Propositions

1. Adsorption in the biofilm followed by sedimentation comprises the major removal mechanism for E.Coli in a Rotating Biological Contactor (RBC).

   This dissertation.

2. Since the major part of the residual E.Coli in the final effluent of Rotating Biological Contactor (RBC) is associated with particles with a size ranging between (0.45 - 4.4 µm), the removal of the colloidal dispersed fraction represents the limiting step in the RBC.

   This dissertation.

3. By applying the Rotating Biological Contactor (RBC), practically all serious drawbacks of stabilization pond systems as post-treatment of anaerobically pre-treated domestic sewage can be overcome, and therefore this system looks extremely attractive for application in developing countries. Because of the main shortcomings of stabilization ponds such as very long hydraulic retention time and low biomass concentrations, large land area requirements for pond construction are needed Yu *et al.*, (1997).

   Yu H, Tay J.H. and Wilson F. (1997) A sustainable municipal wastewater treatment process for tropical and subtropical regions in developing countries. Wat. Sci. Tech. Vol. 35, No. 9, pp. 191-198.

4. Based on the results concerning the so-called minimum water quality for the production of paper, managers of the paper industry should be convinced that water re-use is not only desirable from an environmental point of view but also is economically represent a quite feasible option (Allen *et al.*, 1999).

   Allen, L., Polverari, M., Levesque, B., Francis, W. (1999) Effect of system closure on retention- and drainage-aid performance in TMP newsprint manufacture. Tappi Journal, Vol. 82, No. 4.

5. Although the occurrence of sorption of ammonia in/on activated sludge and biofilms usually is ignored, it may represent a very important phenomenon at alternating process operational conditions (Nielsen,1996).

   Nielsen, P.H. (1996) Adsorption of ammonium to activated sludge. Wat. Res. Vol. 30, No. 3, pp. 762 - 764.

6. Regarding the significantly higher investments and operational costs needed for complying with the far too strict standards of the World Health Organisation (WHO) for E.Coli in treated wastewater, it is for developing countries highly questionable

whether they should attempt to comply with these standards, particularly also considering the prevailing situation of public health risks in these countries and regarding the many other priorities they have for spending the available scarce funds.

7. Poor people are hungry due to a serious lack of practically all primary needs, the rich due to a shameless unsuitable greed .

Propositions belonging to the thesis entitled "The biorotor system for post-treatment of anaerobically treated domestic sewage"

Ahmed Tawfik Ibrahim
Wageningen, 8 January 2002.

THE BIOROTOR SYSTEM FOR POST-TREATMENT OF ANAEROBICALLY TREATED DOMESTIC SEWAGE.

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THE BIOROTOR SYSTEM FOR POST-TREATMENT OF ANAEROBICALLY TREATED DOMESTIC SEWAGE.

AHMED TAWFIK IBRAHIM

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ABSTRACT:

Tawfik A. I. (2002). The biorotor system for post-treatment of anaerobically treated domestic sewage. Ph-D Thesis, Wageningen University & Research Centre, Wageningen, The Netherlands.

This thesis describes the evaluation of the applicability of biorotor system for post-treatment (polishing) of different effluent qualities of an UASB reactor treating raw domestic sewage, with emphasis on the elimination of various COD fractions, ammonia and E.Coli.

The removal mechanism of E.Coli from UASB effluent using a RBC has been investigated. The results obtained revealed that an adsorption process and sedimentation comprise the most important removal mechanism of E.Coli in the biofilm. Die-off is relatively minor importance as removal mechanism in a RBC system.

The performance of an anaerobic versus aerobic RBC system treating a high quality UASB reactor effluent was investigated at the same HRT and OLR. The results obtained indicated that the removal efficiency of the COD fractions and of E.Coli fractions found in the aerobic RBC significantly exceeds that of the anaerobic unit. Therefore, the results of our investigations strongly support the use of an aerobic RBC as a post-treatment step of UASB reactor effluents.

When applying a single and two stage aerobic RBC at the same OLR of 14.5 g COD_{total} $.m^{-2}.d^{-1}$ and at a HRT of 2.5 h., but at different temperatures of 24 and 17 °C respectively, both systems provided the same residual effluent values for COD_{total} (72 mg l^{-1}), for $COD_{suspended}$ (16 mg l^{-1}), for $COD_{colloidal}$ (5 mg l^{-1}) and for $COD_{soluble}$ (51 mg l^{-1}). Moreover, also the removal efficiency of E.Coli was almost the same, viz. amounting to 94 %. However, the ammonia removal in the single stage RBC amounted to 50 % of which 71 % was nitrified, compared to only 23 % in the two-stage system. This better performance can be attributed to the higher temperature of the wastewater during the operation of the single stage RBC system. In view of these results, we recommend to use a single stage RBC system for COD removal and for a partial nitrification and E.Coli removal at OLR of 14.5 g COD_{total} $.m^{-2}.d^{-1}$ and at HRT of 2.5 h for post-treatment of a high quality UASB reactor effluent.

We investigated the use of anoxic reactor followed by a segmental two stage aerobic RBC for nitrogen removal from the nitrified effluent. The results obtained reveal that the introduction of an anoxic reactor as a 1st stage combined with recirculation of the nitrified effluent of the 2nd stage RBC is accompanied with a conversion of nitrate into ammonia,

at least in case the content of COD $_{biod.}$ in the UASB effluent is low. Therefore, the introduction of a separate anoxic reactor for denitrification as final post-treatment step can not be recommended in such a situation.

In one of the experiments the UASB reactor was operated at two different operational temperatures viz. of 30 and 11°C resulting in quite different COD $_{biod.}$ concentrations in the UASB effluent. For the post-treatment of this highly different effluent a single stage RBC was operated at a constant HRT of 1.25 h., consequently at COD $_{biod.}$ loading rates of 17.7 and 36.8 g m^{-2}. d^{-1}. The results clearly show that the residual values of COD fractions and E.Coli are significantly lower at the lower imposed COD $_{biod.}$ loading rate of 17.7g COD $_{biod.}$ m^{-2}. d^{-1}. We also compared the efficiency of the two-stage RBC system for this highly different UASB effluent, viz. once again at the same HRT (2.5 h) and at COD $_{biod.}$ loading rate of 9 and 18 g m^{-2}. d^{-1}. The results reveal that with the two-stage RBC system the residual values of distinguished COD fractions in the final effluent were almost the same, but the residual value of E.Coli in the final effluent amounted to 3.4 x 10^5 at the higher COD $_{biod.}$ loading rate and to 7.6 x 10^4 /100 ml at the lower one. Moreover, the calculated nitrification rate in the 2nd stage of two stage RBC system dropped from 1.56 to 1.1 g NO_3-N.m^{-2}.d^{-1} with an increase the COD $_{biod.}$ loading rate from 11.3 to 16 g m^{-2}.d^{-1}. The results clearly demonstrate that the introduction of a well performing UASB reactor not only improves the nitrification rate but also the E.Coli removal in the post-treatment system.

We compared the performance of the single with that of a two-stage RBC for the treatment of poor quality UASB reactor effluent. The results obtained showed that the COD fractions and the E.Coli content in the final effluent of a two stage were lower than in the effluent of the single stage RBC. Moreover, The calculated nitrification rate in the single stage was much lower compared with the two stages RBC. Based on these results we recommend a two stage RBC system for post-treatment of poor quality UASB reactor effluent. The two-stage system was operated at different HRT's and OLR's in order to assess better design criteria for the system. The removal efficiencies for the various COD fractions decreased only slightly when decreasing the HRT from 10 to 2.5 h., and increasing the OLR from 6.45 to 24 g COD $_{total}$ m^{-2}.d^{-1}. However, the overall nitrification efficiency and E.Coli were negatively affected when increasing the loading conditions in the range investigated. The results found for E.Coli removal revealed that the major part of suspended E.Coli (>4.4 µm) was eliminated by sedimentation or by adsorption in the biofilm of the 1st stage (99.66 %). However, E.Coli present in the colloidal fraction (< 4.4 - > 0.45 µm) was eliminated in the 2nd stage of two stage RBC (99.78 %). Based on these results we recommend for the treatment of a poor UASB effluent quality the use of two stages RBC system for the removal of COD fractions and

ammonia and for a partial removal of E.Coli at HRT of 10 h and OLR of 6.45 g COD $_{total}.m^{-2}.d^{-1}$.

