_{col})_{hydrolyzed}$$

Where $(COD_{sol})_{biodegradable}$ is the anaerobically biodegradable fraction of the applied soluble COD. This fraction is estimated to be 62% according to the maximum methanogenesis% of Bennekom membrane filtered sewage achieved by Elmitwalli *et al.* (2001). For the computation of available COD, two assumptions were made: *(1)* the biodegradability of soluble COD under anaerobic and integrated conditions is equal. *(2)* all the suspended and colloidal COD that was hydrolyzed is biodegradable.

13. $$SRT = \frac{\sum_{i=1}^{i=8} V_i \times X_i}{\left(\sum_j V_j \times X_j \middle/ t \right)}$$

Where; $\Sigma V_i \times X_i$ is the total amount of sludge mass contained by the reactor in gVS. V_i is a reactor segment volume (l), where the effective volume of the reactor is divided into eight segments according to sampling taps. X_i is the segment sludge concentration taken as the average concentration (gVS.l^{-1}) of samples discharged from the segments bounding taps. The rate of sludge discharging was calculated as the sum of V_j(l)$\times X_j$ (g.l^{-1}) divided by the total operational time (t) in days. V_j is the discharged sludge volume on day j and X_j is its concentration.

14. $$Specific\ sludge\ production = \frac{\left(\left(\sum_{i=1}^{i=8} V_i \times X_i\right)_{II} - \left(\sum_{i=1}^{i=8} V_i \times X_i\right)_{I}\right) + \left(\sum_{j} V_j \times X_j\right)_{II}}{Total\ COD\ removed}$$

Where; subscripts I and II refer to the end of periods I and II. Total COD removed is the total COD removed over period II.

15. $$\chi = \frac{\phi \times \eta \times c}{CST}$$

Where; χ is the filterability constant ($Kg^2.m^{-4}.s^{-2}$), ϕ is a dimensionless characteristic constant of the apparatus and is equal to 0.794, η is the viscosity of water at the temperature of the sludge sample used in the CST test, at 25°C η equals 0.894×10^{-3} ($kg.m^{-1}.s^{-1}$), *c* is the sludge total solids concentration ($kg.m^{-3}$) and *CST* is the capillary suction time (s).

4. Results

4.1 Nitrogen, COD and VFA removal efficiencies

During the first period the UASB reactor was operated at an organic loading rate (OLR) ranging between 1.1 and 2.2 $KgCOD_t.m^{-3}.d^{-1}$. During the second period, operation was at an OLR ranging between 1 and 1.9 $KgCOD_t.m^{-3}.d^{-1}$ and at nitrate loading rate (NLR) of 0.05 $KgNO_3$-$N.m^{-3}.d^{-1}$. Two days after launching the integrated process operation 99% removal of nitrate was achieved and an average removal efficiency of 98% prevailed afterward over the second period. No nitrite accumulation was detected. Average nitrogen content of the produced biogas during period I and II was 15.3±4.3% and 20.3±3.6%, respectively.

The average ammonium production (Amm_{Pr}) percentage during period I was 19±8% compared to 35.5±11% prevailing during period II, indicating that DNRA might be taking place. The percentage of removed nitrate that was recovered as nitrogen gas was limited to 66±28%, indicating also that nitrate was partly reduced to ammonium through DNRA. A nitrogen mass balance carried out at the end of period II and based on one week average reactor performances, wherein average COD/NO_3-N ratio was 33, showed 99% removal of nitrate accompanied by 12% removal of TN. Since 18% of influent's TN content was in form of nitrate, TN removal efficiency over 18% is expected if removed nitrate was totally denitrified. Achieving only 12% TN removal shows that nitrate was partly reduced to ammonium.

By means of a mass balance over ammonium, the percentage of nitrate that was reduced to ammonium can be estimated. Ammonium concentration in the effluent was increased by 1.7 gNH_4-$N.d^{-1}$, relative to its concentration in the influent. Due to hydrolysis, organic nitrogen concentration was decreased by 1.2 $gN.d^{-1}$. After taking into account the ammonium incorporated into biomass synthesis, a discrepancy of 0.83 $gN.d^{-1}$ was found between ammonium concentration increase and organic nitrogen concentration decrease. This incremental discrepancy was attributed to the DNRA process and it represented 33% of the removed nitrate that amounted to 2.5gNO_3-$N.d^{-1}$.

The average mean values of effluent COD fractions and VFA, in addition to the UASB removal efficiencies during periods I and II, are presented in Table 2. Results show that operating under integrated conditions (period II) didn't induce significant difference (P >0.05) in removal efficiency of suspended and colloidal fractions of applied COD. Nevertheless, operating under integrated conditions affected the removal efficiency of soluble fraction (P< 0.05).

Table 2: COD and VFA effluent concentrations and removal efficiencies achieved during periods I and II.

	Period I		Period II	
Parameter	Effluent concentration ($mg.l^{-1}$)	Removal %	Effluent concentration ($mg.l^{-1}$)	Removal %
COD_t	158±28 (121-223)	71	161±38 (122-235)	68
COD_{sol}	100±17 (75-124)	45	110±12 (86-124)	31
COD_{ss}	14±12 (8-49)	94	9±8 (0-28)	95
COD_{col}	44±9 (32-62)	58	42±26 (11-90)	62
VFA-COD	13±9 (3.52-29)	71	20±17 (1.29-46)	66

Doubling the up flow velocity under integrated conditions (period III) maintained 99% removal of nitrate accompanied by Amm_{Pr} percentage of 13.7%. The nitrogen content of the produced biogas was 21.6%. The percent of removed nitrate that was recovered as nitrogen gas was 76%. The COD removal efficiency was not deteriorated, achieving a total COD removal efficiency of about 69%. Removal efficiencies of soluble, suspended and colloidal fractions of applied COD were about 47%, 97% and 74%, respectively.

4.2 Methane production, hydrolysis and acidification

The methane content of the produced biogas during periods I and II was 74.3±5.1% and 70.6±3.5%, respectively, corresponding to a methane production of 0.24±0.08 and 0.23±0.08 $m^3CH_4.KgCOD_{removed}^{-1}$ during periods I and II, respectively. Moreover, as shown in Table 3 the degree of methanogenesis prevailing during period II is comparable to that prevailing during period I. Nevertheless, hydrolysis and acidification percentages prevailing during period II were significantly higher than those prevailing during period I.

Table 3: Average values for hydrolysis, acidification and methanogenesis during period I and II.

Parameter (%)	Period I	Period II
Hydrolysis	72± 27	85± 20
Acidification	61±17	73±13
Methanogenesis	56±13	53±12

The COD mass balance presented in Figure 2 shows that over period I, 28% of applied COD was found in the effluent and 56% was transferred into methane. Over period II, 31% of applied COD was found in the effluent and 53% was transferred into methane. Based on anoxic yield (Y_{anox}) of 0.35 $gCOD_{biomass}.gCOD_{removed}^{-1}$, the percentage of applied COD consumed in the catabolic and anabolic reactions concerning nitrate reduction was 16%. This percentage was calculated based on the estimation that 67% of nitrate reduced was reduced to nitrogen gas (denitrification) and 33% was reduced to ammonium (DNRA), according to the total nitrogen balance carried out at the end of period II. These nitrate reduction partitioning percentages are fortified by the percent of removed nitrate that was recovered as nitrogen gas, i.e. 66%.

During period I, the COD accumulating in the reactor ($COD_{accumulated}$) was estimated to be 9% of the applied COD, while during period II the COD accumulating in the reactor was negligible, as all the removed suspended and colloidal COD was hydrolyzed and presumably degraded. Accordingly, COD_{solids} during period II comprises only the COD that corresponds to biomass growth.

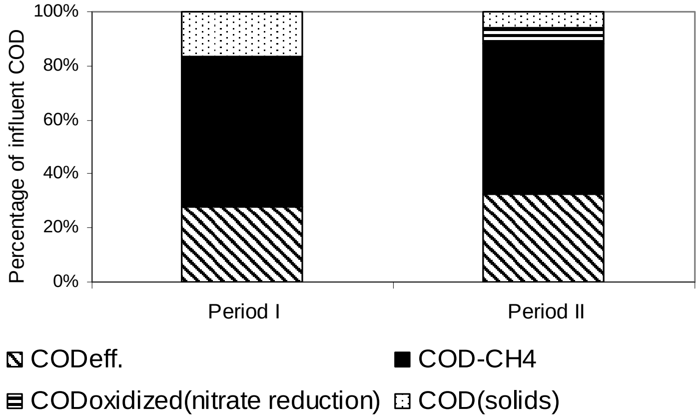

Figure 2: COD mass balance for the two experimental periods

4.3 Characteristics of retained sludge

Denitrification activity and physical characteristics of sludge obtained at reactor heights of 25 cm, 55 cm and 85 cm was assessed at the end of periods I and II, whereas the specific methanogenic activities were characterized at the end of period I (day 62) and after 77, 98 and 122 days of operation.

The profiles of total and volatile solids content of sludge retained in the reactor at the end of periods I and II are presented in Figure 3.

Additional sludge characteristics assessed on sludge obtained at different reactor heights are shown in Table 4. Sludge retained up to 85 cm showed no deterioration on the SVI in consequence of integrated process operation. Apparently, the settling characteristics of the sludge were not affected by the integrated methanogenesis-denitrification process. Moreover, the increase in filterability constants shows that the dewaterability of sludge was positively affected by the integrated operation.

As a result of integrated process operation the concentration of the sludge bed became more evenly distributed over the reactor's height in comparison with the decreasing trend from bottom to top of sludge bed prevailing within period I. The constancy of VS/TS ratio at the end of period II in comparison with period I indicates that the stability of retained sludge wasn't deteriorated as a consequence of integrated process operation. Moreover, the constancy of VS/TS ratio over the sludge bed height indicates that the sludge has a similar degree of stabilization over the height of the sludge bed.

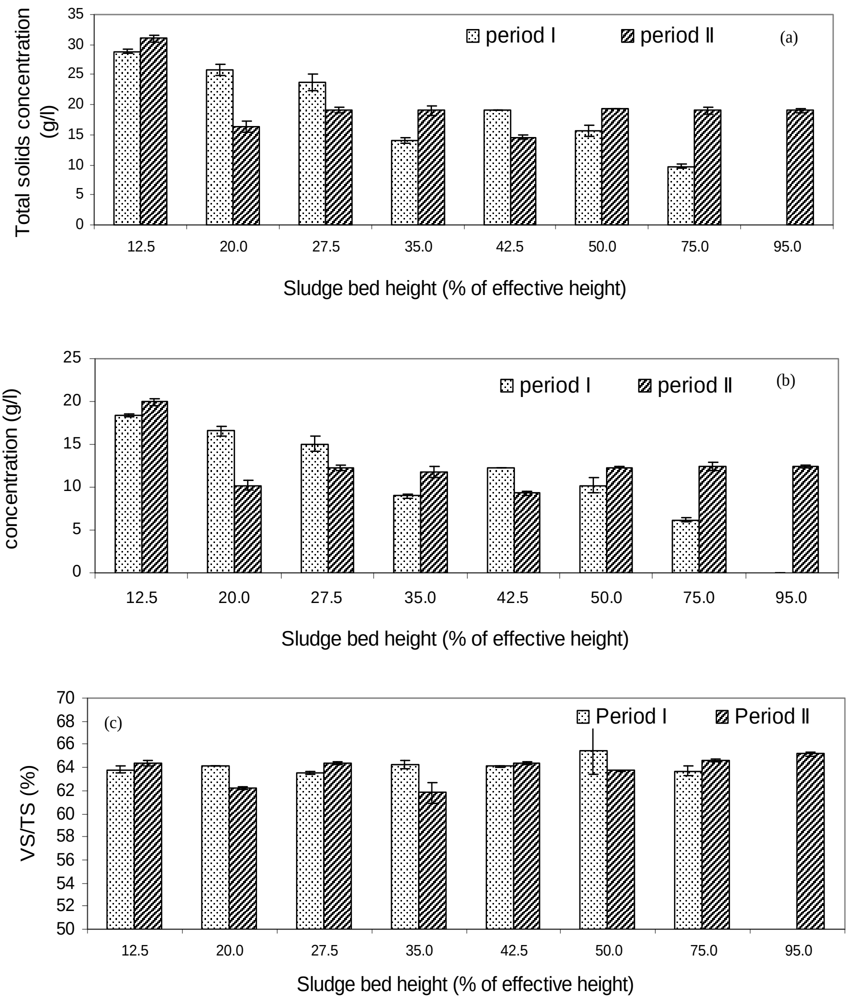

Figure 3: UASB sludge profiles at the end of periods I and II as TS (a), VS (b) and VS/TS ratio (c).

Table 4: Characteristics of retained sludge in the UASB reactor by end of periods I and II at different reactor heights.

	Period I			Period II		
	Sludge obtained at;			Sludge obtained at;		
	25 cm	55 cm	85 cm	25 cm	55 cm	85 cm
SVI ($ml.gTS^{-1}$)	40± 0.8	39± 0.0	44± 0.0	43± 0.8	44± 1.23	23± 0.0
Filterabilty constant $\chi \times 10^5$ ($Kg^2.m^{-4}.s^{-2}$)	9± 0.04	14± 0.1	23± 0.3	18± 0.54	24 ± 1.0	38± 1.8
COD/VS ($gCOD.gVS^{-1}$)	1.6	2.0	1.5	1.6	1.9	1.8

Sludge production during period II operation was 0.11 $gVS.gCOD_{removed}^{-1}$, which is comparable to values reported in literature for UASB reactors operated under strict methanogenic conditions (Mahmoud *et al.*, 2004 and Goncalves *et al*., 1999). The relatively low production can be attributed to the fact that under integrated process conditions approximately all entrapped solids were hydrolyzed whereas the contribution of the high growth-yield denitrifiers in the sludge production is low due to the low nitrate load applied. Estimated SRT during period I operation was 243 days, compared to 300 days for period II operation.
The denitrification activity of the sludge obtained at 25 cm height from the bottom of the reactor at the end of period I was insignificant (Figure 4a) and accumulation of nitrite took place within the five hours period of the test. Assays incubated with sludge obtained at heights of 55cm and 85 cm showed a slight reduction in nitrate concentration within 120 min and 180 min, respectively. This reduction was accompanied by nitrite accumulation (Figures 4b and 4c) and it was followed by a sudden reduction in nitrite and nitrate concentration.
The denitrification activity of the sludge obtained at the end of period II was, as expected, higher compared to the sludge obtained at the end of period I. As shown in Figure 5 two reduction rates were distinguished and hardly any accumulation of nitrite took place. The SDA expressed as (NO_3+0.6 NO_2)-N uptake rate and based on the second reduction rate are 2.32, 3.42 and 3.55 $mgN.gVS^{-1}.h^{-1}$ for the sludge harvested at 25 cm, 55 cm and 85 cm reactor heights, respectively.
The SMA of sludge obtained from different reactor heights along the progress of the experiment is shown in Figure 6. The change in SMA was assessed relative to the SMA of the sludge that was obtained exactly before the start of the integrated process.The SMA of sludge obtained at 55 cm and 85 cm reactor heights was respectively reduced by 60% and 68% after two weeks of integrated process operation (day 77), and by 18% and 35% after five weeks of integrated process operation (day 98). For the sludge obtained at 25 cm, a 54% increase in SMA took place after five weeks of integrated process operation and 36% of increase was maintained after approximately eight weeks of integrated operation (day 122). For sludge obtained at 55 cm, 37% increase in SMA was prevailing after eight weeks of integrated process operation, compared to 66% reduction for sludge obtained at 85 cm. Based on SMAs and VS concentration profiles, the potentiality for methane production of sludge retained in the reactor was about 125 gCH_4-$COD.d^{-1}$ at the end of period I, compared to 134 gCH_4-$COD.d^{-1}$ at the end of period II. Apparently, there is no reduction in SMA of the accumulating sludge during integrated process conditions.