The effluent of two stages still cannot be used for unrestricted irrigation purposes, at least according to the standards provided by the WHO with respect to the E.Coli content. Therefore, in order to meet these (very stringent) standards, we investigated the use of a three stage RBC-system for post-treatment of an effluent from a rather poorly performing UASB reactor. This three stage RBC was first operated at a HRT of 3.0 h. Under these conditions the E.Coli count in the final effluent was still too high. However, when applying an HRT of 10 h., the E.Coli content complied almost the WHO standards for unrestricted irrigation purposes. Therefore, when such high removal efficiency for E.Coli really would be required, the best solution is to use three independent stages at HRT of 10 h., which then obviously implies very significantly investment and operational costs.

CONTENTS

1

Introduction

GENERAL INTRODUCTION

BACKGROUND

In many countries of the world, the problem of providing adequate water supplies has become a very critical issue, as the demand for water increases in direct proportion to the explosive increase of population and the rapid development of industry. To meet these tremendous needs worldwide, elaborate policies targeting towards the ultimate goal of maximising the benefits obtained from every drop of water should be established.

Generally, most of the developing countries depend on agricultural activities for the development of their economy. A situation which seems to continue for the coming years. Looking to the future of the increasing water demands, whether for drinking purposes, industrial development or agricultural expansion, it is quite obvious that integrated management programs for the available water resources is required. Meanwhile the responsible authorities should urgently consider a strategy for the proper management and development of non-conventional water resources.

The reuse of treated domestic wastewater in agriculture is a global practice of particular importance to developing countries. Wastewater reuse provides both an additional supply of water for irrigation and a low-cost source of nutrients and organic material which, can act as crop fertiliser and soil conditioner. The World Health Organisation has stated that the removal of pathogenic organisms is the principal objective for treatment of wastewater that is destined for reuse in agriculture (WHO, 1989).

The degree of treatment required varies according to the specific reuse application and associated water quality requirements. Therefore, it becomes more and more important to develop appropriate wastewater treatment processes, which combine high treatment efficiency with low capital investment and maintenance costs, as well as simple operation requirements.

Anaerobic treatment processes which represent a sustainable treatment option, are efficient for the removal of organic matter, suspended solids and intestinal nematode eggs from domestic wastewater. Anaerobic treatment processes don't consume energy, but in fact produce useful energy in the form of biogas (Zeeman and Lettinga,

1999). Moreover, they represent systems that can be applied at any scale and almost anywhere.

Despite these advantages, anaerobic processes cannot always produce effluents that can comply with the standards for reuse in irrigation purposes viz. the standards set by WHO for pathogens. Therefore, a post-treatment step is of great importance to achieve the required standards. The main objectives of the post-treatment are to remove pathogens and residual organic matter (colloidal particles), and to promote the removal of components, which are rarely affected by the anaerobic treatment such as nutrients (Lettinga, 1996 and Collivignarelli *et al.*, 1990), although latter is only some during non -growth seasons.

TREATMENT OF THE ANAEROBIC EFFLUENT.

The post-treatment system to be selected strongly depends on the characteristics of the anaerobic effluent and on the standards set by the authorities for reuse of treated wastewater. In general two situations can be distinguished with respect to the required effluent quality i.e.

- for discharge into surface water where generally strict effluent standards apply for the parameters, such as BOD, SS, NH_4^+-N, NO_2—N, NO_3-N, phosphates and pathogens,
- for irrigation where generally only pathogens, organic matter, BOD (COD), and SS have to be reduced. But in some cases, e.g. during specific periods of the growth season also restriction may apply for the NH_4^+-N and PO_4^{-3} content.

In order to satisfy the different standards, post-treatment processes have been developed and / or investigated for control of the nitrogen content by nitrification-denitrification, of sulphide content by sulphide oxidation, and the phosphate by chemical treatment, SS by precipitation, BOD by biological treatment, and the pathogen content by biological oxidation and/or disinfection (Bovendeur *et al.*, 1990; Buisman *et al.*, 1990; Penetra *et al.*, 1999; Collivignarelli *et al.*, 1990 and El-Gohary 1998).

1. Pathogenic bacteria removal.

Because of their size, freely dispersed pathogenic bacteria may be considered as living colloidal particles. Usually they have a net negative surface charge in the pH range of natural waters (Loder and Liss, 1985). Although biofilms are also negatively charged at this pH range, it is still possible that pathogenic bacteria become adsorbed on the biofilm. Extra-cellular Polymeric Substances (EPS) excreted by organisms provide the possibilities for sorption of pathogenic bacteria on/in the biofilm. When approaching a biofilm - pathogenic bacteria may attach to a polymeric

molecule on the biofilm, thus forming a "bridge" between the biofilm and pathogenic organism.

The precise mechanism by which bacteria of faecal origin become entrapped in the biofilm systems is not yet clearly understood. In general, the removal of pathogenic bacteria is a combination of physical and biological reactions. Physical processes include adsorption (attachment to biofilm, algal biomass or duck weed surface) and sedimentation (when pathogenic bacteria adsorb to or become entrapped by particles or flocs). Biological removal mechanisms for pathogen include antibiosis, exposure to biocides, predation, and attack by lytic bacteria, natural die-off and competition for limiting nutrients or trace elements (Green *et al.,* 1997). In addition also certain climatological factors such as sun light, temperature and wind may be responsible for the elimination of pathogen.

Table 1. Summary of recent results for pathogenic bacteria removal in post-treatment of UASB effluent by using different systems.

Process or configuration	HRT	Influent	Effluent	%R	Guideline of WHO (1989) 1000 /100 ml for unlimited irrigation.	References
Integrated Pond system 10 ponds (2 duckweed, 3 algae ponds and 5 duck weed ponds)	4.2 days	($6.3*10^5$-$1.3*10^6$)	($3.3*10^2$-$0.5*10^3$)	(99.9-99.99)	The final effluent being suitable for unrestricted irrigation.	Van der steen *et al.,* (1999)
Waste Stabilisation Pond (WSPS) 1 facultative + 3 maturation Ponds	20.0 days	($9.2*10^6$)	($6.1*10^2$)	(99.998)	The final effluent being suitable for unrestricted irrigation.	Dixo *et al.,* (1995)
Algal Pond	10.0 days		($2.9*10^2$)		The final effluents being suitable for unrestricted irrigation.	El-Gohary (1998)
Lemina Pond	10.0 days		($3.1*10^4$)		The final effluents being unsuitable for unrestricted irrigation.	
Rotating Biological contactor			($2.7*10^3$-$3.2*10^4$)		The final effluents being unsuitable for unrestricted irrigation.	
Waste stabilisation ponds	20 days		$2.5*10^5$		The final effluents being unsuitable for unrestricted irrigation.	Ghosh *et al.,* (1999)

It is therefore, of prime importance to understand the mechanism of pathogen removal from anaerobic effluent in post-treatment systems; e.g. biofilm reactors in order to develop the optimum design parameters for selected the post treatment system. Recently, different systems have been used for removal of pathogenic bacteria from the UASB effluent (Table 1). In the following section different mechanisms for the removal of pathogens will be discussed.

a) Adsorption

The adsorption of pathogenic bacteria in/on a biofilm has been studied in several systems. Ueda and Horan (2000) used a membrane bioreactor to achieve indigenous bacteriophage removal from settled domestic sewage. The membrane alone demonstrated a poor phage removal efficiency, but the removal efficiency increased after the formation of biofilm. This has also been proved by LeChevallier *et al.,* (1987) who isolated a large variety of different heterotrophic bacteria (including potentially pathogenic bacteria) from the biofilm. Also investigations carried out by Van Der Drift *et al.,* (1977) indicated that the removal of Escherichia Coli from wastewater treated with activated sludge is a two-step process. In the first step, a rapid adsorption of bacteria on the sludge flocs takes place. This is followed by a slower elimination of bacteria, presumably due to predation by ciliated protozoa. Omura *et al.,* (1989) studied the removal of coliform bacteria, enterococcus bacteria, and coliphages in two sewage treatment plants, one using the activated sludge process and the other using a high-rate trickling filter. Removal efficiencies of Coliform bacteria, Enterococcus bacteria, and Coliphages were 91.6 %, 97.0 %, and 96.6 % respectively, in the activated sludge system, and 96.4 %, 98.3 % and 81.5 %, respectively, in the trickling filter. The removal could mainly be attributed to adsorption on the biomass in the activated sludge and on the slime in the trickling filter. They also found evidence that the die-off rate of the microorganisms seems to play a minor role in the count reduction. Likewise the investigation of Feachem (1978) which showed that the removal, of various types of pathogenic bacteria and helminthes in trickling filters ranged from 0 to 2 $\log_{10}$ unit reductions. Bio-growth of films in slow sand filters was found to be essential for the effective removal of pathogenic bacteria (Schuler *et al.,* 1991). According to the results of Banks and Bryers (1992) the development of biofilms on glass, polycarbonate, and granular activated carbon surfaces enhances the removal of pathogenic bacteria.

Two Rotating Biological Contactors (RBC), a bench scale and a pilot-plant scale were investigated by El-Zanfaly and El- Abagy (1987) for treatment of domestic sewage in the lab. and natural conditions respectively. The removal efficiencies for total coliform (97.82 %), faecal coliform (99.74 %) and faecal streptococci (97.93 %) in the pilot-scale RBC were much better than that found for the lab-scale reactor where the removal values were 92.76 % for total coliform, 90.97 % for faecal coliform and 87.69

% for fecal streptococci. The higher removal of pathogen in the pilot-plant RBC system could be attributed to the positive effect of the environmental factors such as sunlight. However, residual bacterial densities were still high enough to represent a potential health hazard (10^9 for total coliform, 10^6 for faecal coliform and 10^6 MPN-index / 100 ml for faecal streptococci).

b) Sedimentation

It is known that some of the pathogenic bacteria are associated with the coarse suspended solids. Therefore, sedimentation plays an important role for removal of faecal coliform e.g. from surface waters (Mitchell and Chamberlin, 1978). Gannon *et al.,* (1983) observed an accumulation of viable feacal coliform bacteria on the sediment surface in Ford Lake, Mich., and concluded that sedimentation represents an important mechanism for the removal of faecal coliforms bacteria from the water phase. Auer and Niehaus (1993) studied the distribution pattern of faecal coliform bacteria on particles of seven size classes in surface water. The majority of the faecal coliform bacteria were found to be associated with two particle classes, 0.45 - 1 and 6 - 10 µm. To simplify the analysis, all particles were assigned to one of these two groups: small (0.45 - 10µm) and large (> 10µm). On the average 90.5 % of the faecal coliform bacteria were found to be associated with small particles category and 9.5% were associated with the large one.

c) Predation

Pathogenic bacteria are considered part of the food chain in biological wastewater treatment systems. They are predated by higher organisms, especially protozoa and nematodes, which are strict aerobic organisms.

RBC systems generally consist of several segmental stages, which are operated, in series and therefore they can be considered plug-flow systems. Therefore, the biofilm produced on the last bio-discs is usually rich in protozoan and metazoan organisms. Most of the research carried out by Landon-Arnold (1985) was on RBC protozoa and metazoa. The colonisation dynamics of ciliated protozoa in a full-scale activated sludge plant and in a RBC pilot plant was investigated by Madoni (1994) in an attempt to quantify their presence in terms of biomass. Ciliate biomass reached values of 250 mg/l (dry weight) in the activated sludge and 314 µg/cm^2 (dry weight) in the RBC biofilm. The ciliate biomass/volatile solids ratio was over 9.0 % for the activated sludge and 12 to 19 % for RBC biofilm. This emphasises the importance of using a rotating biological contactor system for the removal of pathogenic bacteria.

Several researchers suggested that flocculation and/or predation by protozoa represent important factors for the removal of E.Coli. Heukelekian and Roudolfs (1929) already presented circumstantial evidences, suggesting the responsibility of ciliated protozoa for the removal of E.Coli. Curds *et al.,* (1968) demonstrated that

ciliated protozoa are responsible for a significant reduction in the total viable counts of bacteria in activated sludge effluents. The removal of E.Coli from sewage in two bench – scale activated sludge plants was investigated by Curds and Fey (1969) in which protozoa were absent in the sludge. Ciliated protozoa were added to one of the plants in order to assess their importance The half-life time of E.Coli in the plant without protozoa was 16 h, while it was only 1.8 h in presence of protozoa. Curds (1992) demonstrated that ciliates play an important role in the removal of dispersed bacterial growth by predation in aerobic biological wastewater treatment processes such as activated sludge, percolating filters and rotating biological contactors. Decamp and Warren (1998) has demonstrated a similar role of ciliates in the root zone in constructed wetlands (Reed Beds) for E.Coli removal. Predation includes the bacterivorous activity of nematodes, rotifers and protozoa and was considered by Green *et al.,* (1997) as an important factor for the removal of bacteria from wastewater's in constructed wetlands. It is clear that protozoa play an important role in the removal of E.Coli from domestic sewage at low organic loading rate.

d) Die off.

The direct impact of the presence of dissolved oxygen in water on E.Coli removal is not well known. However, dissolved oxygen might have a positive influence on the removal of organic matter and consequently on the growth of competing (and possibly predating) organisms. Hanes *et al.,* (1964) studied coliform die-off in a 1% dilution of three types of sewage at three different DO concentrations at 20 °C. After an initial lag phase, they found that the average die-off rate (d^{-1}) of coliform in the three types of diluted sewage were -0.19 at DO = 0.3 - 0.4 mg/l, -0.53 at DO = 7.6 - 8.0 mgl^{-1} and -0.44 at DO = 37.8 - 37.9 mgl^{-1}. The presence of oxygen appeared to increase coliform die-off. However, higher DO concentrations than the normal saturation value did not affect the die-off rate. On the other hand, Pearson *et al.,* (1987) found no effect of aeration on the die-off rate of faecal coliform isolates under starvation conditions at 25 °C. Other researchers such as Maes (1986) however reported a considerable degree of die-off both of coliforms and faecal streptococci directly related to the degree of oxygenation. Ohgaki *et al.*, (1986) found that the concentration of coliphage during daytime decreased under high concentrations of dissolved oxygen results from photosynthesis. On the other hand, the concentration of coliphage increased after sunset due to the drop in the dissolved oxygen concentration to zero as a result of respiration of algae. A strong influence of DO on the inactivation of faecal coliforms in WSPs was observed by Curtis *et al.,* (1992). Dissolved oxygen dependence on sunlight inactivation strongly suggested that a photo-oxidative process be involved. Likewise Davies-Colley *et al.,* (1997) found that sunlight inactivation of E.Coli is strongly dependent on DO and on pH > 8.5 in Waste Stabilisation Pond (WSP).

The effect of aeration on the die-off rate of different bacterial indicators in the wastewater lagoon of La Velles (Salamanca, Spain) has been investigated by Fernandez *et al.,* (1992). They found evidence that partial aeration of the lagoons increased the die-off of total coliforms, faecal coliforms, faecal streptococci and sulphite reducing clostridia from 92, 91, 95 and 17 % to 99, 99, 97 and 53 % respectively. Dissolved oxygen obviously is therefore, indispensable for the growth of strictly aerobic organisms. Since these organisms are competitors to, or predators of enteric organisms, the presence of oxygen could be considered a favourable parameter for pathogen die-off.

The influence of pH on the pathogenic bacteria removal depends on the nature of the bacterial surfaces and ionic strength of the solution (Harvey, 1991). The pH affects bacterial surface zeta potential due to dissociation of the carboxylic and amino groups located on the bacterial cell wall (Gannon *et al.,* 1991). The effect of pH on bacteria removal also depends on the iso-electric point of the bacterial species. Since the pH of domestic wastewater generally is close to 7.0 (Canter, 1985), the effect of pH on the removal of bacteria is minimum. Saqqar and Pescod (1992) found that the rate of faecal colifoms die-off increases with increasing pH in the oxidation ponds. Likewise Pearson *et al.,* (1987) and Mills *et al.,* (1992) found that pH values around 9.0 or more increased faecal coliform die-off particularly under nutrient deficient conditions in waste stabilisation ponds. Rangeby *et al.,* (1996) reported that the die-off rate, K_d in wastewater stabilisation pond system treating domestic wastewater was high at higher pH values.