5. Discussions

The results of our present research have shown that the integration of denitrification and methanogenesis in UASB reactors for simultaneous removal of carbon and nitrogen from domestic wastewater is feasible over prolonged periods of time. In addition to conventional denitrification of nitrate to nitrogen gas, the applied COD/NO_3-N ratio in a range of 20-38 resulted in a partial reduction of nitrate to ammonium via the DNRA process. The latter obviously reduces the effectiveness of the reactor in terms of achieving a high degree of nitrogen removal. The occurrence of the DNRA process was revealed by the increased ammonium production under integrated process conditions compared to its production under strict methanogenic condition.

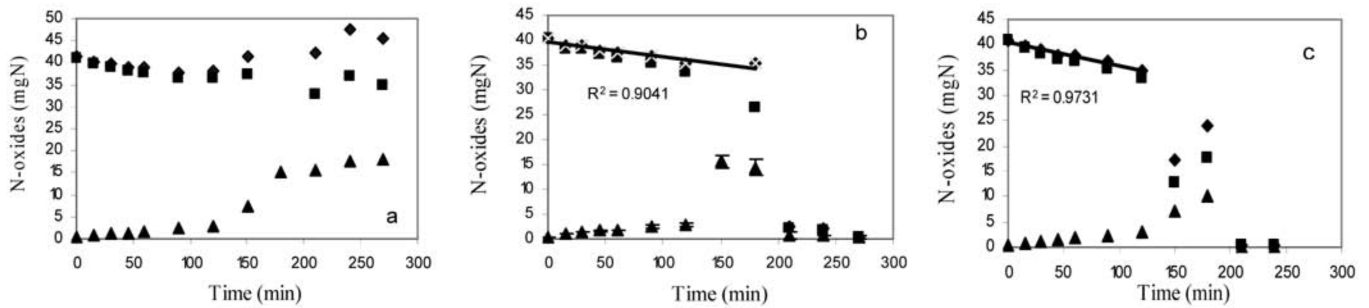

Figure 4: The concentration of N oxides at the SDA test with sludge obtained from reactor height of 25cm (a), 55cm (b) and 85cm (c) at the end of period I ◆ (NO_3+0.6NO_2), ■ (NO_3), ▲ (NO_2).

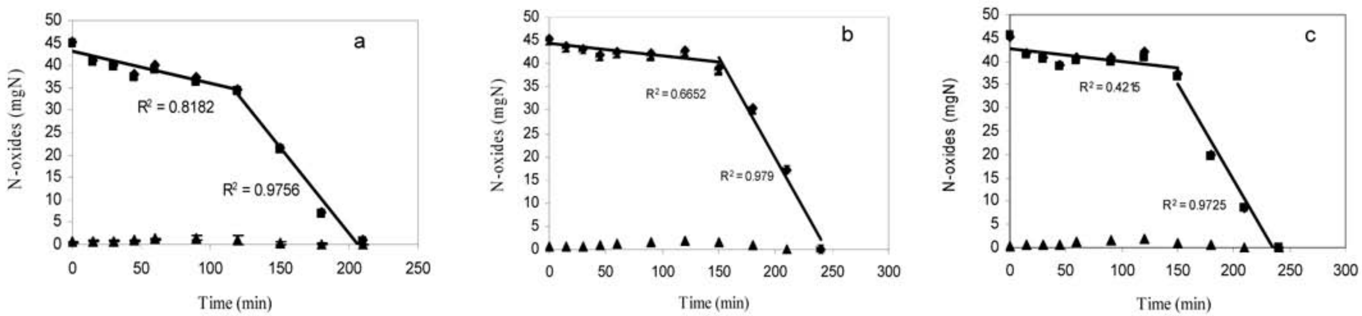

Figure 5: The concentration of N oxides at the SDA test with sludge obtained from reactor height of 25cm (a), 55cm (b) and 85cm (c) at the end of period II. ◆ (NO_3+0.6NO_2), ■ (NO_3), ▲ (NO_2).

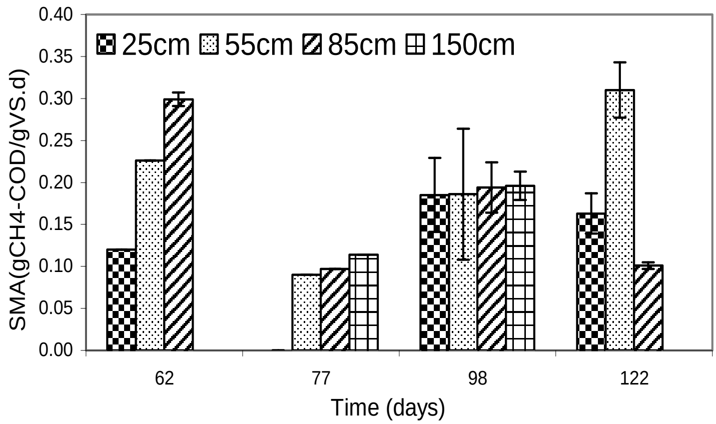

Figure 6: SMA of sludge obtained at different reactor heights over time

Moreover, the relatively low recovery of 66% of removed nitrate as nitrogen gas emphasizes the partial reduction of nitrate via DNRA. The nitrogen mass balance carried out at the end of the integrated period showed that 33% of applied nitrate was reduced via the DNRA process. Many researchers have previously stated that DNRA is controlled by the ratio of available carbon to electron acceptor as well as the nature of carbon source (Akunna *et al*., 1992 and Tugtas and Pavlostathis, 2007). It must be noted that denitrification is a more competitive process than DNRA when the COD/NO_3-N ratio is low. Jun *et al.* (2005), who studied the treatment of raw domestic sewage in up flow sludge blanket reactor (USB) and aerated bio-filter (ABF) with recirculation of nitrified aerobic effluent to the USB reactor applying 1:1 and 2:1 recycle ratios, have achieved complete denitrification of the applied nitrate in the USB reactor at COD/NO_3-N ratios of 38 and 20. Although, the previously mentioned research was carried out with the same range of COD/NO_3-N ratios as it is in this research, the results regarding DNRA vs. denitrification processes were not compatible. Such contradicting results call for further studies to clearly specify the factors that favor the denitrification process in the integrated removal of carbon and nitrogen from domestic wastewater.

In consequence of integrating the denitrification process in an anaerobic reactor, the degree of methanogenesis of the applied COD is expected to diminish. The reason is on one hand the decrease in available COD for methane production as a result of allocating part of applied COD to the denitrification process and on the other hand the expected inhibitory effects of nitrate and its denitrification intermediates on methane production capacity. Regarding the former, the results show that the average degree of hydrolysis prevailing under integrated process conditions increased by 18% compared to operation under strict methanogenic conditions. Henceforth, taking into consideration that the biodegradable fraction of soluble COD for the same type of sewage as used in this experiment is 62% (Elmitwalli *et al*., 2001), the COD available under strict methanogenic and integrated conditions was 69% and 78% of applied COD (equation 12), respectively. Consequently, the apparently enhanced hydrolysis under integrated process conditions results in increased soluble COD availability. Therefore, allocating

16% of applied COD to the nitrate reduction processes didn't result in significant reduction of COD available for methane production. Accordingly and in addition to the fact that the retained sludge SMA was not affected by integrated process operation, the overall methane production rate of the UASB reactor was hardly different between strict methanogenic and integrated conditions. It must be realized that the average influent total COD concentration was even a little lower in period II (Table 1).
It is worth mentioning that the SMA profile over the reactor height shows that the activity of sludge retained in the bottom of the reactor improved. Taking into consideration that possibly entrapped solids would accumulate at the bottom section of the reactor, the SMA increase of the bottom sludge reflects the higher hydrolytic activities.
Integrated process operation didn't lead into deterioration in sludge stability since the VS/TS ratio of total accumulating sludge mass was maintained at 64%. Moreover, the SVI measured at the end of the strict methanogenic and integrated periods were comparable, indicating that no deterioration on settlablity took place. SVIs computed within this research are comparable with those reported by Mahmoud *et al.* (2004) (33 $ml.gSS^{-1}$) for wasted sludge from the UASB reactor of a UASB-digester system operated for treatment of the same type of wastewater at HRT of 6 h and temperature of 15°C. Also Elmitwalli *et al*. (2002/a) reported similar values of 34 $ml.gSS^{-1}$ for discharged sludge from anaerobic hybrid reactors that treat domestic wastewater operating at an HRT of 4 h and 13°C. However, they are higher than values reported by Halalsheh *et al*. (2005), who found values as low as 5-12 $ml.gTS^{-1}$ for sludge accumulating in a UASB reactor that treated strong domestic wastewater with a COD content of about 1500 $mg.l^{-1}$ at an HRT ranging between 23-27 h at average ambient temperatures of 15°C at winter times and 25°C at summer times.
The filterability constants of sludge cultivated under the two operational periods are higher than values reported in literature for the same type of sewage (Mahmoud *et al.*, 2004 and Elmitwalli *et al*., 2002/a). This likely can be attributed to the characteristic of seed sludge used within this research, which most probably resulted in sludge particles with a lower specific surface area and sludge bed characterized by a higher porosity and permeability coefficient. Unfortunately, the particle size distribution of the accumulated sludge was not determined to validate the aforementioned explanation. In comparison with strict methanogenic sludge, integrated sludge was characterized by a higher dewaterability. This might be explained by the higher degree of hydrolysis prevailing under integrated process conditions, which may have resulted in an increase in the average radius of the sludge particles due to biomass growth over hydrolyzed substrate (Elmitwalli *et al*., 2001). Consequently, the sludge particle's specific surface area is reduced and thus the permeability coefficient is increased. This postulation is supported by the fact that sludge obtained at the bottom section of the reactor showed the highest increase in filterability constant, i.e. from 9×10^{-5} to 18×10^{-5} $Kg^2.m^{-4}.s^{-2}$. Noteworthy, sludge accumulated at the bottom of the reactor is expected to exhibit the highest hydrolytic activities due to accumulation of the easily settleable solids coming from the primary sludge, which was part of the influent.

6. Conclusions

- The presented results illustrate the high potentiality of simultaneous removal of carbon and nitrogen form domestic wastewater in a UASB reactor.

- Although denitrification was the main nitrate reduction pathway, partial reduction via DNRA was detected. Henceforth, for successful integrated operation further studies should be carried out to specify the operational conditions that favor the reduction of nitrate via the denitrification process.
- Compared to operation under strictly anaerobic condition, integrated operation at a COD/NO_3-N ratio ranging between 20 and 38 didn't result in significant reduction in methanogenesis. This can be attributed to the higher degree of hydrolysis prevailing under integrated process conditions and to the preservation of sludge SMA.
- Integrated operation didn't lead into deterioration of the flocculent sludge settleability features. Therefore, we do not expect to experience sludge detainment problems in consequence of long-term integrated operation.
- No deterioration of dewaterability features of the retained sludge were encountered in consequence of integrated process operation.

7. Acknowledgment

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Chapter 5

Integrating methanogenesis and denitrification processes in EGSB reactors treating domestic wastewater for adjusting effluent nitrogen levels

Integrating methanogenesis and denitrification processes in EGSB reactors treating domestic wastewater for adjusting effluent nitrogen levels

Kassab Gh., Koetse E., Klapwijk B., Fayyad M. and van Lier J.B.

Abstract
The integration of methanogenesis and denitrification processes was studied in EGSB reactors at liquid up flow velocities of 4.5 $m.h^{-1}$ (period I) and 8 $m.h^{-1}$ (period II). Two 4.9 l reactors were employed in the study; the first was operated under integrated denitrification and methanogenic conditions, whereas the second was operated under strict methanogenic conditions to set a reference for assessing the impacts of integrated process operation. The two reactors were initially inoculated with methanogenic granular sludge, and denitrification was launched in the integrated reactor by incorporating synthetic nitrate in the feed. At a COD/NO_3-N ratio of 20, 100% and 95% nitrate removal efficiency was achieved in the integrated reactor during period I and II, respectively. Denitrification was the main nitrate reduction pathway. Liquid shear stress induced by 4.5 $m.h^{-1}$ and 8 $m.h^{-1}$ up flow velocity was not enough to prevent the accumulation of fluffy biofilm on the surface of the integrated granules and consequently deteriorating their settleability. Nevertheless, periodic increase in the applied upflow velocity or carrying out "internal reversed" recirculation of integrated granular sludge resulted in substantial improvement on sludge settleability. The methanogenic activity of granules cultivated in the integrated denitrifying-methanogenic EGSB reactor was almost similar to the activity of granules cultivated in the strict methanogenic EGSB reactor for the two experimental periods. The latter indicates that similar organic loading rates can be applied under methanogenic and integrated conditions.

Keywords: Denitrification, methanogenic, domestic wastewater, granular sludge, nutrient and EGSB.

1. Introduction

Treatment by means of sequential anaerobic-aerobic processes introduces cost efficiency to domestic wastewater management (Lettinga, 1996 and von Sperling and Chernicharo, 2005). The anaerobic stage reduces the organic load on the subsequent aerobic stage by 60-80% whereas it is characterized by relatively low construction and operational costs, low excess sludge production, production of energy in form of biogas and applicability at small and large scales (Hallalsheh, 2002). In the aerobic stage, organics that haven't been degraded in the anaerobic stage will be removed and oxidation of ammonium to nitrate/nitrite via nitrification will take place. If effluent nitrogen level adjustment is required, the nitrified aerobic effluent can be partially recycled to the anaerobic stage for integrated methanogenesis and denitrification processes. Particularly at relatively low operational temperatures treating concentrated sewage, such recirculation would be feasible, since in these cases the anaerobic reactor is limited by the maximum applicable organic loading rate (OLR) in stead of the hydraulic flow regime (van Lier, 2008). Domestic wastewaters contain high fractions of suspended solids and at low to moderate temperatures the application of single-stage anaerobic systems show accumulation of non-active suspended solids in the sludge bed due to the slow hydrolysis of entrapped solids (Zeeman and Lettinga, 1999 and Uemura and Harada, 2000). Therefore, to achieve adequate treatment, long HRTs must be applied (Zeeman and Lettinga, 1999). The rate of solids accumulation obviously correlates with the concentration of the municipal domestic sewage and sewage temperature, requiring even longer HRTs under e.g. Middle East conditions. Alternatively, suspended solids are separated from the raw sewage before its application to a high-rate methanogenic reactor. Separation of suspended solids can be achieved by plain settling in a primary clarifier or by applying two anaerobic stages in series, in which the functionality of the primary clarifier is in fact combined with that of a sludge digester. In such approach, the first anaerobic stage is designed for either (van Lier *et al*., 2001): *(i)* physical entrapment of suspended solids (Elmitwalli, 2000); *(ii)* entrapment, hydrolysis and acidification of solids (Wang, 1994) and *(iii)* pre-digestion of solids including methanogenesis (Sayed and Fergala, 1995). Accordingly, a treatment strategy that consists of an anaerobic pretreatment stage followed by a methanogenic polishing reactor and finally by an aerobic stage is considered a high-potential concept for concentrated domestic wastewater treatment at low to moderate temperatures. The second stage anaerobic reactor receives a clarified influent characterized by a high $COD_{soluble}/COD_{suspended}$ ratio and may consist of an expanded granular sludge bed system as proposed by others (van der Last and Lettinga, 1992 and Sayed and Fergala, 1995). Recirculation of nitrified effluent from the aerobic step to adjust effluent nitrogen levels can be introduced in either of the two stages. However, based on the expected sludge characteristics in the first and second anaerobic stage, a combination with the second expanded bed stage has the highest perspectives, because of the prevailing high hydraulic shear forces controlling biomass detachment (Figure 1).