2. Colloidal particles removal.

The removal of colloidal particles, which represent 20 – 30 % of the total COD in domestic sewage is the limiting factor in an anaerobic treatment process (El-mitwalli, 2000). As a result, effluents of anaerobic treatment systems contain relatively high concentrations of colloidal particles.

In removing colloidal particles contrary to anaerobic reactors, aerobic systems such as aerobic biofilm are quite effective. According to published research, there are two possible removal mechanisms (Table 2 and Fig. 1).

According to the hypothesis No. 1, (Table 2) surface adsorption is the first step in the sequence of degradation of non-diffusible organic matter in biofilms .The adsorbed substrate would then be hydrolysed to diffusible substrate on the surface of the biofilm. It is generally assumed that no bulk liquid hydrolysis takes place. The hypothesis is based on experimental results in experiments with activated sludge systems, originally obtained using soluble starch as a model substrate (Banerji *et al.,* 1968). In biofilm reactors, experimental support for the hypothesis was found in a

methanogenic fluidised bed reactor treating full milk (Sprouse and Rittmann 1990). They also found no indication of bulk liquid hydrolysis in their reactor system.
In the more recent new hypothesis (Table 2), the importance of surface adsorption is assumed to be negligible. Instead, bulk liquid hydrolysis is suggested to be the important step of the degradation mechanism. The mechanism of degradation is described as follows: the microorganisms in the biofilm produce free and membrane-bound extracellular hydrolytic enzymes. The free extracellular enzymes diffuse out of the biofilm into the bulk liquid. Due to the hydrolytic enzyme activity in the bulk of the liquid, non-diffusible substrate is hydrolysed into diffusible substrates ("diffusible" meaning "diffusible in the biofilm"). The diffusible substrate diffuses into the biofilm where it is hydrolysed by membrane-bound extracellular enzymes to degradable substrate ("degradable" meaning that the molecules are small enough to be taken up by the microorganisms).

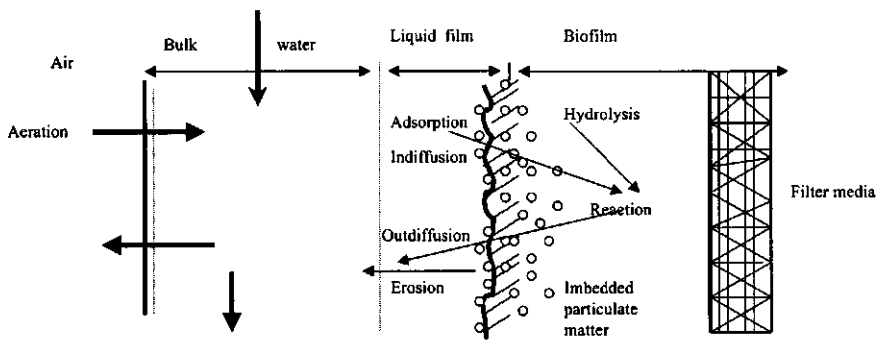

Fig. 1 Schematic illustration of the phenomena involved with fixed-biofilm performance (Harremoës and Gönenc, 1983).

Table 2. Hypothesis of removal of colloidal particles in biofilm system.

Hypothesis No. 1 (Harremoes, 1993; Bouwer, 1987)	Hypothesis No. 2 (Larsen and Harremoes, 1994).
(1) adsorption onto the biofilm surface	(1) bulk liquid hydrolysis
(2) hydrolysis on the biofilm surface	(2) diffusion into the biofilm
(3) diffusion into the biofilm	(3) hydrolysis within the biofilm
(4) degradation within the biofilm	(4) degradation within the biofilm

3. Nitrogen removal

In wastewater treatment processes microorganisms remove part of the nitrogen, because it is required for growth. The amount of nitrogen assimilated during oxidation

of carbonaceous material has been generally estimated to be around 5% of the oxygen demand (that is, BOD: N = 20: 1) (Clark and Viessman 1965). The consequence is two-fold: nitrogen must be present for biological oxidation of carbonaceous material, and ammonia removal occurs during biological treatment of wastewaters because of assimilation.

The principle of biologically induced nitrogen removal in wastewater treatment facilities is based on the activity of autotrophic nitrifying bacteria and of heterotrophic denitrifying bacteria, which are capable to oxidise and reduce sequentially nitrogen from ammonia to nitrate and to nitrogen gas respectively. Nitrification is the oxidation of ammonia to nitrate, and denitrification is the reduction of nitrate to nitrogen gas.

Nitrification is used to control levels of ammonia in wastewater effluents, but for control of total nitrogen levels in wastewater effluents both nitrification and denitrification must be implemented. The importance of nitrogen control in wastewater effluents is due to its impact on receiving water bodies. As ammonia becomes oxidised to nitrate, the dissolved oxygen level is decreased. Deionized ammonia-nitrogen at concentrations of 0.25 to 0.30 mgl^{-1} are lethal to fish within 14 to 21 days (Smart, 1979). Nitrate is readily available for assimilation by plants, causing algal blooms (Sawyer and Mc Carty 1967). Also, nitrate can cause methemoglobinemia for infants when contaminated water is used as a drinking water supply source (Gruener and Shuval 1973). On the other hand nitrate in treated wastewater is very important for irrigation purposes for its fertilising value.

a) Nitrification.

The oxidation of ammonia to nitrate proceeds via nitrite and is performed sequentially by the autotrophic bacteria *Nitrosomonas* SP and *Nitrobacter* SP. The overall oxidation of ammonia by these organisms is given by the following equation:

$$NH_4^+ + 2O_2 + 2HCO_3^- \rightarrow NO_3^- + 2H_2CO_3 + H_2O \qquad (1)$$

The nitrification activity and bacterial growth rate are influenced by factors like pH, alkalinity, oxygen, temperature and organic loading rate as well as the presence of inhibitory compounds (e.g. sulphides). The different factors may have a direct influence on the enzyme activity and growth rate or an indirect effect through the biomass / biofilm structure, the diffusion rates, the solubility of oxygen etc. Also, synergistic effects between some parameters may occur. It is, therefore difficult to evaluate the effect of the different factors separately.
Typical nitrification rates in biofilm processes, defined by the apparent removal rate are summarised in Table 3a.

Alkalinity and pH

During the oxidation of ammonia carbonate is utilised for neutralisation (Eq. 1), leading to the carbonic acid (CO_2) production. This microbiologically induced change in the carbonate buffering system, results in the destruction of bicarbonate alkalinity at a rate of 7.1 mg (as $CaCO_3$)) per mg of ammonia oxidised (EPA, 1975). The bicarbonate alkalinity is reduced by about 0.14 meq per mg ammonia oxidised to nitrate. In biofilm processes, Siegriest and Gujer (1987) found that the nitrification rate declines, if the residual alkalinity is below about 1.5 $meql^{-1}$ (The relationship between residual alkalinity and nitrification rate however, depends on the biofilm thickness due to the diffusion effect).

Table 3a. Nitrification rates assessed in different biofilm systems treating raw sewage.