The major constraints with this strategy are the inhibitory effects of nitrate and its denitrification intermediates on methane production (Akunna *et al*., 1992 and Chen and Lin, 1993) and the occurrence of dissimilatory nitrate reduction to ammonia (DNRA), which represents a step back in the nitrogen removal process (Akunna *et al*., 1993&1994 and Chapter 4). On the other hand, by introducing the nitrified effluent in the second anaerobic stage, the COD/NO_3 ratio will possibly drop to levels in which the occurrence of the DNRA process is not very likely.

Hendriksen and Ahring (1996) and Lee *et al*. (2004) have shown that the inhibitory effects of nitrate and its denitrification intermediates can be avoided in UASB reactors operated with granular sludge. In such type of sludge, the denitrifiers grow on the granules' surface where nitrate is sufficient and methanogens grow in the inner part where nitrate is deficient, i.e. a spatial separation of the two trophic groups. Nevertheless, owing to the growth of denitrifiers in form of fluffy biofilm on the granules' surface, the denitrifying/methanogenic granules (integrated granules) grew to be buoyant and tend to washout (Hendriksen an Ahring, 1996). Detaching part of this fluffy biofilm can improve the integrated granules settleability. The relatively high liquid up flow velocities applied in expanded granular sludge bed (EGSB) reactor can induce such detachment forces. Another obvious plus point for applying the EGSB reactor, which can be operated at up flow velocities reaching 8-10 $m.h^{-1}$, is that it can easily accommodate the extra hydraulic load brought about by the recycling flows from the aerobic stage.

The purpose of the present research is to investigate the feasibility of integrating methanogenic and denitrification processes in EGSB reactors operated with granular sludge for the treatment of pre-settled domestic wastewater, simulating the secondary anaerobic polishing stage. Our present work describes the start up of the denitrification process in the EGSB reactor, the performance of the EGSB reactor regarding carbon and nitrogen removal, and finally assessing the effect of integrated process operation on the sludge biological activities and physical properties. Moreover, the inhibitory effects of different nitrate concentrations on the acetate-utilizing methanogens within anaerobic granular sludge in batch cultures are investigated.

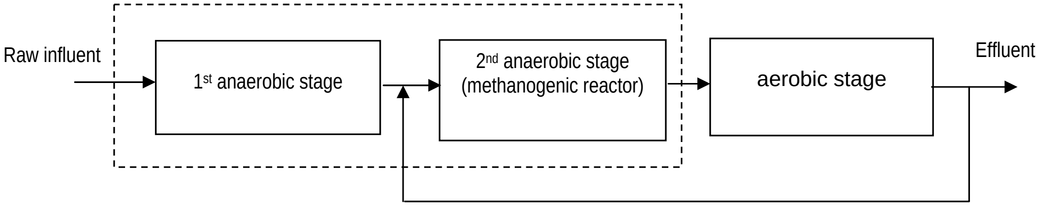

Figure 1: process configuration of sequential anaerobic-aerobic treatment with simultaneous removal of carbon and nitrogen in the secondary anaerobic stage.

2. Materials and Methods

2.1 Effect of nitrate on methane production in batch cultures

2.1.1 Preparation of batch cultures

The test was carried out with two types of sludge: 1) methanogenic granular sludge obtained from a full scale UASB reactor that treats paper mill wastewaters at Eerbeek (The Netherlands), and 2) “integrated” granular sludge obtained from the EGSB reactor operated under integrated denitrification and methanogenic conditions within this research. The sludge obtained from the full scale UASB reactor served as microbial seed for the EGSB reactor, hence it will be nominated as the seed granular sludge.

Seed granular sludge

Prior to the performance of batch tests the sludge was acclimatized to mesophilic conditions in lab-scale EGSB reactor fed with synthetic wastewater consisting of acetate, butyrate, propionate and glucose at a ratio of 1:1:1:1 and operated at an OLR of 2.5 $KgCOD.m^{-3}.d^{-1}$ and HRT of 6 h. About 1.5 g of granular sludge corresponding to a concentration of 0.9 $gVS.l^{-1}$ was added to twelve 315 ml capacity serum bottles. To reactivate the granular sludge after storage at 4°C, 0.6 ml of 150 $gCOD.l^{-1}$ glucose stock solution was added in addition to macro and micro nutrients, phosphate buffer and yeast extract added according to van Lier (1995). To achieve anaerobic conditions the headspaces were flushed with N_2-CO_2 (80:20 in terms of volume) for 5 minutes. Afterward, the serum bottles were incubated at 30°C for 4 days using a shaker (Buhler VKS 75, orbital shaker) at 80 rpm. After four days, the volatile fatty acids (VFA) were analyzed for depletion, after which acetate stock solution was added to each bottle to achieve an initial COD concentration of 1.5 $gCOD.l^{-1}$. Then, five duplicates of the serum bottles were injected with potassium nitrate stock solution corresponding to a nitrate initial concentration of 10, 37.5, 75, 150 and 300 mgNO_3-N.l^{-1}, resulting in COD/NO_3-N ratios of 150, 40, 20, 10 and 5, respectively. The sixth duplicate didn't receive any nitrate and served as control. Afterwards all bottles were filled with demineralized water to 150 ml and the gas in the headspace was replaced by Ar-CO_2 (90:10 in terms of volume). Overpressure in the head space was amended to reach atmospheric pressure by a needle. Subsequently, the whole set was incubated at 30°C with shaking at 80 rpm.

Integrated granular sludge

The test was carried out under the same procedure followed with the seed granular sludge, except that incubation with acetate and potassium nitrate immediately took place and it was carried out in 1000 ml capacity serum bottles with incubation medium of 300 ml.

2.1.2 Monitoring and analytical methods

Within the batch tests inoculated with seed granular sludge, the gas composition (CH_4, CO_2, N_2 and O_2) was periodically determined by Fisons 8000 gas chromatograph equipped with Teflon column (1.5m×2mm) connected in parallel to a steel column (1.2m×2mm). The injected sample volume was 100 μl, the carrier gas was helium and the oven and TCD detector temperatures were 40 and 100°C, respectively. Gas pressure was measured, prior to gas composition analysis, by means of digital pressure meter (GMH 3150, Greasing electronics). In the batch tests, inoculated with integrated granular sludge, methane percentage in the produced biogas was measured by HP 6890 gas chromatograph equipped with particle trap column (2.5m× 0.53mm). The injected sample volume was 100 μl, the carrier gas was helium and the oven and FID detector temperatures were 120 and 250°C, respectively. Gas pressure was measured by means of WTW OxiTop® measuring heads (OC 110), in which overpressure developed in the serum bottles is measured and stored for the whole incubation period once started.

2.2 Integration of denitrification and methanogenesis in EGSB reactor

Two identical EGSB reactors with a total effective volume of 4.9 l were utilized. Height and inner diameter of each reactor was 1.57 m and 0.06 m, respectively. The two reactors were operated in two different periods (period I & period II). During each period, one of the reactors received nitrate, to integrate denitrification and methanogenic processes and, was henceforth, nominated the integrated reactor. The second reactor was kept operating under strict methanogenic conditions to serve as blank reactor, nominated the methanogenic reactor. During the first period, a gas liquid solid separator (GLSS) was installed at the top of the reactors and connected to a gas meter through a hydraulic seal. During the second period, the GLSS was replaced by a 1 mm sieve installed at the effluent port for easier handling of accumulated scum. Influent was supplied by a peristaltic pump at the bottom of the reactor. The desired up flow velocity was achieved by recirculating effluent from the top of the reactors through a port below the effluent port to a T-connection with the influent stream. Operational temperature was kept at 25±2 °C, using a thermostat bath-circulator (Julabo F25). During period I the two EGSB reactors were operated for 112 days. Within the first 45 days, both reactors were operated under sole methanogenic conditions and on day 46, the integrated process was launched in the integrated reactor by incorporating synthetic nitrate in the feed. During period II the two EGSB reactors were operated for 135 days and the integrated process was immediately initiated by incorporating synthetic nitrate in the feed.

2.2.1 Operational conditions

The operational conditions applied during the two experimental periods are shown in Table 1. The two EGSB reactors were inoculated with sludge originating from a full scale UASB reactor treating paper mill wastewater with total solids concentration of 164 mgTS.g wet sludge^{-1} and volatile solids concentration of 123 mgVS.g wet sludge^{-1}, respectively. The specific methanogenic activity (SMA) of the inoculum sludge was 0.35 gCH_4-COD.gVS^{-1}.d^{-1}.

Table 1: Operational conditions of the EGSB reactors.

Parameter	Period I		Period II	
	Integrated reactor	Methanogenic reactor	Integrated reactor	Methanogenic reactor
OLR (KgCOD$_t$.m^{-3}.d^{-1})	6.6 (5.1-9.1)	6.6 (5.1-9.1)	2.7 (1.4-3.6)	2.7 (1.4-3.6)
SLR[1] (gCOD$_t$.gVS^{-1}.d^{-1})[1]	0.22	0.22	0.25	0.25
Flow rate (m^3.d^{-1})	0.08	0.08	0.03	0.03
HRT (h)	1.5	1.5	3.9	3.9
Up flow velocity (m.h^{-1})	4.5	4.5	8.0	8.0
NLR (gNO_3-N.gVS^{-1}.d^{-1})[2]	0.01	-	0.01	-
COD$_t$/NO_3-N	20 (15-26)	-	20 (9-26)	-

[1]SLR = sludge loading rate, calculated based on the average total COD (COD$_t$) during the experimental period and initial amount of granular sludge added.
[2]NLR = nitrate loading rate, calculated relative to initial amount of granular sludge added.

2.2.2 Wastewater characteristics

Wastewater employed in this research was the effluent of a 26 m^3 settler with a settling time of two hours. This settler receives sewage from the wastewater treatment plant of Bennekom, The Netherlands, after passing screens and grit chamber. Main characteristics of this settled sewage based on 24 h composite samples over the two distinct experimental periods are shown in Table 2. Nitrified effluent was simulated by applying synthetic nitrate in form of KNO_3 pumped from a 25 l container maintained in 4°C.

2.2.3 Analysis

Gas

The biogas production was monitored daily using a wet test gas meter (Schlumberger, Dordrecht, The Netherlands). The gas composition of CH_4, CO_2, N_2 and O_2 was analyzed using Fison 8000 gas chromatograph equipped with Teflon column (2*25m×0.53mm) connected in parallel to a steel column (30m×0.53mm). The injected sample was 100 μl, the carrier gas was helium and the oven and TCD detector temperatures were 40 and 100°C, respectively.

Wastewater

Twice per week 24 h composite samples of the reactor's influent and effluent of both systems was taken. The samples were analyzed for total COD (COD$_t$), paper filtered

COD (COD_{pf}), soluble COD (COD_{sol}), volatile fatty acids (VFA) and ammonium, nitrate and nitrite concentration. Solid fractionation for COD_{pf} test was performed using Schleicher & Schuell filter paper (595 1/2, pore size 4.4μm). COD_{ss} is considered the difference between COD_t and COD_{pf}. The COD_{sol} was determined using Schleicher & Schuell membrane filter (ME 25, pore size 0.45μm). Colloidal COD (COD_{col}) is considered the difference between COD_{pf} and COD_{sol}. The differentiated COD fractions were measured using Dr. Lange cuvette tests (LCK 514 & LCK 314), where organic material in the sample is oxidized by potassium dichromate in acid conditions and presence of catalyst (Ag^+). The reduced quantity of chromate is determined photometrically and related to the COD of the sample. VFA were determined by HP 5890A gas chromatograph equipped with Supelco port (11-20 mesh) coated with 10% Fluorrad FC 431. The temperature of the injector, the column and the FID detector were 200, 130 and 280 °C. Ammonium, nitrate and nitrite concentration was determined using Segment Flow Analyzer (Skalar 1520).

Table 2: Characteristics of pre-settled domestic wastewater used in the experiment

Parameter ($mg.l^{-1}$)	Total COD	Soluble COD	Suspended COD	Colloidal COD	VFA-COD	NH_4-N
Period I	412±63 (315-565)*	209±38 (159-305)	40±26 (10-94)	162±41 (59-210)	79±25 (48-139)	47±5 (38-54)
Period II	446±87 (221-588)	189±43 (108-265)	68±37 (2-163)	189±38 (82-288)	96±28 (44-165)	50±9 (26-60)

*Values between brackets are the minimum and maximum values.

Sludge

Sludge samples obtained from the bottom of the two reactors, were analyzed for total solids (TS), volatile solids (VS), granular strength, settling characteristics, specific denitrification activity (SDA) and specific methanogenic activity (SMA). The sludge volume index (SVI) of flocculent sludge developed in the integrated reactor was measured as well.

TS, VS and SVI were determined according to the Standard Methods (APHA, 1995). Granular strength was determined as the resistance against axial compression forces according to Hulshoff Pol *et al*. (1986). However, fluffy biofilm accumulated on the surface of integrated granules made it impossible to specify the breakage point. In order to have an indication whether the strength of the interior of the integrated granules was affected, they were washed to detach the fluffy biofilm and the strength test proceeded according to the procedure presented by Hulshoff Pol *et al*. (1986). Settling characteristics of decanted samples of granular sludge were determined with a sedimentation balance that recorded the weight of the settled sludge as a function of the sedimentation time within a water column of 3.75 m height. (Hulshoff Pol *et al*., 1986).

Since in the integrated reactor, denitrifiers did not only grow on the surface of the granules but also as flocculent sludge, for the SDA a distinction has been made between granules and flocculent sludge by manually sieving the integrated sludge on 500 µm sieve. Tests were carried out separately on sieved sludge with an apparent flocculent appearance and the sludge that didn’t pass the sieve with a granular appearance. The sieving was carefully done so as not to detach the biofilms that accumulated on the surface of the granules.