Process or configuration	Nitrification rate ($g/m^2.d$)	Organic loading or influent Conc.	References
Submerged biological filter			Al-Haddad *et al.*, (1991); Rusten (1984) and Schlegel (1988).
1-stage	0.65	1 $gCOD/m^2.d$	
1-stage	0.5	1 $gBOD/m^2.d$	
2-stage(1st stage)	1.32	-	
2-stage(2^{nd} stage)	1.58	-	
2-stage	1 - 3.5	BOD 5 - 18 mg/l	
4-compartment	1 - 4.5	COD6.3 - 50 mg/l	
Rotating biological contactor			Surampalli and Baumann (1989)
4-stage	0.3	COD 80-210 mg/l	
4-stage	0.2 - 0.6	COD240-690mg/l	
4-stage+suppplemental aeration	0.83 - 1.4	COD80- 210 mg/l	
4-stage+suppplemental aeration	1.0	COD240- 90mg/l	
Trickling filter			Parker *et al.*, (1989)
1-stage	0.65	1 g $COD/m^2.d$	
Tertiary stage	2.1 - 2.9	BOD < 10 mg/l	
Biofilm with membrane oxygenation			Yamagiwa *et al.*, (1998)
1-stage	1.9	11.6 g $BOD/m^2.d$	
Fixed bed biofilters	0.84	2.5 $gCOD/m^2.d$	Canziani *et al.*, (1999)

As the nitrification process reduces the alkalinity and increases the carbonic acid concentration, the pH of the wastewater may drop to values around 6.0, which adversely affects the rate of nitrification. This decrease in pH can be minimised by stripping CO_2 from the wastewater by aeration, or by ensuring the presence of excess alkalinity (EPA, 1975). The reported optimum pH covers a wide range, but there is a general consensus that the rate of nitrification decreases when the pH declines. Sawyer *et al.*, (1973) and Engel and Alexander (1958) have reported that

the optimum pH values for nitrification ranges from 8.0 to 9.0, and from 7.0 to 9.0, respectively. Painter (1970) stated that nitrification processes cease at or below pH 6.3 to 6.7. Poduska and Andrews (1974) have shown that an abrupt drop of pH from 7.2 to 5 reduced significantly the oxidation rate of ammonia, however, by adjusting the pH to around 7, the nitrification rate restored to its original value. Also the activity in biofilm processes has been shown to drop dramatically below pH about 7 (Boller *et al.,* 1994). A low pH may result in nitrite accumulation in the biofilm since nitrous acid (HNO_2) may inhibit the *Nitrobacter*, whereas NH_3 at high pH may inhibit both *Nitrosomonas* and *Nitrobacter* (Anthonisen *et al.,* 1976).

Temperature

A primary environmental condition for optimal rates of nitrification is the temperature. Temperature optima for nitrification are generally reported by various researchers to be around 30 °C with a range varying from 28 to 35 °C (Buswell *et al.*, 1954). Temperature influences both the heterotrophic and autotrophic microorganisms. The nitrification rate is more temperature sensitive than conversion of organic matter (Busch, 1971). Nitrification rates decrease by about 50% for each 10 °C drop below about 30 °C (EPA, 1973 and 1975).

Residual organic matter.

Reduced nitrification rate occurs in the presence of biodegradable organic matter, which is typically a result of competition between heterotrophic and autotrophic bacteria. In biofilm reactors, heterotrophic biofilm layers will cover the nitrifying bacteria, resulting in oxygen limitations for the nitrifying bacteria (Okabe *et al.,* 1995). To ensure an efficient nitrification, it is favourable to divide the process into two or more steps, where the major part of the organic matter is removed in the first step. Gilmore *et al.,* (1999) investigated the nitrification efficiency using a pilot-scale, two-stage (the first stage for carbon oxidation, and the second for ammonia oxidation) fixed film biological aerated filter (BAF). The system was fed with domestic sewage. The overall nitrification efficiency exceeded 90 % when the organic loading to the second stage was below 0.6 $kg.m^{-3}.d^{-1}$. The nitrification efficiency started to deteriorate at higher OLR's. According to Hanaki *et al.,* (1990) a high OLR results in lower nitrification efficiency because of the inhibitory effect of heterotrophic cells. The growth of heterotrophs decreases the density of nitrifiers in the aerobic part of the biofilm, and at very high OLR nitrification ceases (Bovendeur *et al.,* 1990). From the results of the studies carried out by Boller *et al.,* (1987) using RBCs operated under different OLR, it was concluded that nitrification starts at an organic load of 15 $gCOD.m^{-2}.d^{-1}$ and was fully developed at about 8.0 $gCOD\ m^{-2}.d^{-1}$. However according to Boongorsrang *et al.,* (1982) the COD loading rate should be less than 2.54 $g\ m^{-2}.d^{-1}$ for nitrification in a rotating disc contactor. Bovendeur *et al.,* (1990)

found a nitrification rate of 0.576 g NH_4^+-N $m^{-2}.d^{-1}$ at COD loading rate = 0, and it decreased by 0.015 g NH_4^+-N $m^{-2}.d^{-1}$ at every additional g $m^{-2}.d^{-1}$ of COD removed in a biofilm system. Hem *et al.,* (1994) studied the effect of the OLR on the nitrification in a moving bed biofilm reactor. An OLR of 2 - 3 g total BOD_7 $m^{-2}.d^{-1}$ resulted in a nitrification rate in the range 0.3 - 0.8 g NO_3-N $m^{-2}.d^{-1}$. While at an OLR of 1 - 2 g total BOD_7 $m^{-2}.d^{-1}$, it was in the range of 0.7 - 1.2 gNO_3-N $m^{-2}.d^{-1}$ and it almost stopped at an OLR exceeding 5 g tot BOD_7 $m^{-2}.d^{-1}$.

Dissolved oxygen.

Kiff (1972) found that a low DO level reinforces the inhibitory effect of high OLR on the nitrification process. However this phenomenon has not yet been well described or clarified. Alleman (1985) and Jayamohan et al., (1988) reported accumulation of nitrite during the nitrification process at low DO level. Hem *et al.,* (1994) found a first order correlation between the nitrification rate and the oxygen concentration up to about 15 mgO_2l^{-1} in a moving bed process with a homogenous nitrifying biofilm.

Sulphide content

The nitrification efficiency dropped by 28 %, 67 % and 76 % at sulphide concentrations of 1, 5, and 10 mgSl^{-1} respectively (Beccari *et al.,* 1980). Bentzen *et al.,* (1995) found that the nitrification efficiency of the biofilters increased by 10 % when the concentration of sulphide was reduced from about 5.5 to 2.0 mgSl^{-1}. The extent of sulphide inhibition is supposed to depend on the composition of the biomass, the degree of acclimatisation of the biomass, the concentration of sulphide, and the content of other inorganic and organic compounds in the wastewater. Results of the studies carried out by ÆsØy *et al.,* (1998) showed that sulphide concentration of 0.5 mgl^{-1} exerted considerable negative effect on the nitrification activity. They found that the sulphide together with relatively high concentrations of organic matter in the septic wastewater caused a 30 – 40 % reduction of the nitrification capacity. In order to eliminate the problems of a high sulphide load of the first stage of a multi-stage RBC-reactor, a preliminary sulphide-sulphur conversion pre-treatment step could be implemented by using the system developed by Buisman *et al.,* (1990). Such a pre-treatment step only needs around 15 min contact time.

Recirculation.

Effluent recirculation is a common practice for wastewater treatment using trickling filters. It has been emphasised that recirculation could be used successfully to dilute the RBC influent organic carbon and hence increase nitrification. Klees and Silverstein (1992) demonstrated in a pilot - scale-rotating biological contactor that recirculation improved nitrification at all hydraulic loading rates applied. This

improved nitrification could be attributed to the dilution of influent biodegradable organic carbon (BOD_5) as a result of mixing secondary plant effluent with the influent of the second stage.

Predation

The capacity of a biofilm process to nitrify can be affected by biological factors, such as grazing of nitrifying bacteria by predators. Parker *et al.*, (1989) were able to increase the nitrification rate in a trickling filter by 50 – 100 % by using a cross-flow support medium to enhance oxygen transfer in combination with regular floodings and back washings of the filter. The repeated flooding removed some of the larger metazoa (larvae) from the filter, which was assumed to be a reason for the increased nitrification. However, the study carried out by Parker *et al.*, (1990) on full-scale systems indicated that measures taken to control the micro-fauna also may influence other important parameters affecting nitrification, such as oxygen and ammonium transfer. Some studies dealing with the interactions between protozoa and nitrifiers in other systems than biofilm processes have shown that protozoa may enhance nitrification (Griffiths, 1989; Verhagen and Laanbroek, 1992). Theoretically, the grazing of protozoa and metazoa should be more negative for the slow growing nitrifiers than for the fast-growing heterotrophs. However, the interactions between predators and microflora are complex (Curds, 1975), so for instance some of the phagotrophs (protozoa and metazoa feeding bacteria) graze specifically on just a few bacterial species (Curds 1975). Griffiths (1989) could not find any protozoa grazing specifically on nitrifiers, and he attributed this to the fact that nitrifiers grow in large agglomerates, which are more difficult to ingest than freely dispersed single cells. Verhagen and Laanbroek (1992) indeed found some flagellates that grazed specifically on dispersed free nitrifiers, possibly because of their larger cell size. Nevertheless Lee and Welander (1994) found that predators such as rotifers exerted negative effect on the growth of nitrifiers in aerobic biofilm processes, at least in systems not limited by oxygen or ammonium transfer. A selective suppression of predators can increase the nitrification capacity of such biofilm processes significantly.

Hydraulic loading conditions

A high hydraulic loading rate using wastewater with moderate BOD_5 concentration is unfavourable for nitrification. According to a literature review published by EPA, (1973) a detention time of 60 minutes is required for nitrification in biofilm system to reduce ammonia concentration from 25 to 30 mgl^{-1} down to 2.5 to 5.0 mgl^{-1}. For complete ammonia removal a HRT of 1.5 h is required at pH 8.5 and 7.0 hrs at pH 6.5. Using a RBC as a secondary treatment unit, Antonie and Koehler, (1971) reported that approximately 60 minutes would be required to accomplish 85 % ammonia nitrification. At a higher hydraulic loading, an RBC unit would need a

minimum of 1.5 days detention time for nearly complete ammonia removal, (lue-Hing *et al.*, 1976). An unfavourable factor for nitrification is high BOD_5 concentration in the RBC influent. Bacteria that remove carbonaceous BOD grow at much faster rate than nitrifiers. Consequently, they outgrow and eliminate the nitrifiers in the RBC unit because no biological sludge is recycled. According to Antonie *et al.*, (1972) the BOD_5 limit in RBC system for successful nitrification is 30 mgl^{-1}, and according to Antonie and Koehler (1971) 14 to 20 mgl^{-1}.

b) Nitrate reduction.

Three types of microbial nitrate reduction can be distinguished (Tiedje, 1988), viz. two dissimilatory nitrate reduction at different environmental conditions, i.e. respiratory denitrification and dissimilatory nitrate reduction to ammonium and even assimilatory nitrate reduction. However since most anaerobic environments are characterised by high concentrations of ammonium and organic nitrogen, the latter process is suppressed or made quantitatively insignificant. Dissimilatory processes differ from the assimilatory process by the fact that the cell does not use the reduced nitrogen. Rather, the nitrogen oxide serves as an electron acceptor for the cell's metabolism. Since the dissimilatory processes are inhibited by oxygen, they occur only in anaerobic environments. The main features of these three processes are summarised in Table 4 a.

Table 4a. Biological nitrate reduction mechanisms.

Process	Pathway of free intermediates	Regulated by	Groups of organisms possessing process
Assimilatory mechanism			
Assimilatory nitrate reduction	$NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$	NH_4^+, organic N	Plants, fungi, algae, bacteria
Dissimilatory mechanisms			
Denitrification	$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$	O_2	Aerobic bacteria also capable of anaerobic growth with NO_3^- or NO_2^-
Dissimilatory nitrate reduction to ammonium	$NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$	O_2	Anaerobic and facultatively anaerobic bacteria

Dissimilatory nitrate reduction to ammonium.

Both the assimilatory and dissimilatory nitrate reduction processes result in ammonium production, but the regulation of the two pathways is different (Table 4 a). Oxygen remains unaffected by ammonium dissimilatory pathway, while the opposite

applies for assimilatory reduction. Therefore, the dissimilatory pathway suits well in anaerobic environments. Furthermore, in anaerobic environments where the availability of electron acceptors often restrict the metabolism, the transfer of eight electrons per nitrogen in the conversion of nitrate to ammonium makes this step one of the most favourable electron acceptors in anaerobic environments (Tiedje *et al.*, 1982).
The major criterion that characterise dissimilatory nitrate reduction to ammonium is that ammonium is produced in excess over the amount needed for growth. The easiest way to distinguish this process experimentally from assimilatory reduction route is to use 15 NH_4^+ plus organic 15 N production from 15 NO_3^- in the presence of sufficient ammonium (e.g. 1 m M) to suppress nitrate-assimilating pathways.

The optimum COD/NO_3-N ratio for denitrification depends on the nature of the carbon source. It has been found that in the presence of non-fermentable organic compounds denitrification is likely the major nitrate reduction route. While, in the presence of fermentable organic carbon compounds, ammonification is obtained (Akunna, 1993; Akunna *et al.*, 1993). Besides, high COD/NO_3-N ratios have been reported to promote ammonification whereas low ratios will lead to denitrification (Akunna *et al.*, 1992). Experiments of Akunna *et al.*, (1993) showed, however, that nitrate reduction to ammonium in methanogenic sludge not previously adapted to nitrate, also depended upon the nature of the carbon source available. With glucose and glycerol, 50 % of the nitrogen originating form nitrate was found as ammonium, whereas in the presence of acetic or lactic acid 100 % of the nitrate was denitrified to nitrogen gas.

Organisms identified in the process of dissmilatory nitrate reduction are listed in Table 4 b.

Table 4 b. Organisms reported to dissimilate nitrate or nitrite to ammonium (DNRA).

Facultative anaerobes	Typical habitat	References
Escherichia coli	Soil, wastewater	Bleakley and Tiedje (1982), Cole, (1978)
Citrobacter sp.	Soil, wastewater	Smith, (1983)
Salmonella typhimurium	Sewage	Satoh *et al.*, (1981)
Klebsiella species	Soil, wastewater	Satoh *et al.*, (1981); Bleakley and Tiedje (1982); Cole and Brown (1980)
Enterobacter (Aerobacter) aerogenes	Soil, wastewater	Bleakley and Tiedje (1982)

Denitrification

The process of denitrification, the reduction of nitrate to nitrogen gas proceeds via the production of intermediates as HNO_2, NO_2 and N_2O. Denitrification requires the presence and availability of an electron donor (COD) which can be organic material or compounds like sulphide or hydrogen. To achieve an efficient and smooth biological denitrification the following conditions should be fulfilled: (1) the pH value of the solution should be between 7-9, because pH values lower than 7.2 lead to N_2O production. (2) the presence and availability of nutrients and trace elements. Application of the process of denitrification in pre and post treatment schemes is summarised in Table 5a.

Effect of oxygen

It is known that the presence of dissolved oxygen in denitrification reactors leads to a reduction of the denitrification capacity, due to the fact that oxygen penetrates into the anoxic zone of the biofilm (Laursen *et al.,* 1992). Oxygen strongly suppresses denitrification; aerobic denitrification rates are only 0.3 – 3 % at the anoxic conditions (Tiedje, 1988). The concept
of pre-denitrification and successive nitrification by applying recycling of aerated nitrate rich water is widely used, both with activated sludge systems or biofilters. The main disadvantage
of this concept is that the required high recirculation rate will cause aerobic conditions in the denitrification reactor, and then DO is used as electron acceptor for the removal of organic matter instead of nitrate oxygen, according to the three component diffusion theory of Hagedorn *et al.,* (1993).
Recently microbiologists (Robertson and Kuenen, 1984) found evidence that both nitrifiers as well as denitrifiers have a much higher physiological variety than expected. For instance, many denitrifiers are capable of denitrifying under aerobic condition. Zart *et al.,* (1995) proved that *Nitrosomonas europea* and *Nitrosomonas* eutropha are able to denitrify in the presence of small amounts of oxygen. Furthermore many heterotrophs were found to be capable to nitrify while many of these nitrifiers are able to denitrify aerobically as well as anaerobically (Castigntti and Hollocher, 1984). In fact, some researchers even demonstrated the ability of some strains to denitrify under fully aerobic conditions viz. *Thiosphaera Pantotropha, Alcaligenes faecalis* (Robertson *et al.,* 1990), *Pseudomonas nautica* (Bonin *et al.,* 1991), and *Pseudomonas sp.* (Thomas, *et al.*, 1994).

Effect of pH

A literature review published in 1975 by Francis and Callahan indicated that the optimal pH range for denitrification is between 7.5 and 8.5. The process for converting nitrate to nitrite is less sensitive to a pH drop than the conversion of nitrite into nitrogen gas.