The SDA tests were performed in batch mode in vessels of 2 l working volume, equipped with side ports for sampling. Incubation was carried out in 2 l medium. Acetate that corresponds to 250 mg.l^{-1} was added in addition to macro and micro nutrients, phosphate buffer and yeast extract added according to van Lier (1995). The batches were inoculated with either 10 g of methanogenic or integrated granules or with 200 ml flocculent sludge. The SDA of the scum layer was assessed as well using 300-400 ml scum layer sludge. At t=0 of the experiment, nitrate corresponding to a concentration of 40 mgNO_3-N.l^{-1} in form of KNO_3 was added. Batches were flushed with nitrogen for two minutes before taking the first sample. Hereafter, batches were incubated at 30°C under shaking (200 rpm) conditions. Liquid samples were taken every 15 minutes for the first hour and every 30 minutes thereafter. The duration of the test was approximately 4 to 5 hours. The samples were paper filtered (4.4μm Schleicher& Schuell) directly after collection and the concentration of nitrate and nitrite was determined in a Segment Flow Analyzer (Skalar, 1520) instantly after the end of the test.
The SMAs of the seed sludge and the granules that developed in the integrated and methanogenic reactors were determined. A sample of integrated granular sludge was obtained by manually decanting the reactor sludge, except for the test carried out after 39 days of integrated operation during period II, where the integrated granules were separated from flocculent sludge by sieving using a 500 μm sieve. In addition to the SMA of flocculent sludge developed in the integrated reactor, the SMA of accumulated scum in each reactor was determined as well. The test was performed in 1 liter bottles, equipped with a side port for gas sampling and which were closed with Oxi-Top® measuring heads (WTW OxiTop® Control, OC 110). Incubations were done in 300 ml medium containing: 1.2 g acetate-COD.l^{-1}, 2 gVS.l^{-1} sludge, macro nutrients, micro nutrients, phosphate buffer and yeast extract added according to van Lier (1995). To achieve anaerobic conditions, bottles were flushed with nitrogen gas for two minutes. Afterwards, the bottles were incubated using a rotary shaker at 120 rpm and 30°C. The biogas composition CH_4, CO_2, N_2 and O_2 was determined using 100 μl samples and was analyzed using Fison 8000 gas chromatograph operated under the conditions presented in section 2.2.3 (gas analysis). Methane production was computed by multiplying the methane percentage in the produced biogas by the prevailing pressure inside the bottles at the time of carrying out the composition analysis.

3. Calculations

3.1 Effect of nitrate on methane production in batch cultures

Nitrogen recovery percentage and methane inhibition percentage were calculated based on equations 1 and 2.

$$1.\ \textit{Nitrogen recovery}\ \% = \frac{N_{2\,gasphase}}{NO_3 - N_{reduced}} \times 100$$

$$2.\ \textit{Inhibition}\% = \left(1 - \frac{SMA}{SMA_{control}}\right) \times 100$$

3.2 Integration of denitrification and methanogenesis in EGSB reactor

Nitrate recovery percentage as nitrogen gas was calculated using equation 3, where nitrate$_{reduced}$ refers to the nitrate removed in the EGSB reactor. Stripped nitrogen ($N_{2\ stripped}$) is the calculated amount of nitrogen released in the biogas due to stripping of dissolved nitrogen in the influent. It was calculated based on the difference between the nitrogen saturated concentration in influent stream at ambient temperature and nitrogen saturated concentration inside the reactor at 25°C using Henry's law for dissolved gases. Methanisation percentage was calculated using equations 5 and 6 where $COD_{CH4,\ gas\ phase}$ is the COD equivalent to the actual amount of collected methane in the biogas and the $COD_{CH4,soluble}$ is the COD equivalent to saturated concentration of dissolved CH_4 in water, calculated by Henry's law. Percentages of hydrolysis within the integrated reactor were calculated based on equation 7 where Y_{anox} is the anoxic yield taken as 0.40 $gCOD_{biomass}.gCOD_{removed}^{-1}$ (Mara and Horan, 2003). Hydrolysis percentage in the methanogenic reactor was calculated based on equation 8. Mass balances based on total COD removal for the integrated and methanogenic reactors were estimated based on equations 9 and 10, respectively. COD available for methanogenesis and denitrifiers was estimated based on equation 11. Assuming that COD available was either used by denitrifiers or methanogens, COD available for methane production was distinguished based on equation 12.

3. $$Nitrogen\ Recovery\ \% = \frac{N_{2\ gas\ phase} - N_{2\ stripped}}{(NO_3 - N)_{reduced}} \times 100$$

4. $$COD_{oxidized\ (denitrification)} = 2.86 \times (NO_3 - N)_{reduced}$$

5. $$COD_{CH_4} = COD_{CH_4,\ gasphase} + COD_{CH_4,\ soluble}$$

6. $$Methanogenesis\% = \frac{COD_{CH_4}}{COD_{t,influent}} \times 100$$

7. $$Hydrolysis\ \%\ (integrated) = \frac{\frac{COD_{CH_4}}{1-y_{anar}} + \frac{COD_{oxidized\ (denitrification)}}{1-y_{anox}} + COD_{sol,\ effluent} - COD_{sol,\ influent}}{COD_{t,\ influent} - COD_{sol,\ influent}}$$

8. $$Hydrolysis\ \%\ (methanogenic) = \frac{\frac{COD_{CH_4}}{1-y_{anar}} + COD_{sol,\ effluent} - COD_{sol,\ influent}}{COD_{t,\ influent} - COD_{sol,\ influent}}$$

Mass balances
Integrated reactor

9.
$$COD_{influent} = COD_{effluent} + COD_{CH_4} + COD_{oxidized\,(denitrification)} + COD_{accumulated} + COD_{biomass}$$

Methanogenic reactor

10. $$COD_{influent} = COD_{effluent} + COD_{CH_4} + COD_{accumulated} + COD_{biomass}$$

Where;

$$COD_{accumulated} = \left(COD_{ss} + COD_{col}\right)_{removed} - \left(COD_{ss} + COD_{col}\right)_{hydrolyzed}$$

$$\left(COD_{ss} + COD_{col}\right)_{hydrolyzed} = Hyrolysis\,\% \times \left(COD_{ss} + COD_{col}\right)_{influent}$$

11. $$COD_{available} = \left(COD_{sol}\right)_{biodegradable} + \left(COD_{ss} + COD_{col}\right)_{hydrolyzed}$$

12. $$COD_{available\ methane\ production}\ (methanogenic\ reactor) = COD_{available}$$

$$COD_{available\ methane\ production}\ (integrated\ reactor) = COD_{available} - \frac{COD_{oxidized\,(denitrification)}}{1 - y_{anox}}$$

4. Results

4.1 Effect of nitrate on methane production in batch cultures

Effect of nitrate and its denitrification intermediates on methane production was studied for five different initial nitrate concentrations using seed granular sludge and integrated granular sludge as inoculum (Table 3). Strikingly, nitrogen gas started to be produced after one day of incubation in all cultures incubated with nitrate and seed granular sludge, in spite of the fact that the UASB reactor from where the seed granules were obtained treats nitrate free wastewaters. Moreover, the nitrogen balance shows that nitrate was truly denitrified, since a good recovery of reduced nitrate to nitrogen gas was observed (Table 3). Nitrogen recoveries deviating from 100% are ascribed to sampling errors and errors in used analytical procedures.

In comparison with the control culture, addition of nitrate temporarily inhibited methane production for the two types of granules. Generally, inhibition periods increased with increasing nitrate concentrations and the SMA of the integrated granules was much more severely affected by nitrate additions than the SMA of the seed granules. However, it is worth mentioning that the inhibition periods prevailed with the integrated granules were lower than those prevailed with the seed granules. The latter can be likely attributed to the fact that consumption of nitrate using integrated granules was more rapid due to its higher SDA of 3.5 $mgNO_x\text{-}N.gVS^{-1}.h^{-1}$ compared to seed granules' SDA of 2.3 $mgNO_x\text{-}N.gVS^{-1}.h^{-1}$. The SMA calculated based on methane production following the suppression period were significantly (*$p<0.1$*) and negatively correlated to the nitrate concentration applied.

Table 3: Nitrogen balance and residual specific methanogenic activities of the seed and integrated granular sludge.

		Initial COD/NO_3-N ratio (nitrate concentration applied, mg NO_3-N.l^{-1})				
	Control	150 (10)	40 (37.5)	20 (75)	10 (150)	5 (300)
<u>*Seed granular sludge*</u>						
Nitrogen balance						
Reduced NO_3-N, mg		1.5	5.6	11.3	22.5	45.0
Produced N_2, mg		1.5	5.5	12.6	23.2	45.4
Recovery %		100.0	98.0	111.5	103.1	100.8
Methane production						
SMA, gCH_4-COD.gVS^{-1}.d^{-1}	0.35	0.34	0.28	0.26	0.19	0.10
Inhibition, %		2.9	20.0	25.7	45.7	71.4
<u>*Integrated granular sludge*</u>						
Methane production						
SMA, gCH_4-COD.gVS^{-1}.d^{-1}	0.42	0.38	0.24	0.14	0.03	0.01
Inhibition, %		9.5	42.9	65.7	92.9	97.6

4.2 Integration of denitrification and methanogenesis in EGSB reactor

4.2.1 Bioreactor performance

During period I, nitrate removal efficiency of 95.5% was achieved within the first 24 hours after launching the integrated process, accompanied by a 38% increase in nitrogen content in the biogas. An average nitrate removal of 95±3% was maintained for the next 66 days of operation. During period II nitrate was completely removed from day one onwards and complete removal was maintained throughout the whole period. No nitrite accumulation was detected during the two experimental periods; average nitrite concentration in the integrated effluent didn't exceed 0.1 mg.l^{-1} in period I and was below detection limit in period II. During the first period the average NH_4-N_{eff}/ NH_4-N_{inf} ratio was 1.02 and 1.07 for the integrated and methanogenic reactors, respectively. During the second period the NH_4-N_{eff}/ NH_4-N_{inf} ratio was 0.99 and 1.05 for the integrated and methanogenic reactors, respectively. Average percentage of nitrogen recovery during periods I and II was 94% and 140%, respectively. Over 100% nitrogen recovery prevailing during the second period is most probably due to air intrusion and possibly underestimation of stripped nitrogen.

The performance of the two reactors with regard to different COD fractions and VFA is illustrated in Table 4. The difference in performance between the integrated reactor and the methanogenic reactor regarding soluble COD removal during the two experimental periods was statistically insignificant *(paired t-test, P< 0.1*). However, with respect to suspended and colloidal COD removal, the integrated reactor showed significantly better performance during the two experimental periods *(paired t-test, P< 0.1*). Regarding the VFA removal rates, no significant difference was found in the performance of the two reactors during the first period, however, during the second period the methanogenic reactor significantly performed better (Paired t-test, *P<0.1*).

Relative to the amounts of COD removed during period I, methane recovered in the biogas amounted to 0.13 and 0.23 $m^3CH_4.KgCOD_{removed}^{-1}$ for the integrated and methanogenic reactors, respectively. During period II, methane recovered in the biogas relative to the amounts of COD removed amounted to 0.10 and 0.20 $m^3CH_4.KgCOD_{removed}^{-1}$ for the integrated and methanogenic reactors, respectively.

Table 4: Effluents COD and VFA concentrations and achieved removal efficiencies in the integrated and methanogenic reactors during period (I) and (II).

	Period I				Period II			
	Integrated reactor		Methanogenic reactor		Integrated reactor		Methanogenic reactor	
Parameter ($mg.l^{-1}$)	Effluent concentration	Removal (%)	Effluent concentration	Removal (%)	Effluent concentration	Removal (%)	Effluent concentration	Removal (%)
COD_t	256± 47 (150-314)	38	271± 27 (226-311)	34	245± 38 (148-344)	45	268± 40 (167-333)	36
COD_{sol}	104± 21 (64-129)	50	102± 7 (89-111)	51	93± 24 (54-138)	51	93± 27 (39-141)	48
COD_{ss}	17± 8 (2-30)	59	26± 14 (11-52)	34	27± 19 (0-92)	60	34± 17 (0-77)	51
COD_{col}	136 ± 29 (69-174)	17	143 ±24 (96-187)	12	125 ± 28 (71.6-189)	34	142 ±35 (74-207)	24
VFA-COD	23± 10 (10-42)	71	20 ±11 (6-42)	74	31± 11 (16-63)	68	22 ±7 (10-33)	77

4.2.2 Sludge characteristics

During the two experimental periods sludge inoculated in the integrated reactor progressively changed in appearance and color, wherein, fluffy biofilm of brownish color grew on the granules' surface. Moreover, sludge of flocculent structure developed. The VS concentrations and the VS/TS ratios of integrated and methanogenic granules through out the two experimental periods were comparable (Figures 2 and 3), with integrated granules' VS concentration and VS/TS ratio being slightly less.

SDA measurements for integrated granules, sampled after 24 days of integrated operation during period I, showed in comparison with seed granular sludge an increase from 2.3 to 3.2 mg NO_x-N.$gVS^{-1}.h^{-1}$. SDA measurements for integrated granules after 28 days of integrated operation during period II didn't show any increase, while measurements taking place after 130 days of integrated operation showed a SDA of 3.05 mgNO_x-N.$gVS^{-1}.h^{-1}$. Additionally, the flocculent sludge developed within the integrated reactor sludge bed during the two experimental periods had an SDA of 8.7 and 11.2 mgNO_x-N.$gVS^{-1}.h^{-1}$ measured after 24 and 28 days of integrated operation for periods I and II, respectively. The aforementioned results clearly show that denitrifiers in the integrated reactor were growing on the surface of the granules and in form of flocculent sludge as well. The SVI of flocculent sludge that developed in the integrated reactor within period II was 67 $ml.gTS^{-1}$. The growth of denitrifiers on the surface of granules made them fluffy and buoyant, thus, having tendency to washout as previously reported by Hendriksen and Ahring (1996) and Lee *et al*. (2004). Expansion of the sludge bed started to take place after one week of integrated operation during the two experimental periods. Expansion continued till 32 days of integrated operation during period I and 60 days during period II, when the buoyancy of accumulated sludge was enough to cause flotation of part of the sludge as a piston on the top of the reactor. Under the aforementioned condition, the reactor operation was temporarily stopped and the floating piston was broken up and degassed using a bar for stirring. Afterwards, to minimize the washout of granules, during period I, a short period of 2-5 minutes of high superficial velocity up to 8-10 m.h^{-1} was occasionally applied in order to detach the fluffy biofilm growing on the surface of granules, and consequently improving the granular sludge settleability.

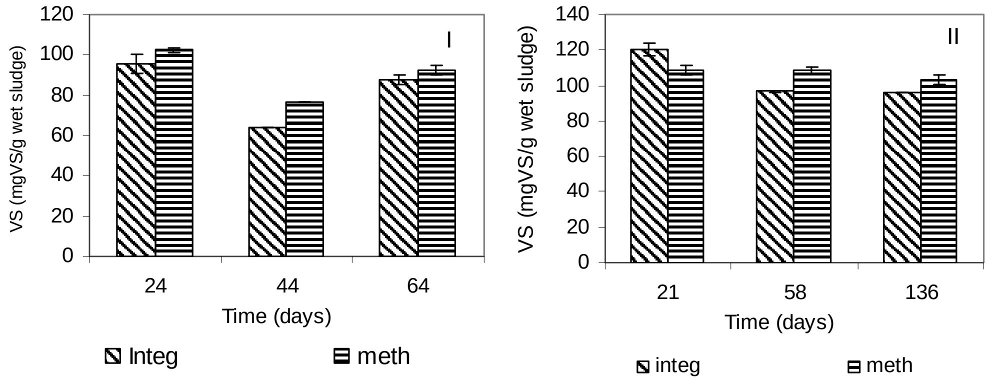

Figure 2: Evolution of integrated and methanogenic granules VS concentration during periods (I) and (II), time corresponds to integrated operation periods.

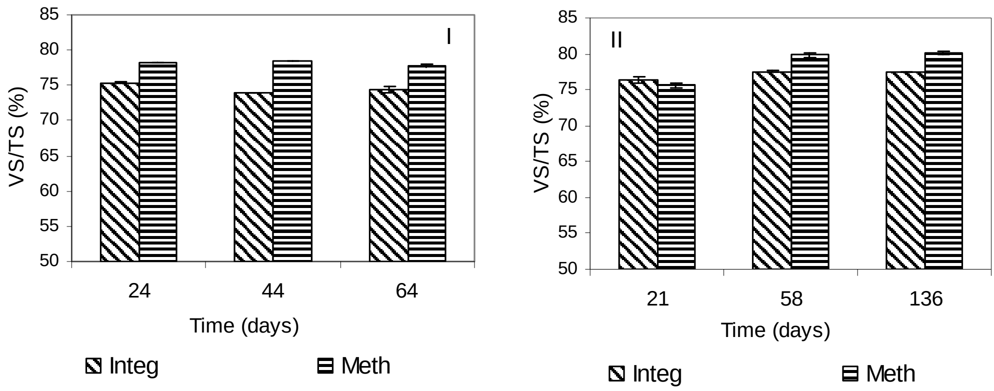

Figure 3: Evolution of integrated and methanogenic granules VS/TS ratios during periods (I) and (II), time corresponds to integrated operation periods.

During period II, the recirculation flow employed to achieve the 8 m.h^{-1} up flow velocity was almost approaching the max capacity of the effluent recycling pump, so a slight increase in up flow velocity didn't result in appreciable detachment of the fluffy bio-film and improvement on their settleability. In order to detach the fluffy biofilm from the granules a reversed internal recirculation of the sludge bed for less than two minutes was carried out. This reversed recirculation was achieved by reversing the direction of effluent recycling pump. The SDA of integrated granules operated for 24 days without reverse recirculation was 3.5 mgNO$_x$-N.gVS^{-1}.h^{-1}, while after reverse recirculation its SDA was 2.1 mgNO$_x$-N.gVS^{-1}.h^{-1}, indicating the loss of denitrifying sludge from the granules' surface.

In addition to accumulation of flocculent sludge in the sludge bed, scum accumulated under the GLSS and at the top of the integrated reactor during the two experimental periods. As shown in Table 5 the scum SDA is comparable to that of flocculent sludge, indicating that the scum is a floating flocculent sludge that contains a significant part of the removed suspended and colloidal matters next to the washed out granules. In the methanogenic reactor, a very thin layer of flocculent sludge accumulated at the top of the granular sludge bed, whereas a scum layer accumulated under the GLSS and at the top of the reactor. This minimal amount of flocculent sludge developed in the methanogenic reactor may contain denitrifying bacteria as well, which are grown on the nitrate present in the applied settled wastewater at a concentration of 0.3-0.8 $mgNO_x\text{-}N.l^{-1}$. The assessed SDA of accumulated scum in the methanogenic reactor during periods I & II support the aforementioned conclusion (Table 5). During the course of the experiment, accumulated scum was pumped away consistently to avoid deterioration of the removal efficiency of COD_{ss} and COD_{col}.

Table 5: Characteristics of flocculent sludge developed in the integrated reactor and scum accumulated in the integrated and methanogenic reactors during periods (I) and (II).

	Period I		Period II	
	Integrated reactor	Methanogenic reactor	Integrated reactor	Methanogenic reactor
VS/TS %				
o Scum	75±1	77±2	77[1]±4	78[1]±5
o Flocculent sludge	75±2	-	76±2	
COD/VS				
o Scum	-	-	2.1[1]± 0.3 (1.8-2.4)	2.1[1]± 0.3 (1.7-2.5)
SDA ($mgNO_x$-N.g $VS^{-1}.h^{-1}$)				
o Scum	10.0[2], 9.2[3]	2.3[2], 2.3[3]	12[4], 9.7[1,4]	5.3[4]
o Flocculent sludge	8.7[2]	-	11.2[5],8.5[6]	-
SMA (gCH_4-COD.g$VS^{-1}.d^{-1}$)				
o Scum	No M.A[7], 0.1[8]	No M.A[7,8]	0.1[10]	0.03[10]
o Sieved scum[1]	0.05[9]	-	0.03[10], No M.A[11]	No M.A[10,11]
o Flocculent sludge	No M.A[7]			

[1] The test was carried out on scum that passed 500μm sieve.
[2,3] Measured after 22 and 62 days of integrated operation, respectively
[4] Measured after 125 days of integrated operation.
[5,6] Measured after 27 and 135 days of integrated operation, respectively.
[7,8] Measured after 37 and 63 days of integrated operation, respectively.
[9] Measured after 75 days of integrated operation.
[10,11] Measured after 113 and 136 days of integrated operation, respectively.
No M.A: Methane was below detection limit in the biogas produced within the SMA batches.

In order to assess the effect of integrated process operation on the methanogenic activity, the SMA of integrated and methanogenic granules were periodically tested. As shown in Figure 4, in comparison with methanogenic granules, the SMA of integrated granules was reduced by 11% and 20% after 39 and 64 days of period I integrated operation, respectively, and by 12% and 3% after 28 and 133 days of period II integrated operation, respectively. At the end of period II the sludge SMA of both reactors was distinctly higher compared to the previous values. During the two experimental periods no disintegration of granules took place in either of the reactors. Apparently no growth of methanogens, exterior to the granular structure took place as shown by SMA deficiency in the flocculent sludge that developed in the integrated reactor as well as in the sieved scum (passing 500μm sieve) accumulated in both reactors (Table 5). Nevertheless, along the two operational periods washed out granules were present in the scum of the integrated reactor, elevating its SMA (Table 5).

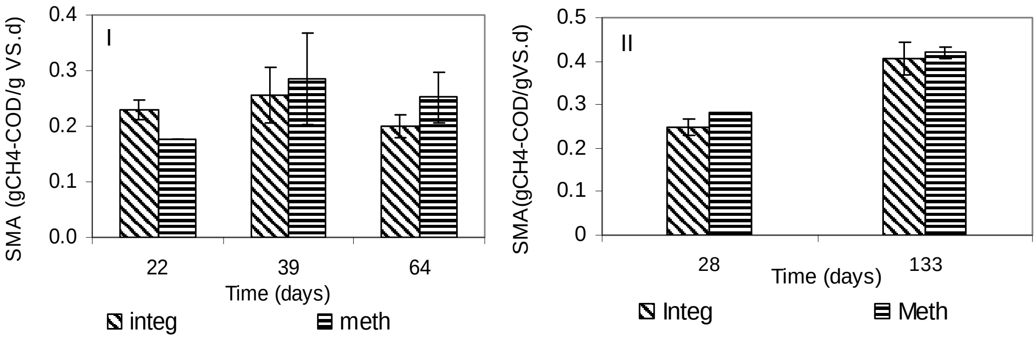

Figure 4: Evolution of integrated and methanogenic granules specific methanogenic activities (SMA) during periods (I) and (II), time corresponds to integrated operation periods.

To evaluate the effect of integrated process operations on the physical characteristics of the sludge, the granules' strength and settleability were assessed. As shown in Figure 5, during the two experimental periods, integrated and methanogenic granules displayed lower strength compared to the inoculum granular sludge, which showed a maximum strength or resistance to a compression pressure of 609±18 KN.m^{-2}. During period I, the strength of the integrated and methanogenic granules deteriorated with time, and integrated granules' strength were consistently lower than that of the methanogenic granules. In period II, the methanogenic granules strength showed better stability compared to the integrated granules strength, which continued deteriorating in time. Nevertheless, compared to period I integrated and methanogenic granules grown during period II were characterized by higher granule strength.

By the end of each experimental period, the settling characteristics of granules that developed in both reactors have been tested. As shown in Figure 6 integrated granules that developed in period I, showed substantially poorer settleability compared to the methanogenic granules. In period II, the settleability of integrated granules improved substantially. However, compared to the methanogenic granules the settleability of integrated granules was still consistently inferior.

4.2.3 Balances

COD mass balance presented in Figure 7 shows that during period I, 65% and 67% of the influent COD was found in the effluent and only 23% and 11% was transferred into methane for the methanogenic and integrated reactors, respectively. Over period II, 61% and 56% of influent COD was found in the effluent and 21% and 11% was transferred into methane for the methanogenic and integrated reactors, respectively. Based on a denitrification yield of 0.40 $gCOD_{biomass}.gCOD_{removed}^{-1}$, 18%

and 21% of the COD applied to the integrated reactor was consumed in the catabolic and anabolic reactions of the denitrification process for periods I and II, respectively.

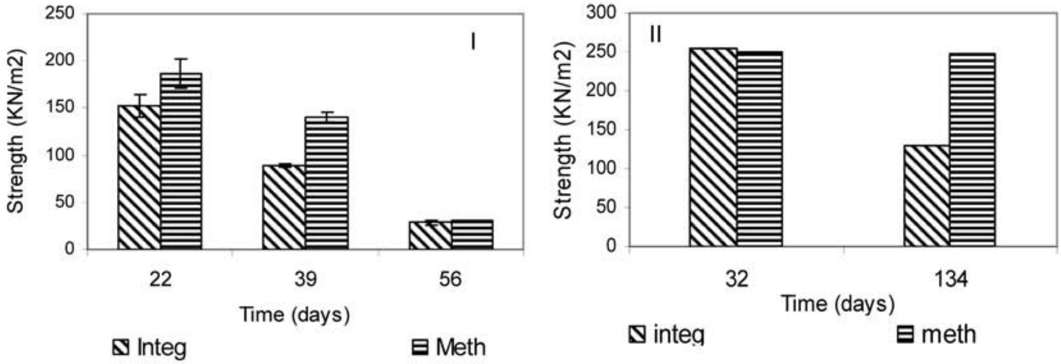

Figure 5: Evolution of integrated and methanogenic granules strength during periods (I) and (II), time corresponds to integrated operation periods.

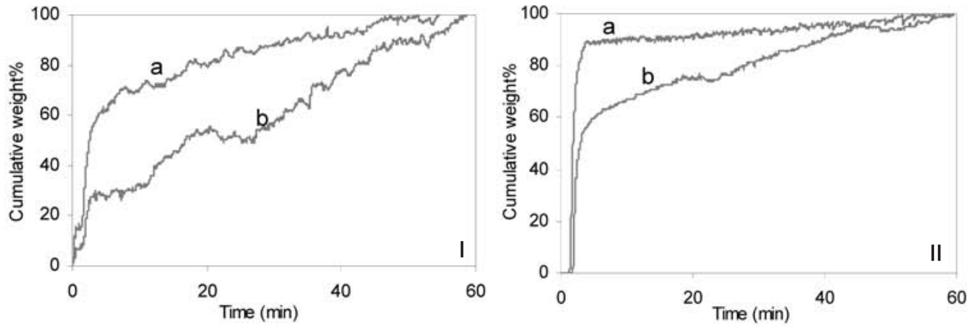

Figure 6: Settling characteristics of methanogenic granules (a) and integrated granules (b) at the end of periods (I) and (II).

Hydrolysis percentages of 20% and 17% were achieved in the integrated reactor during periods I and II, respectively. These hydrolytic activities were most probably resulting from entrapment of removed COD_{ss} and COD_{col} in the developed flocculent sludge. In spite of that 22% and 25% of applied suspended and colloidal COD was removed in the methanogenic reactor during periods I and II, respectively, no hydrolytic activities were taking place. This can be explained by the fact that the solids removed in the methanogenic reactor were mostly not entrapped in the sludge bed but accumulated as scum layer. During period I, the sum of accumulated COD and COD incorporated in biomass synthesis presented 13% and 11% of applied COD for the methanogenic and integrated reactors, respectively. These values were calculated based on bacterial yields of denitrifiers and methanogens of 0.40 and 0.1 $gCOD_{biomass}.gCOD_{removed}^{-1}$, respectively. During period II, the aforementioned sum represented 14% and 21% of applied COD for the methanogenic and integrated reactors, respectively. Taking into consideration that the biodegradable fraction of soluble COD for the same type of pre-settled sewage as used in this experiment is 54% (van der Last and Lettinga, 1992), 28% and 37% of applied COD to the methanogenic and integrated reactors during period I were respectively bio-available. During period II, 30% and 39% of applied COD to the methanogenic and integrated reactors were bio-available. The elevated hydrolytic activities in the integrated reactor increased the percentage of available COD. Nevertheless, allocating part of applied COD to the denitrification process in the integrated reactor decreased the available COD for methane production to 19% and 17% during periods I and II, respectively. The reduction on hydrolysis percentage that took place during period II wasn't associated with reduction in bio-availability percentage, because the influent applied during period II had a slightly higher percent of COD_{sol}/COD_{t}.

During period I, COD consumed in methane production was 67% of the COD available for its production in the integrated reactor compared to 91% in the methanogenic reactor. During period II, COD consumed in methane production was 69% of the COD available for its production in the integrated reactor compared to 80% in the methanogenic reactor. Operating the methanogenic reactor on slightly higher initial SLR and a higher upflow velocity, and consequently a higher washout of granules during period II in comparison with period I, have resulted in a reduction in the percentage of COD consumed in methane production relative to the COD available for its production. Nevertheless, the gap in the so called percentage between the integrated and methanogenic reactor was reduced during period II in comparison with period I. This can be attributed to the fact that the reversed recirculation, performed during period II to detach the fluffy biofilm from the granules' surface and consequently minimizing their washout rate, was more efficient than the measures taken during period I, i.e. a periodic increase in the upflow velocity.

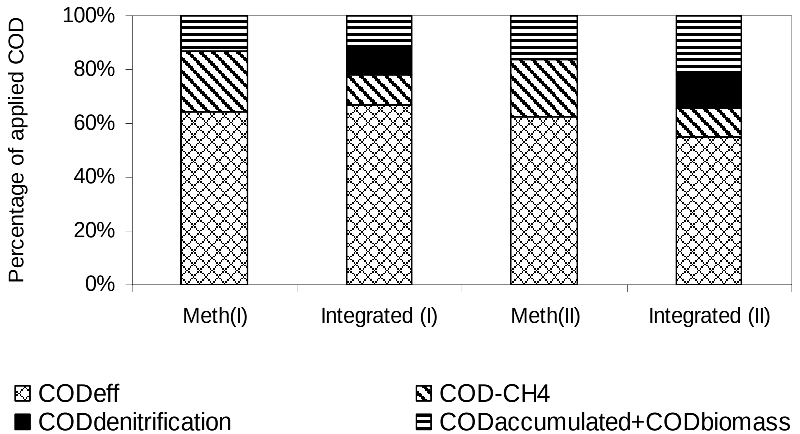

Figure 7: COD mass balance over integrated and methanogenic reactors during periods I and II.

5. Discussion

5.1 Effect of nitrate on methane production in batch cultures

Our present results show that anaerobic sludge, which has never been exposed to nitrate, immediately exerts denitrifying activity if nitrate is added as electron acceptor. Results indicate that a periodic on-off mechanism for periodic N-removal in anaerobic sewage treatment plants is a feasible strategy.

However addition of nitrate to anaerobic and integrated granular sludge led to a reversible inhibition of methane production, confirming previous observations made with a salt marsh sediments (Balderston and Payne, 1976), anoxic rice field soil (Kluber and Conrad., 1998 and Chidthaisong and Conrad, 2000) and anaerobically digested sludge (Akunna *et al*., 1994). Inhibition periods and percentages correlated positively with the concentration of nitrate applied as have been shown clearly by others (Akunna *et al.,* 1994 and Roy and Conrad*,* 1999). Inhibition periods were reduced with sludge characterized by a higher denitrification activity, likely because nitrate is more rapidly consumed.

The higher methane production inhibition percentages encountered with integrated granules relative to the percentages encountered with seed granules, illustrates that inhibitory effects of nitrate and its denitrification intermediates was not reduced upon long term exposure to integrated denitrification and methanogenic processes.