Effect of temperature

Reports concerning the influence of temperature on the process are contradictory. Stensel *et al.,* (1973) observed no changes in the rate of denitrification when increasing the temperature from 20 to 30 °C, Cooper and Smith, (1983) reported a two fold increase in the denitrification rate for each 10 °C increase in temperature between 15 °C and 60 °C. The optimum temperature was found to be 60 °C.

Effect of biodegradable organic matter

The efficiency of the denitrification process in the anoxic zone depends strongly on the amount of readily metabolised biodegradable organic matter. Often organic matter is a rate limiting parameter. An external carbon source may be added to achieve a high denitrification rate. For domestic sewage only values exceeding 14 mg COD/mgN (or 4.5 mgTOC/mgN) in the influent will keep denitrification efficiencies above 90 %. From 4 to 6 g COD is required to denitrify 1 g of NO_3-N with ethanol and methanol as substrate in a post-denitrification processes (Henze *et al.,* 1997). Obviously when oxygen and nitrite are present, more carbon source is required for complete denitrification. The effect of oxygen and nitrite can be accounted for, by expressing it in terms of NO_2-N equivalent i.e. 1.0 g NO_2-N to 0.6 g NO_3-N.eq and 1 g O_2 is equal to 0.35 g NO_3-N eq. In this specific case at least 65 mg/l of COD will be required in order to have complete denitrification.

Denitrification with anaerobic effluent.

The changes in wastewater composition due to anaerobic pre-treatment will influence the nitrogen removal process. The denitrification rate in a biological process depends mainly on the activity of denitrifiers of the sludge and the available carbon source. The denitrification rates of the sludge depend on the fraction of denitrifying biomass present in the influent. Henze (1989) found that the biomass, which resulted from the raw wastewater, has a high denitrification rate as compared to the biomass that resulted from the pre-treated wastewater. The more organic matter is removed from the wastewater in the pre-treatment step, the less denitrification capacity is left. The resulting denitrification rate depends on the amount of the various organic fractions left after the pre-treatment. The denitrification capacity of the solution generally is strongly reduced by the pre-treatment, which means that generally an additional carbon source is needed in order to obtain the required removal of nitrogen. Sulphates in the raw wastewater play a significant role in pre-denitrification, because they are reduced in the anaerobic step. They are deoxidised by nitrates consequently they then, reduce the organic matter need for denitrification. This process is mediated by *Thiobacillus* denitrificans depending on the concentration. They can be completely

Table 5a. Pre and post-denitrification in different systems with different carbon sources.

Process or configuration	Pre or post denitrification	Denitrification rate	Carbon source and temp.(°C)	References
Fixed-bed biofilters	Pre-denitrification	0.65 g $N/m^2.d$	Raw domestic sewage	Canziani *et al.*, (1999)
bmerged up-flow biofilters	Pre-denitrification	1.0 $KgNO_3$-$N/m^3.d$(70%)	Settled municipal wastewater	Ninassi *et al.*, (1999)
Submerged up-flow biofilters	Pre-denitrification	1.2 $KgNO_3$-$N/m^3.d$ (85%)	Settled municipal wastewater + methanol	
Activated sludge	Pre-denitrification	10 g NO_3-N/(kgVSS.h)	Settled sewage	Lynga and Balmer (1992)
Activated sludge	Pre-denitrification	1-2 mg NO3-N/gMLVSS h.	Settled sewage (COD = 130 mg/l)	Plaza *et al.*, (1991)
Activated sludge + trickling filter + activated sludge	Post-denitrification	Nitrate reduced from 15-35 to 2-5 mg/l.	Excess sludge from a high loaded activated sludge plant	Schreff and Wilderer (1998)
	Pre-denitrification	50%	Settled sewage	
Rotating Biological Contactor	Pre-denitrification	83-99%	Acetic acid (ground water)	Bandpi *et al.*, (1999)
Moving bed biofilm	Post-denitrification	1.6 $gN/m^2.d$	acetate	Odegaard, (1992)
Moving bed biofilm	Post-denitrification	1300 $gN/m^3.d$	Ethanol (15 °C)	Rusten *et al.*, (1996)
	Post-denitrification	600 $gN/m^3.d$	Methanol (15°C)	
Suspended biomass	Post-denitrification	700 $gN/m^3.d$	Ethanol (15°C)	Nyberg *et al.*, (1996)
	Post-denitrification	200 $gN/m^3.d$	Methanol(15°C)	
Submerged filter	Post-denitrification	5000 $gN/m^3.d$	Methanol (13°C)	Tandberg and Ydstebo, (1992)
Continuous filter	Post-denitrification	2400 $gN/m^3.d$	Methanol (10-14°C)	Andersson *et al.*, (1991)
Continuous filter	Post-denitrification	1000 $gN/m^3.d$	Methanol (10°C)	Upton, (1993)
Continuous filter	Post-denitrification	2700 $gN/m^3.d$	Methanol (20°C)	Koopman *et al.*, (1990)
Fluidised bed	Post-denitrification	6000 $gN/m^3.d$	Methanol (11-25°C)	Semon *et al.*, (1996)
Fluidised bed	Post-denitrification	4300 $gN/m^3.d$	Methanol (20°C)	Gasser *et al.*, (1973)
Fluidised bed	Post-denitrification	7400 $gN/m^3.d$	Methanol (20°C)	Jeris and Owens, (1975)
Fluidised bed	Post-denitrification	3000 $gN/m^3.d$	Methanol (9°C)	Cooper and Wheeldon, (1980)
Fluidised bed	Post-denitrification	3000 $gN/m^3.d$	Methanol (7°C)	Hansen and Kirkegaard, (1981)

deoxidised in the anoxic step, thus reducing the oxygen need in the oxic step. The oxidation of H_2S, present in the effluent of the UASB reactor with recycled NO_3 can be achieved in the pre-denitrification process (Kuhl and Orgensen 1992). The removal of nitrogenous and organic substances and oxidation of sulphides, increase the redox potential in the pre-denitrification process which will positively affect the subsequent nitrification process (Collivignarelli *et al.,* 1990). The recirculation of the nitrified effluent to the UASB effluent will result in a shift in bacterial population. The recycled nitrate stimulates growth of bacteria, which can utilise nitrate for the oxidation of sulphide to sulphur or sulphate. *Thiobacillus* denitrificans, *Thiomicrospira* denitrificans and *Thiosphera Pantotropha* are examples of sulphur – oxidising denitrifying bacteria. The use of nitrate for the prevention of hydrogen sulphide production has been investigated by Bentzen *et al.,* (1995).

A biological system consisting of segmental set of anaerobic, anoxic and aerobic reactors may represent a highly efficient and feasible treatment scheme for the removal of carbon and nitrogen pollution (Collivignarelli *et al.,* 1991). The anaerobic module reactors will take care for the removal of biodegradable compounds. Nitrate reduction to molecular nitrogen is accomplished in the anoxic reactor and ammonia oxidation to nitrate in the aerobic reactor. References to this system for sewage treatment have been previously reported (Morgan *et al.,* 1994). Recent results dealing with pre-denitrification using anaerobic effluent as carbon source are shown in Table 5 b.

HOW TO IMPROVE THE PRE-DENITRIFICATION PROCESS IN BIOFILM SYSTEMS AFTER ANAEROBIC PRE-TREATMENT?

The efficiency of Pre-denitrification in biofilm systems treating anaerobically pre-treated sewage can be potentially improved in a number of ways:

- by allowing a high biodegradable COD content in the UASB effluent, consequently applying higher loading rates to the UASB- reactor (e.g. lower operational temperatures).
- According to Werner and Kayser (1991) it is possible to use the methane released from the UASB reactor as external carbon source to achieve a high denitrification rate. Methane represents an inexpensive and readily available alternative reducing agent. However, only little work has been carried out in this filed because early studies (Sollo *et al.,* 1979) showed relatively low denitrification rates, although according to earlier work of Davies and Pretorious (1973) specialised forms of bacteria are not needed for using methane as a carbon source for denitrification. They isolated several bacteria from methanol-enriched culture that were found to be capable to grow and denitrify with methane as the sole carbon source. These were identified as *Alcaligenes* spp, *Achromobacter* .