5.2 Integration of denitrification and methanogenesis in EGSB reactor

Results presented in this study illustrate the possibility of simultaneous removal of carbon and nitrogen from pre-settled domestic wastewater in EGSB reactors. Denitrification started immediately in spite of the fact that granular seed sludge to our knowledge was never exposed to nitrate before. Denitrification was the main nitrate reduction pathway and no accumulation of nitrite was detected. The slight occasional production of ammonium in the integrated reactor within periods I and II was always accompanied by equivalent production in the methanogenic reactor, indicating that the production was due to degradation of organic nitrogen rather than dissimilatory nitrate reduction to ammonium (DNRA). Apparently, compared to the results achieved with the single stage UASB reactor (Chapter 4), the lowered COD/NO_3 levels in the EGSB reactor was sufficient to suppress the DNRA process.

Flocculent sludge developed in the integrated reactor induced better entrapment of solids. Consequently, compared to the methanogenic reactor higher removal efficiencies for the colloidal and suspended fractions of applied COD was achieved during the two experimental periods. Entrapment of solids in the integrated reactor stimulated their hydrolysis. Consequently, 50% and 37% of COD allocated to the denitrification process was derived from the hydrolyzed suspended and colloidal COD during periods I and II, respectively.

The high concentration of VFA in effluents of the two reactors during the two experimental periods- even at the beginning of the experiments- means that either the specific sludge loading rates applied were over the removal capacity of the two reactors or that the apparent Monod half saturation constants *(*K_m values) for VFA removal are relatively high. Granular sludge is generally characterized by high apparent K_m values resulting from mass transfer limitation from the bulk towards the core of the granules (van Lier *et al*., 1996). The fact that the VFA load applied on the two reactors during the two experimental periods is significantly lower than the VFA conversion capacity of the two reactors, and that increasing the HRT during period II

at approximately the same SLR didn't result in improvement of the VFA removal efficiency, indicate that mass transfer resistance was limiting the VFA removal.
The fact that the SMA of integrated granules was comparable to that of the methanogenic granules can be attributed to the minimization of inhibitory effects induced by nitrate and its denitrification intermediates on methane production by the habitat segregation of methanogens and denitrifiers, i.e. denitrifiers grew on the granules' surface where nitrate is sufficiently present, whereas methanogens grew in the inner part where nitrate is deficient. It can also be attributed to the reduction in inhibitory effects brought about by dilution in the EGSB reactor (Zhang and Verstraete, 2001) and the partial compensation of COD consumed in the denitrification process by the elevated hydrolytic activities.
In comparison with the methanogenic reactor the efficiency of the integrated reactor in transferring the COD available for methane production into methane was less. Since the SMA of integrated granules was not significantly deteriorated during the two experimental periods, the aforementioned deficiency in the integrated reactor performance might be attributed to progressively operating the reactor under an increasing SLR resulting from the washout of granules.
Results of the period I experiment have shown that the growth of denitrifiers on the surface of granules in form of fluffy bio-film enhanced the tendency of biomass washout. Therefore, within period II the up flow velocity was increased from 4.5 $m.h^{-1}$ to 8 $m.h^{-1}$ to increase liquid shear stress and consequently enhance the detachment of fluffy biofilm and improve the settleability of granules. Nevertheless, this increase in the upflow velocity didn't result in improvement of integrated granules detainment in the EGSB reactor. However, periodic increase in the applied upflow velocity or carrying out internal reversed recirculation of integrated granular sludge shears off part of the fluffy biofilm and substantially improves granules settleability. The proposed approach raises the possibility of discharging the denitrifiers without jeopardizing the loss of the granules with a high methanogenic activity.
Since the strength of integrated granules was not significantly lower than that of the methanogenic granules, disintegration of granules is not expected as a result of long-term integrated operation.

6. Conclusions

- Our present results clearly show the potentiality of integrating denitrification and methanogenesis processes in an EGSB reactor for simultaneous removal of carbon and nitrogen from pre-settled domestic wastewater.
- Carrying out the integrated process in an EGSB reactor at COD/NO_3-N ratio of 20, resulted in complete reduction of nitrate via the denitrification process.
- At up flow velocities of 4.5 $m.h^{-1}$ and 8 $m.h^{-1}$ growth of fluffy bio-film on the surface of granules enhanced the tendency of biomass washout and sludge bed flotation. Nevertheless, periodic increase in upflow velocity or internal reversed recirculation of integrated granular sludge resulted in substantial improvement on sludge settleability.
- Of practical interest is the preservation of SMA in the sludge grown under integrated conditions. Thus, if the sludge detainment problems encountered with integrated granules are resolved, EGSB reactors under integrated conditions can be operated at organic loading rates comparable to those under strict methanogenic conditions.

7. Acknowledgments

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Chapter 6

General summary, conclusions and recommendations

Combined carbon and nitrogen removal in integrated anaerobic/anoxic sludge bed reactors for the treatment of domestic sewage

General summary, conclusions and recommendations

The removal of nitrogen from wastewater has become an important part of the overall treatment process due to the significant impact of nitrogen compounds on the environment and the requirements to meet local standards for discharge and/or agricultural reuse.

Nitrogen removal processes can be grouped in two main categories, biological and physical-chemical processes. Considering the relatively low nitrogen concentrations in domestic wastewater, biological processes are much more feasible than the physical-chemical processes. The current challenge is to incorporate biological nitrogen removal in efficient, sustainable and cost effective organic matter removal systems.

Treatment of domestic wastewater by means of sequential anaerobic-aerobic systems shows interesting perspectives as a sustainable treatment approach (von Sperling and Chernicharo, 2005). Anaerobic stage(s) ascertain 60-80% reduction in the organic load applied on the subsequent aerobic stage. The generally applied anaerobic systems are characterized by low construction and operational costs, low excess sludge production, low or zero energy consumption, production of energy in form of biogas and applicability in small and large scales (Lettinga, 1996 and Foresti, 2002). In the aerobic stage, organics that haven't been degraded in the anaerobic stage(s) will be removed and oxidation of ammonium to nitrate via nitrification will take place. Compared to conventional aerobic technologies the sequential anaerobic-aerobic system is lower in power consumption, lower in excess sludge production and less complex in operation (van Haandel and Lettinga, 1994 and Vieira *et al*., 2003). Effluent nitrogen level adjustment can be incorporated by circulating part of the nitrified aerobic effluent to the anaerobic stage for denitrification to take place. Wherein, part of the organic carbon content in the wastewater serves as carbon source for the denitrification process and the rest is converted to methane.

This thesis describes an investigation on the applicability and effectiveness of integrating anaerobic digestion and denitrification processes in a single sludge system. The integrated concept is of particular interest for the treatment of high-strength domestic wastewater and is accomplished by means of sequential anaerobic-aerobic system. The anaerobic pre-treatment can consist of a single anaerobic stage or two anaerobic stages, conditioned mainly by the wastewater characteristics, the prevailing ambient temperatures and the scale of application.

Effluent nitrogen level adjustments using the single anaerobic pre-treatment stage can be incorporated by circulating part of the nitrified aerobic effluent to the influent of the anaerobic stage, integrating denitrification and anaerobic digestion (Figure 1). In the sequential treatment system consisting of two anaerobic stages followed by an aerobic stage (Figure 2), nitrogen level adjustments can be incorporated by partially circulating the nitrified aerobic effluent to the secondary anaerobic stage (methanogenic stage). In the course of this thesis, treatment configurations in which denitrification and anaerobic digestion takes place in a single sludge process was referred to as integrated systems, and the anaerobic reactor in which denitrification and methanogenesis takes place was referred to as the integrated reactor.

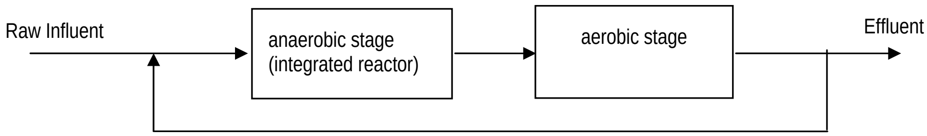

Figure 1: Single stage anaerobic-aerobic integrated system.

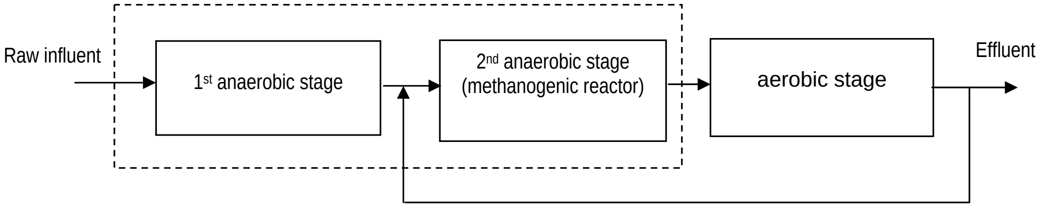

Figure 2: Two stage anaerobic-aerobic integrated system.

To put the sequential anaerobic-aerobic treatment options on view and to state their feasibility and efficiency in domestic wastewater treatment, a review of researched sequential anaerobic-aerobic treatment configurations is given in **Chapter Two**. The reviewed systems are classified according to the mode of growth in the aerobic system i.e. suspended growth versus attached growth. The output of this review shows that the contribution of anaerobic treatment in the sequential systems' overall performance with regard to carbon removal can be as high as 80%. Accordingly, up-to date literature results (re-)confirm the fact that preceding aerobic treatment with anaerobic pre-treatment significantly reduces power consumption and excess sludge production.

The integrated systems are of particular interest for the treatment of concentrated domestic wastewater, i.e. COD concentration higher than $1 g.l^{-1}$ owing to two reasons. Firstly the volumetric design of the anaerobic reactor won't be limited by the hydraulic loading rate (van Lier, 2008), and thus a spare volumetric capacity is available to accommodate the re-circulated flow. Secondly, limitation in nitrate reduction owing to inadequate COD levels is not expected. The second reason is crucially applicable in the integrated system that consists of two anaerobic stages followed by an aerobic stage.

The main concerns associated with the integration of denitrification and anaerobic digestion in single stage reactors are the inhibitory effects of nitrate and its denitrification intermediates on methane production (Akunna *et al*., 1994 and Chen and Lin, 1993). An additional concern is the occurrence of dissimilatory nitrate reduction to ammonia (DNRA) (Akunna *et al*., 1993), which in fact signifies a step back in the nitrogen removal process.

Using a CSTR inoculated with anaerobic sludge of flocculent nature and fed with synthetic wastewater containing glucose as the only source of carbon, Akunna *et al*. (1992) showed that at COD/NO_x-N ratios ranging between 8.86 and 53, denitrification and methane production occurred simultaneously. Their results clearly indicate that an integrated concept can be applied in sludge bed systems operated with flocculent sludge. Since the sludge aggregates developing in anaerobic sludge bed reactors operated on raw domestic sewage are expected to be of flocculent nature, the results presented by Akunna *et al*. (1992) highlight the potential viability of an integrated system consisting of single anaerobic stage followed by an aerobic stage. Thus far, the upflow anaerobic sludge blanket (UASB) reactor is the most widely and successfully used high rate anaerobic system for domestic wastewater treatment. Therefore, the first technical part of this research is focused on studying the potentiality of integrating denitrification and methanogenesis processes in single stage UASB reactors operated with flocculent sludge, simulating the integrated reactor of a single stage anaerobic-aerobic integrated system.

In a lab scale UASB reactor inoculated with flocculent sludge and operated on synthetic wastewater, simulating concentrated sewage with a COD content of 1.5 $g.l^{-1}$, the integration of denitrification and methanogenesis processes was studied (**Chapter Three)**. To set a reference for performance, the UASB reactor was initially operated under strict anaerobic conditions at an average organic loading rate (OLR) of 3.2 $KgCOD.m^{-3}.d^{-1}$. Hereafter, the reactor was operated under integrated anaerobic-denitrifying conditions by means of nitrate incorporation in the feed. The applied nitrate loading rate (NLR) was 0.15 $KgNO_3\text{-}N.m^{-3}.d^{-1}$, whereas the applied OLR was maintained at 3.2 $KgCOD.m^{-3}.d^{-1}$.

Under integrated conditions, 92% of applied COD and 97% of applied nitrate were removed. Denitrification was the main nitrate reduction pathway. Yet, 12% of applied nitrate nitrogen was reduced via DNRA, for the apparent reason that glucose was a constituent in the carbon substrate medium (Akunna *et al*., 1994). Integrated operation at a COD/NO_3-N ratio of 23 resulted in using 18% of applied COD in nitrate reduction processes. COD removed and not consumed in nitrate reduction processes was converted to methane. Upon launching the integrated process, the reduction in methanogenesis percentages coincided with the reduction in COD available for methane production. Thus, indicating that methane production in the UASB reactor was not distressed by the denitrification process inhibitory effects. An increase in the biomass production from 0.07 to 0.1 $gVS.gCOD_{removed}^{-1}$ was estimated upon shifting from anaerobic to integrated conditions.

Having the process-technical feasibility of integrating denitrification and methanogenesis demonstrated with synthetic wastewater in lab scale UASB reactors, a semi-technical scale UASB reactor was employed for carbon and nitrogen removal from raw domestic wastewater (**Chapter Four**). Similar to the previous experiment, the UASB reactor was initially operated as reference under strict anaerobic condition at an average OLR ranging between 1.1 and 2.2 $KgCOD.m^{-3}.d^{-1}$. Hereafter, the reactor was operated under integrated anaerobic-denitrifying conditions by means of nitrate incorporation in the influent. The applied

NLR was 0.05 KNO_3-N.m^{-3}.d^{-1}, whereas the OLR was ranging between 1 and 1.9 KgCOD.m^{-3}.d^{-1}.

Under integrated conditions, results show that nitrate was completely removed and an average total COD removal efficiency of 68% was achieved. However, nitrate was not completely denitrified, as 33% of applied nitrate was reduced via DNRA process. Compared to operation under strictly methanogenic conditions, integrated operation at a COD/NO_3-N ratio ranging between 20 and 38 didn't result in significant reduction in observed methanogenesis. This can be attributed to two factors. Firstly, under integrated process conditions, an increased degree of hydrolysis prevailed that apparently mitigated the reduction in COD available for methane production resulting from COD allocation to nitrate reduction processes. Secondly, the sludge specific methanogenic activity (SMA) preserved under integrated conditions that most likely resulted from the fact that a substantial fraction of the methanogenic biomass was shielded from the oxidized N-compounds in the interiors of the flocculent biofilm.

Most interestingly, integrated operation didn't lead to deterioration of the flocculent sludge settleability features. Thus, sludge detainment problems in consequence of long term integrated operation are not expected. Integrated operation also didn't lead into deterioration of the flocculent sludge dewaterability features.

Results presented in Chapters Three and Four illustrate the high potentiality of simultaneous removal of carbon and nitrogen from domestic wastewater in UASB reactors operated with flocculent sludge, indicating the viability of a single stage anaerobic-aerobic integrated system. However, in terms of achieving a high degree of nitrogen removal, the effectiveness of the system was reduced due to partial reduction of nitrate via the DNRA process. Nitrate reduction via the DNRA process is more favorable at high COD/NO_3-N ratios, i.e. generally above 20. Therefore, if nitrogen level adjustment is required, the preference between a single stage anaerobic-aerobic system and a two-stage anaerobic-aerobic system should include the expected COD/NO_3 ratio at the influent of the integrated reactor. Taking into consideration that the COD/NO_3 ratio of the influent applied to the integrated reactor within the single stage anaerobic-aerobic configuration is higher than it is of the influent applied to the integrated reactor within the two stage anaerobic-aerobic configuration.

In the two stage anaerobic-aerobic configuration in which the second anaerobic stage is integrated with denitrification, the volumetric design of the latter stage is likely hydraulically limited. The latter can be attributed to the COD reduction already achieved in the first anaerobic stage. Under such conditions, the expanded granular sludge bed (EGSB) reactor, which can be operated at up flow velocities reaching 8-10 m.h^{-1}, can satisfactorily accommodate the extra hydraulic load brought about by the recycling flow from the aerobic stage. In addition, the high suspended solids load that could possibly negatively affect granulation of integrated biomass, is considerably reduced by the first stage. Another advantage of applying an EGSB reactor as the second stage is that the prevailing high up flow velocities will likely improve "integrated granules" settleability. Many researchers have shown that methanogenesis and denitrification processes can be successfully integrated in UASB reactors operated with granular sludge (e.g. Hendriksen and Ahring, 1996 and Lee *et al*., 2004). However, the denitrifying/methanogenic granules (integrated granules) grew to be buoyant and tend to washout of the reactor. This was attributed to the growth of denitrifiers in the form of fluffy biofilm on the granules surface. However, the high up flow velocities applied in EGSB reactors can detach this fluffy biofilm and consequently enhance integrated granules formation.

The second part of this research is focused on studying the integration of methanogenesis and deitrification processes in EGSB reactors for the treatment of pre-settled domestic wastewater, simulating the integrated reactor of a two stage anaerobic-aerobic integrated system (**Chapter Five**). Two 4.9 l EGSB reactors were employed in the study; the first was operated under integrated denitrification and methanogenic processes, whereas the second was operated under strict methanogenic conditions to set a reference for assessing the impacts of integrated process operation. The two reactors were initially inoculated with methanogenic granular sludge and denitrification was launched in the integrated reactor by incorporating synthetic nitrate in the feed. The experiment was carried in two successive periods, in which the applied upflow velocity in the first and second period was 4.5 $m.h^{-1}$ and 8 $m.h^{-1}$, respectively.

During the first and second period, 95% and 100% nitrate removal efficiency was respectively achieved in the integrated reactor that was operated at a COD/NO_3-N ratio of 20. Nitrate was completely denitrified and no accumulation of nitrite was detected.

In the integrated reactor, the COD consumed in methane production was 67% and 69% of the COD available for its production during the first and second period, respectively. Such low percentages are attributed to integrated granules washout of the reactor. Since the liquid shear stress induced by the 4.5 $m.h^{-1}$ and 8 $m.h^{-1}$ up flow velocities was not enough to prevent accumulation of fluffy biofilm on the surface of the integrated granules.

Of practical interest is the preservation of specific methanogenic activity in the sludge grown under integrated conditions. Thus, if the encountered sludge detainment problems with integrated granules are resolved, EGSB reactors under integrated conditions can be operated at OLRs comparable to those under strict methanogenic conditions.

Results in Chapter Five illustrate the potentiality of integrating denitrification and methanogenic processes in EGSB reactors for simultaneous removal of carbon and nitrogen from pre-settled domestic wastewater. However, for the integrated process to be viable in an EGSB reactor, integrated sludge detainment problems should be resolved. Results presented in Chapter Five showed that periodic application of excessive detachment forces induced either by periodic increase in up flow velocity or by periodic circulation of the sludge bed from the bottom to the top of the reactor, substantially improved integrated granules settleability.

Conclusions

- Integrated denitrification and methanogenesis processes show high potentiality for simultaneous removal of carbon and nitrogen from domestic wastewater.

- The methanogenic activity of sludge, flocculent or granular, cultivated under integrated denitrifying and methanogenic conditions was comparable to the methanogenic activity of sludge cultivated under strictly methanogenic conditions. Accordingly, similar organic loading rates can be applied under methanogenic and integrated conditions.

- In UASB reactors operated under denitrifying and methanogenic conditions at average COD/NO_3-N ratios of 33 nitrate wasn't completely denitrified and it was partially reduced to ammonium via the DNRA process.

- Using the bench scale reactor set-up, liquid shear stress induced by 4.5 $m.h^{-1}$ and 8 $m.h^{-1}$ up flow velocities was not enough to prevent the accumulation of fluffy biofilm on the surface of granules cultivated under integrated denitrifying and methanogenic conditions in EGSB reactor.

- Periodic increase in applied up flow velocity or periodic circulation of sludge bed from the bottom to the top of the reactor, substantially improve the settling characteristics of the granules cultivated under integrated conditions in EGSB reactor.

Recommendations

- The performance of "integrated systems" under the environmental conditions of arid climate countries, still needs to be demonstrated by means of research at pilot scale and/or demonstration scale.

- Further research on the DNRA route is recommended for optimizing the integrated operation for maximum nitrogen removal, i.e. complete nitrate reduction via the denitrification process.

- Measures that perform best in improving integrated granules settleability needs to be explored. Regarding the limitations of down-scaled hydraulics, demonstration of the EGSB reactor set-up at pilot scale is highly recommended.

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Algemene samenvatting, conclusies en aanbevelingen

Gecombineerde koolstof- en stikstofverwijdering in geïntegreerde anaerobe/anoxische slibbedreactoren voor de behandeling van huishoudelijk afvalwater

Algemene samenvatting, conclusies en aanbevelingen

De verwijdering van stikstof uit afvalwater is een belangrijk onderdeel van het totale behandelingsproces, vanwege de aanzienlijke impact van stikstofverbindingen op het milieu en de eis om te voldoen aan lokale normen voor lozing en/of agrarisch hergebruik.
Stikstofverwijderingsprocessen kunnen worden ingedeeld in twee categorieën: biologische en fysisch-chemische processen. Gezien de relatief lage stikstofconcentraties in huishoudelijk afvalwater bieden biologische processen meer mogelijkheden dan fysisch-chemische processen. De huidige uitdaging is om biologische stikstofverwijdering te integreren in efficiënte, duurzame en kosteneffectieve organische stofverwijderingssystemen.

Behandeling van huishoudelijk afvalwater door middel van sequentiële anaerobe-aerobe systemen biedt interessante perspectieven als een duurzame behandelingswijze (von Sperling en Chernicharo, 2005). In de anaerobe fase wordt de organische belasting van de daaropvolgende aerobe fase met 60-80% gereduceerd. De algemeen toegepaste anaerobe systemen worden gekenmerkt door lage bouw- en operationele kosten, een lage overtollig slibproductie, een minimaal energieverbruik, de productie van energie in de vorm van biogas en toepasbaarheid op kleine en grote schaal (Lettinga, 1996 en Foresti, 2002). In de aerobe fase worden organische verbindingen verwijderd die in de anaerobe fase niet zijn afgebroken, en zal door middel van nitrificatie oxidatie van ammonium tot nitraat plaatsvinden. Vergeleken met conventionele aerobe technologieën heeft het sequentiële anaerobe-aerobe systeem een lager energieverbruik, een lagere slibproductie en een minder complexe bedrijfsvoering (van Haandel en Lettinga, 1994, en Viera *et al.* 2003). Aanpassing van de stikstofconcentratie in het effluent aan de heersende normen voor lozing of hergebruik kan in het systeem worden geïncorporeerd door het terugvoeren van een deel van het genitrificeerde aerobe effluent naar de anaerobe fase, waar vervolgens denitrificatie kan plaatsvinden. Een deel van de organische stof in het afvalwater dient dan als koolstofbron voor het denitrificatieproces en de rest wordt omgezet in methaan.

Dit proefschrift beschrijft een onderzoek naar de toepasbaarheid en doeltreffendheid van de integratie van anaerobe afbraak en denitrificatieprocessen in één slibsysteem. Dit geïntegreerde concept is van bijzonder belang voor de behandeling van sterk geconcentreerd huishoudelijk afvalwater en kan worden toegepast door middel van sequentiële anaerobe-aerobe systemen. De anaerobe voorbehandeling kan bestaan uit een enkele trap of twee fysiek gescheiden trappen, afhankelijk van de afvalwaterkenmerken, de heersende omgevingstemperatuur en de schaal van de toepassing.
Het aanpassen van de stikstofconcentratie in het effluent met behulp van de interne anaerobe voorbehandeling kan worden bereikt door het terugvoeren van een deel van het genitrificeerde aerobe effluent naar het influent van de anaerobe fase, waarbij denitrificatie en anaerobe afbraak worden geïntegreerd (Figuur 1). In het

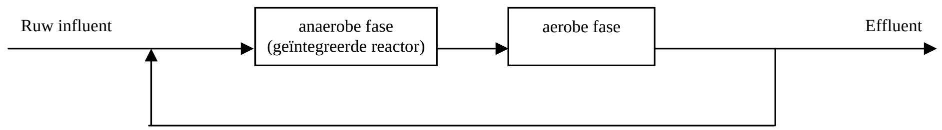
sequentiële behandelingssysteem bestaande uit twee anaerobe trappen gevolgd door een aerobe fase (Figuur 2), kan een verlaging van de stikstofconcentratie worden bereikt door het gedeeltelijk recirculeren van het genitrificeerde aerobe effluent naar de secundaire anaerobe trap (methanogene fase). In dit proefschrift wordt de behandelingsconfiguratie waarbij denitrificatie en anaerobe gisting plaatsvinden in één enkel slibproces aangeduid als *geïntegreerd systeem*, en wordt de reactor waarin denitrificatie en methanogenese plaatsvinden aangeduid als de *geïntegreerde reactor*.

Om inzicht te krijgen in de sequentiële anaerobe-aerobe behandelingsopties en hun haalbaarheid en efficiëntie voor de behandeling van huishoudelijk afvalwater wordt in **Hoofdstuk 2** een overzicht gegeven van onderzochte sequentiële anaerobe-aerobe behandelingsconfiguraties. In het overzicht worden de systemen ingedeeld volgens de wijze van groei in het aerobe systeem, d.w.z. gesuspendeerde groei versus groei op drager. Uit dit vergelijkend onderzoek blijkt dat de bijdrage van de anaerobe behandelingsfase in de prestaties van sequentiële systemen met betrekking tot koolstofverwijdering kan oplopen tot maar liefst 80%. Recente literatuurgegevens bevestigen het feit dat een anaerobe behandeling voorafgaand aan een aerobe behandeling het stroomverbruik en de overtollig slibproductie aanzienlijk vermindert.

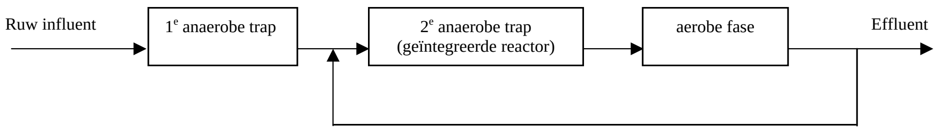
De geïntegreerde systemen zijn om twee redenen van bijzonder belang voor de behandeling van geconcentreerd huishoudelijk afvalwater, d.w.z. met een CZV-concentratie hoger dan 1 $g.l^{-1}$. Ten eerste zal in dat geval het volumetrische ontwerp van de anaerobe reactor niet worden beperkt door de hydraulische belasting (van Lier, 2008) en zal er dus volumecapaciteit beschikbaar zijn voor het recirculatiedebiet. Ten tweede wordt niet verwacht dat er een beperking in de nitraatreductie zal optreden vanwege te lage CZV-concentraties. De tweede reden is cruciaal voor de toepassing van het geïntegreerde systeem dat bestaat uit twee anaerobe trappen gevolgd door een aerobe fase.

De belangrijkste zorgen in verband met de integratie van denitrificatie en anaerobe afbraak in eentraps reactoren zijn de remmende effecten van nitraat en de denitrificatie tussenproducten op de methaanproductie (Akunna *et al.*, 1994 en Chen en Lin, 1993). Een bijkomende zorg is het mogelijke voorkomen van dissimilatorische nitraatreductie tot ammonium (DNRA) (Akunna *et al*, 1993), wat in feite een stap terug is in de stikstofverwijdering.
Met behulp van een CSTR geënt met vlokkig anaeroob slib gevoed met synthetisch afvalwater met glucose als de enige koolstofbron werd door Akunna *et al.* (1992) aangetoond dat denitrificatie en methaanproductie tegelijkertijd optreden bij CZV/NO_x-N ratio's tussen 8.86 en 53. De resultaten tonen duidelijk aan dat een geïntegreerd concept kan worden toegepast in slibbed systemen met vlokkig slib. Aangezien de slibaggregaten die zich ontwikkelen in anaerobe slibbedreactoren gevoed met ruw huishoudelijk afvalwater naar verwachting van vlokkige aard zijn, vestigen de resultaten gepresenteerd door Akunna *et al.* (1992) de aandacht op de mogelijke haalbaarheid van een geïntegreerd systeem dat bestaat uit enkele anaerobe trap gevolgd door een aerobe fase. Tot dusver is de *Upflow Anaerobic Sludge Blanket* reactor (UASB reactor) het meest gebruikte en meest succesvolle hoogbelaste anaerobe systeem voor de behandeling van huishoudelijk afvalwater. Daarom is het eerste technische gedeelte van dit onderzoek gericht op het bestuderen van de mogelijkheden van de integratie van denitrificatie en

methanogenese in eentraps UASB-reactoren bedreven met vlokkig slib, om zo de geïntegreerde reactor van een eentraps anaeroob-aeroob geïntegreerd systeem te simuleren.



Figuur 1: Eentraps anaeroob-aeroob geïntegreerd systeem.



Figuur 2: Tweetraps anaeroob-aeroob geïntegreerd systeem.

De integratie van denitrificatie en methanogenese werd onderzocht in een labschaal UASB-reactor geënt met vlokkig slib en gevoed met synthetisch afvalwater dat geconcentreerd rioolwater met een CZV-gehalte van 1.5 $g.l^{-1}$ simuleert (**Hoofdstuk drie**). Als referentie voor de prestaties van het systeem werd de UASB-reactor eerst onder strict anaerobe omstandigheden bedreven, bij een gemiddelde organische belasting (*Organic Loading Rate*, OLR) van 3.2 $kgCZV.m^{-3}.d^{-1}$. Hierna werd de reactor onder geïntegreerde anaerobe-denitrificerende condities bedreven door middel van het toevoegen van nitraat aan de voeding. De toegepaste nitraatbelasting (*Nitrate Loading Rate*, NLR) was 0.15 $kgNO_3\text{-}N.m^{-3}$, en de toegepaste OLR werd gehandhaafd op 3.2 $kgCZV.m^{-3}.d^{-1}$.

Onder geïntegreerde condities werd 92% van het toegediende CZV en 97% van het toegediende nitraat verwijderd. Denitrificatie was de belangrijkste route voor nitraatreductie. Echter, 12% van de toegediende nitraatstikstof werd gereduceerd via DNRA, mogelijk omdat glucose een bestanddeel van het medium was (Akunna *el al.* 1994). Het geïntegreerd bedrijven van het systeem bij een CZV/NO_3-N ratio van 23 resulteerde in het gebruik van 18% van het toegepast CZV voor nitraatreductieprocessen. Het CZV dat niet via nitraatreductieprocessen verwijderd werd, werd omgezet in methaan. Na de opstart van het geïntegreerde proces kwam de vermindering in het percentage methanogenese overeen met de vermindering van de hoeveelheid CZV die beschikbaar was voor methaanproductie. Dit geeft aan dat de methaanproductie in de UASB-reactor niet negatief werd beïnvloed door remmende effecten van het denitrificatieproces. De verandering in biomassaproductie bij verschuiving van anaerobe naar geïntegreerde omstandigheden werd geschat op een toename van 0.07 naar 0.1 gVS.gCOD $verwijderd^{-1}$.

Na de procestechnische haalbaarheid van de integratie van denitrificatie en methanogenese aangetoond te hebben met synthetisch afvalwater in labschaal UASB-reactoren, werd een semi-technische schaal UASB-reactor gebruikt voor

koolstof- en stikstofverwijdering uit ruw huishoudelijk afvalwater (**Hoofdstuk vier**). Vergelijkbaar met het eerdere experiment werd de UASB-reactor aanvankelijk bedreven als referentie onder strikt anaerobe condities, ditmaal bij een gemiddelde OLR variërend tussen 1.1 en 2.2 $kgCOD.m^{-3}.d^{-1}$. Daarna werd de reactor bedreven onder geïntegreerde anaerobe-denitrificerende condities door middel van de toevoeging van nitraat aan het influent. De toegepaste NLR was 0.05 KNO_3-$N.m^{-3}.d^{-1}$, bij een OLR variërend tussen de 1 en 1.9 $kgCZV.m^{-3}.d^{-1}$.
De resultaten verkregen onder geïntegreerde condities laten zien dat nitraat volledig werd verwijderd en een gemiddelde totale CZV verwijderingsefficiëntie van 68% werd bereikt. Nitraat werd echter niet volledig gedenitrificeerd, omdat 33% van het toegediend nitraat werd gereduceerd via het DNRA proces. Vergeleken met de werking onder strikt methanogene condities, resulteerde geïntegreerde bedrijfsvoering bij een CZV/NO_3-N ratio tussen de 20 en 38 in een vergelijkbare methaanproductie, die kan worden toegeschreven aan twee factoren. Ten eerste trad onder geïntegreerde procescondities een verhoogde mate van hydrolyse op, wat blijkbaar compenseerde voor de verminderde beschikbaarheid van CZV voor methaanproductie als gevolg van CZV-gebruik voor nitraatreductieprocessen. Ten tweede bleef onder geïntegreerde condities de specifieke methanogene activiteit (SMA) van het slib behouden, zeer waarschijnlijk als gevolg van het feit dat een aanzienlijk deel van de methanogene biomassa werd afgeschermd van de geoxideerde N-verbindingen in het binnenste van de slibvlokken.
Van groot praktisch belang is de waarneming dat het geïntegreerd bedrijven niet leidde tot een verslechtering van de bezinkbaarheid van het vlokkig slib. Problemen met slibuitspoeling als gevolg van langdurige geïntegreerde bedrijfsvoering worden daardoor niet verwacht. De geïntegreerde bedrijfsvoering leidde ook niet tot verslechtering van de ontwaterbaarheid van het slib.

De resultaten beschreven in de hoofdstukken drie en vier illustreren het grote potentieel van gelijktijdige koolstof- en stikstofverwijdering uit huishoudelijk afvalwater in UASB-reactoren bedreven met vlokkig slib, en zijn een indicatie voor de haalbaarheid van een eentraps anaeroob/aeroob geïntegreerd systeem. Voor wat betreft het bereiken van een hoge mate van stikstofverwijdering werd de effectiviteit van het systeem echter verlaagd als gevolg van een gedeeltelijke nitraatreductie via het DNRA proces. Hoge CZV/NO_3-N ratio's, normaliter boven de 20, stimuleren nitraatreductie via DNRA. Dit betekent dat bij het bepalen van de keuze voor een ééntraps of tweetraps anaeroob-aeroob systeem de verwachtte CZV/NO_3 in het influent van de geïntegreerde reactor bepalend zal zijn. Hierbij moet rekening worden gehouden met de overweging dat de COD/NO_3 verhouding van het influent van de geïntegreerde reactor in de eentraps anaerobe-aerobe configuratie hoger is dan van het influent van de geïntegreerde reactor in de tweetraps anaerobe-aerobe configuratie.

In de tweetraps anaerobe-aerobe configuratie waarbij de tweede anaerobe trap is geïntegreerd met denitrificatie, is de hydraulische belasting bepalend voor het ontwerp van de tweede trap, aangezien de organische belasting al significant is verlaagd in de eerste trap. De *Expanded Granular Sludge Bed* reactor (EGSB-reactor), die kan worden bedreven bij opstroomsnelheden van 8-10 $m.h^{-1}$ is dan de meest voor de hand liggende configuratie. Bovendien is de hoge belasting met zwevende stof, die de korrelvorming van geïntegreerde biomassa negatief zou kunnen beïnvloeden, in de eerste fase al verminderd. Een ander voordeel van het toepassen van een EGSB reactor voor de tweede trap is dat door de daar voorkomende hoge opstroomsnelheden de bezinkbaarheid van de "geïntegreerde korrels" zou kunnen verbeteren. Veel onderzoekers hebben aangetoond dat methanogenese en denitrificatie met succes kunnen worden geïntegreerd in UASB-reactoren bedreven met korrelig slib (bv. Hendriksen en Ahring, 1996 en Lee *et al.*, 2004). In genoemde onderzoeken gingen de denitrificerende/methanogene slibkorrels (geïntegreerd korrelslib) echter drijven en werden uitgespoeld. Dit werd toegeschreven aan de groei van denitrificeerders in de vorm van een vlokkige biofilm op het korreloppervlak. De hoge opstroomsnelheden in EGSB reactoren kunnen deze vlokkige biofilm verwijderen en daarmee de vorming van stabiel geïntegreerd korrelslib vergroten.

Het tweede deel van dit onderzoek is gericht op het bestuderen van de integratie van methanogenese en denitrificatie in EGSB reactoren voor de behandeling van voorbezonken huishoudelijk afvalwater, een simulatie van een geïntegreerde reactor van een tweetraps anaeroob-aeroob systeem (**Hoofdstuk vijf**). Twee 4.9 1 EGSB reactoren werden gebruikt in de studie; de eerste werd bedreven onder geïntegreerde denitrificerende en methanogene condities, terwijl de tweede bedreven werd onder strikt methanogene condities als referentie om de effecten van geïntegreerde procesvoering te kunnen beoordelen. Beide reactoren werden geënt met methanogeen korrelslib en denitrificatie werd gestart in de geïntegreerde reactor door het toevoegen van synthetisch nitraat in de voeding. Het experiment werd uitgevoerd in twee opeenvolgende perioden, de toegepaste opstroomsnelheden in de eerste en de tweede periode waren respectievelijk 4.5 $m.h^{-1}$ en 8 $m.h^{-1}$.

Tijdens de eerste en de tweede periode, werd respectievelijk 95% en 100% nitraatverwijdering bereikt in de geïntegreerde reactor, die werd bedreven bij een CZV/NO_3-N ratio van 20. Nitraat werd volledig gedenitrificeerd en nitrietaccumulatie werd niet waargenomen.
In de geïntegreerde reactor werd tijdens de eerste en de tweede periode respectievelijk 67% en 69% van de voor methanogenese beschikbare CZV gebruikt voor methaanproductie. De matige methaanvorming is toe te schrijven aan het uitspoelen van geïntegreerde slibkorrels uit de reactor. Blijkbaar was de schuifspanning van de vloeistof veroorzaakt door de toegepaste opstroomsnelheden van 4.5 $m.h^{-1}$ en 8 $m.h^{-1}$ niet voldoende om ophoping van vlokkige biofilm aan het oppervlak van de geïntegreerde korrels te voorkomen.
Van praktisch belang is het behoud van de specifieke methanogene activiteit van het slib gekweekt onder geïntegreerde condities. Indien de slibuitspoelingproblemen worden beheerst, kunnen EGSB reactoren onder geïntegreerde condities worden bedreven bij organische belastingen die vergelijkbaar zijn met die van strikt methanogene reactoren.

De resultaten in hoofdstuk vijf tonen de haalbaarheid aan van geïntegreerde denitrificatie en methanogenese in EGSB reactoren ten behoeve van gelijktijdige verwijdering van koolstof en stikstof uit voorbezonken huishoudelijk afvalwater. Echter, om een succesvolle bedrijfsvoering van een geïntegreerde proces in een EGSB reactor te kunnen garanderen, dienen de slibuitspoelingsproblemen moeten worden opgelost. De resultaten hoofdstuk vijf tonen aan dat het periodieke toepassen van zeer grote afschuifkrachten, hetzij door een periodieke verhoging van de opstroomsnelheid, hetzij door periodieke verplaatsing van het slibbed van onder naar boven in de reactor, de bezinkbaarheid van de geïntegreerde korrels substantieel verbeterde.

Conclusies

- Gelijktijdige verwijdering van koolstof en stikstof uit huishoudelijk afvalwater is mogelijk door het toepassen van geïntegreerde denitrificerende en methanogene processen in één reactor.

- De methanogene activiteit van vlokkig dan wel korrelvormig slib, gekweekt onder geïntegreerde denitrificerende en methanogene condities, is vergelijkbaar met de methanogene activiteit van slib gekweekt onder strikt methanogene condities. Zodoende kunnen onder methanogene en onder geïntegreerde condities vergelijkbare organische belastingen worden toegepast.

- In UASB reactoren die bedreven worden onder denitrificerende en methanogene condities bij een gemiddelde COD/NO_3-N ratio van 33 wordt nitraat onvolledig gedenitrificeerd en wordt deels omgezet in ammonium via het DNRA proces.

- Bij gebruik van semitechnische schaal EGSB reactoren is de afschuifspanning op het slib veroorzaakt door de vloeistofopstroomsnelheden van 4.5 m.h^{-1} en 8 m.h^{-1} onvoldoende om te voorkomen dat er ophoping van vlokkig biofilm plaats vindt aan het oppervlak van korrels, die worden gekweekt onder geïntegreerde denitrificerende en methanogene condities.

- De periodieke verhoging van de toegepaste opstroomsnelheid of de periodieke verplaatsing van het slibbed van onderin naar bovenin de reactor geeft een aanzienlijke verbetering van de bezinkingskarakteristieken van slibkorrels gekweekt onder geïntegreerde condities in een EGSB reactor

Aanbevelingen

- De prestaties van “geïntegreerde systemen” onder de milieucondities van landen met een droog klimaat moet nog worden aangetoond door middel van onderzoek op pilot-schaal en/of demonstratieschaal.

- Verder onderzoek naar de DNRA route wordt aanbevolen voor het optimaliseren van de geïntegreerde bedrijfsvoering voor maximale stikstofverwijdering, d.w.z. volledige nitraatreductie via het denitrificatieproces.

- Er dient nader onderzoek te worden verricht naar het verbeteren van de bezinkbaarheid van geïntegreerde slibkorrels. Gelet op de beperkingen van hydraulische testen op laboratoriumschaal, wordt demonstratie van een EGSB-reactor op pilot-schaal ten zeerste aanbevolen.

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List of Abbreviations

ABF	Aerated bio-filter
ABR	Anaerobic baffled reactor
AF	Anaerobic filter
AH	Anaerobic hybrid reactor
Amm_{pr}	Ammonium production
ANANOX	Anaerobic-anoxic-oxic
AS	Activated sludge
BWKW	Mixture of black water and kitchen waste
COD_{col}	Colloidal COD
COD_{pf}	Paper filtered COD
COD_{sol}	Soluble COD
COD_{ss}	Suspended COD
COD_t	Total COD
CST	Capillary suction time
CSTR	Completely stirred tank reactor
DHS	Down flow hanging sponge reactor
DNRA	Dissimilatory nitrate reduction to ammonium
EGSB	Expanded granular sludge blanket
FFB_{ae}	Aerobic fixed film
FFB_{an}	Anaerobic fixed film
F/M	Food to microorganisms ratio
GLSS	Gas- liquid- solid separator
HRT	Hydraulic retention time
HUSB	Hydrolysis up flow sludge bed
JLR	Jet loop reactor
K_m	Monod half saturation constant
MBBR	Moving bed biofilm reactor
$N_{2\ stripped}$	Stripped nitrogen
NLR	Nitrate loading rate
OLR	Organic loading rate
PROSAB	Brazilian research program on basic sanitation
RBC	Rotating biological contactor
SBR	Sequence batch reactor
SDA	Specific denitrification acitiviy
SMA	Specific methanogenic activity
SRT	Sludge retention time
SuLR	Surface loading rate
SVI	Sludge volume index
TF	Trickling filter
TKN	Total kjeldahl nitrogen
TN	Total nitrogen
TS	Total solids
TSS	Total suspended solids
TVS	Total volatile solids
UABR	Up flow anaerobic biofilm reactor
UASB	Up flow anaerobic sludge blanket
UASR	Up flow anaerobic solids removal
UBAF	Up flow biological anaerobic filter
UBBR	Up flow multi layer bio-reactor

USB	Up flow sludge blanket
VFA	Volatile fatty acids
VS	volatile solids
VSS	Volatile suspended solids
r	Recycle to feed ratio
Y_{anaer}	Anaerobic yield
Y_{anox}	Anoxic yield
χ	Filterability constant
η	Viscosity of water

Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment

CERTIFICATE

The Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment
(SENSE), declares that

Ghada Nassri Kassab

Born on: *20 February 1972* at: *Baghdad, Iraq*

has successfully fulfilled all requirements of the
Educational Programme of SENSE.

Place: *Wageningen* Date: *17 June 2009*

the Chairman of the SENSE board

Prof. dr. R. Leemans

the SENSE Director of Education

Dr. A. van Dommelen

The SENSE Research School declares that Ms. Ghada Nassri Kassab has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 32 ECTS, including the following activities:

SENSE PhD courses:

- Environmental Research in Context
- Research Context Activity: Writing a journalistic article on the feasibility of applying anaerobic technology for pre-treatment of strong domestic sewage in Jordan
- Biological Processes in Environmental Technology
- Basic and Advanced Statistics

Other PhD courses:

- Efficient management of wastewater, its treatment and reuse, in the Mediterranean countries-Jordan, Lebanon, Palestine and Turkey.
- Bio-solids risk assessment and standards development
- Techniques for writing and presenting scientific papers.
- Training of trainers
- Research approaches and strategies

Research and Management Skills:

- Extended literature study, Sequential anaerobic-aerobic treatment for domestic wastewater- A review
- Organization of scientific workshop, International Training Program on Trans-boundary Water Management (TWM) in the Middle East and North Africa (MENA)
  2-13 November 2008, Jordan

Presentations:

- Oral Presentation: Adjusting nitrogen concentrations to agricultural demand in a UASB reactor, 5 October 2005, Punta del Este, Uruguay
- Poster Presentation: Integrating methanogenesis and denitrificaiton in EGSB reactor for adjusting effluent nitrogen level, 10 October 2007, Antwerp, Belgium

Mr. J. Feenstra
SENSE Coordinator PhD Education and Research

Curriculum Vitae

The author of this thesis, Ghada Kassab was born in 1972 in Baghdad/Iraq. In, 1994, she obtained her Bachelor of Science degree in Chemical Engineering from the University of Jordan. In 1999, she was awarded the degree of Master of Science in Environmental and Water Resources Engineering from the Civil Engineering department of University of Jordan. Right after that she started to work as a research assistant in the Water and Environment Research and Study Center/University of Jordan, participating in research projects focused on low cost wastewater treatment. In 2002 she got a scholarship from University of Jordan funded by the Dutch government to study at the Sub-department of Environmental Technology in Wageningen University. From the beginning of 2003 to the mid of 2007 she performed the experimental work as described partially in the thesis.

Her address in Jordan
Water and Environment Research and Study Center/University of Jordan
Zip code 11942
Amman/Jordan
Email: gh.kassab@gmail.com

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