Table 5b. Pre-denitrification results by using anaerobic effluent as carbon source.

Process or configuration	Pre or post denitrification	Nitrate(%R)	Carbon source	References
Anaerobic filter	Pre-denitrification	89 - 98%	Synthetic wastewater	Polprasert and Park (1986)
Modified RBC combined with anaerobic biofilter	Pre-denitrification	90%	Landfill leachate	Hosomi *et al.*, (1991)
RBC	Pre-denitrification	50%	Landfill leachate	
UASB reactor	Pre-denitrification	94 - 97%	glycerol	Loniewska *et al.*, (1985)
Acidogenic reactor	Pre-denitrification	75%	Synthetic wastewater	Rustrian *et al.*, (1999)
UASB reactor	Pre-denitrification	99%	Synthetic wastewater	Hendriksen and Ahring (1996)
Anaerobic biofilter	Pre-denitrification	75%	Domestic sewage	Inamori *et al.*, (1986)
Up-flow Anaerobic filter	Pre-denitrification	74 - 75%	Domestic sewage	Kim *et al.*, (1997)
Anoxic tank	Pre-denitrification	81.2%	Anaerobic baffled reactor effluent	Garuti *et al.*, (1992)
Anoxic biological fluidised bed	Pre-denitrification	95%	UASB effluent	Collivignarelli *et al.*, (1990)
Anoxic Hanging Sponge Cubes biofilter(USHB)	Post-denitrification	84%	Carbon source	Agrawal *et al.*, (1997)

spp, *Pseudomonas* spp and *Bacillus* sp. Micrococcus denitrificans did not grow anaerobically in the presence of methane and nitrate. His laboratory scale-denitrifying unit achieved 50 % denitrification with methane gas as the sole source of organic carbon. Also Zehnder and Brock (1979) reported that organisms like M. *formicicum*, M. *Arbophilicum* and *Methanobacterium* strain AZ oxidise methane fairly well to carbon dioxide as an end product, although far less than M. *thermoautotrophicum, Methanosarcina barkeri*, and *Methanospirillum hungatii*. In the case of *Methanosarcina barkeri,* also methanol and acetate were formed as the end product. The interest in using methane renewed with the discovery that two groups of bacteria are involved in the process viz. methane oxidising methanotrophs and denitrifying methylotrophs utilising methanol produced by the former (Werner and Kayser, 1991). Therefore, oxygen is required to produce the methanol as C source for denitrification. Strict anoxic conditions consequently are counter productive in this case, although should be avoided because too much oxygen will inhibit nitrate removal, due to further oxidation of the methanol produced to carbon dioxide and water. Recently Rajapakse and Scutt (1999) investigated biological denitrification in an attached reactor system using several growth media, denitrifying cultures and natural gas (95

% methane) as carbon source. Nitrate removal efficiencies obtained were up to 93 % at 0.6 m/h and 55 % at 1.6 m/h water filtration rate.

$$CH_4 + O_2 + 2H + \rightarrow H_2O + CH_3OH \xrightarrow{1.5\,O_2} CO_2 + 2H_2O \quad methanotrophs$$

$$CH_4 + O_2 + 2H^+ \longrightarrow H_2O + CH_3OH \begin{cases} \xrightarrow{1.5\,O_2} CO_2 + 2H_2O \ \text{methanotrophs} \\ \xrightarrow{6/5\,NO_3} CO_2 + 3/5\,N_2 + 7/5\,H_2O + 6/5\,OH^- \ \text{methylotrophs} \end{cases}$$

- Another possibility is to use an anaerobic system like UASB reactor for denitrification in combination with methanogens as described by several researchers. In the anaerobic reactor any external electron donor is not required, and denitrification at the inlet of the reactor would improve the COD removal. An up-flow anaerobic filter, followed by a nitrification tank (using entrapped immobilised nitrifier pellets) and an aerobic filter was investigated for sewage treatment by Kim *et al.*, (1997). The overall total nitrogen removal achieved amounted to 75 % at total retention time of 5.5 h, recycling ratio of 0 and recirculation of 25 to 250 % nitrified effluents to the anaerobic filter for denitrification. The denitrification rate ranged between 0.13 and 0.29 kgN/m^3/d. these results are in agreement with those obtained by Inamori *et al.*, (1986) who reported that 75 % nitrogen could be removed in the anaerobic-aerobic biofilter process at a circulation ratio exceeding 1:2. Barber and Stuckey (2000) found that the recirculation of nitrified effluent to the anaerobic baffled reactor treating synthetic sewage achieved a denitrification rates of 0.335 and 0.085 kg NO_3/kg VSS. d.

Nitrification-denitrification processes using new treatment concepts.

Recently, a number of new process configurations for nitrogen removal have been introduced such as SHARON, ANAMOX, DE-AMMONIFICATION and OLAND.

SHARON

The SHARON process; a single reactor high activity ammonia removal over nitrite, developed at the Technical University of Delft (Hellinga *et al.*, 1997), is based on short-circuiting. The denitrification pathway could save both energy and electron donor while also, nitrification could be stopped at nitrite level, (Voets *et al.*, and 1975). Until recently attention to keep nitrification at that intermediate level was not successful, due to the fact that Nitrobacter at normal ambient temperatures rapidly converts nitrite to nitrate. In the SHARON process, careful use is made of the fact that at high temperatures, *Nitrobacter* has a distinctly lower growth rate than

Nitrosomonas. By applying short HRT (1 day) in a completely mixed reactor operated at high temperatures, almost complete wash out of *nitrobacter* can be achieved. By imposing intermittent aeration, both denitrification and concomitant pH control are possible. The overall process presented in the equations illustrates that savings in oxygen supply and electron donor are in the order of 25 and 40 % respectively. The SHARON process should be regarded as a pre-treatment or side-stream treatment system, e.g. for handling of sludge digestion liquor (Hellinga *et al.*, 1997). In processes where nitrite is accumulated at a certain point, one has to pay attention to the fact that nitrite can be involved in side reactions, e.g. forming of nitroanilines in the presence of aniline, nitrite and hydroxyl radicals (Chan and Larson, 1991).

$$NH_4^+ + 1.5\,O_2 \xrightarrow{\text{Nitrification}} NO_2^- + H_2O + 2H^+$$

$$NH_4^+ + 2\,O_2 \longrightarrow NO_2^- + H_2O + 2H^+ \qquad 25\%\ O_2 \text{ saved}$$

$$6NO_2^- + 3CH_3OH + 3CO_2 \xrightarrow{\text{Denitrification}} 3N_2 + 6HCO_3^- + 3H_2O \qquad 40\%\ CH_3OH \text{ saved}$$

$$6NO_3^- + 5CH_3OH + CO_2 \longrightarrow 3N_2 + 6HCO_3^- + 7H_2O$$

ANAMMOX

In 1990, researchers of the Kluyver Laboratory of Biotechnology of Delft introduced a new process which is called ANAMMOX process in which ammonia is converted directly under anaerobic conditions to nitrogen gas with nitrate as the electron acceptor (Van de Graaf *et al.*, 1990). More recently, it became clear that nitrite is the key electron acceptor (Strous *et al.*, 1997). Anaerobic ammonium oxidation is an autotrophic process not requiring any addition of carbon source for denitrification. By combining the ANAMMOX process with a preceding nitrification step, preferably blocked at nitrite level, only a relatively small part of the ammonium needs to be nitrified to nitrite. In this way both oxygen demands for the nitrification reactor and COD demands for the denitrification phase (Strous *et al.*, 1997) are considerably reduced. The detailed biochemistry of this process is still not completely clear. However, since both hydroxylamine and hydrazine can act as electron acceptor, a pathway as outlined in Fig. 2a, b looks possible. Also the organism responsible for these reactions has not fully been characterised. It appears to be an irregularly shaped cell with an unusual morphology (Van de Graaf *et al.*, 1996). Although aerobic nitrifiers were detected in ANAMMOX enrichment cultures, they are considered, not to be responsible for the ANAMMOX process regarding their densities (Van de Graaf *et al.*, 1996). The process is fully operational at lab scale attaining removal rates in the order of 1 kg NH_4^+-N/m^3.d in a fluid bed configuration, and total removal rates of approximately twice that value.

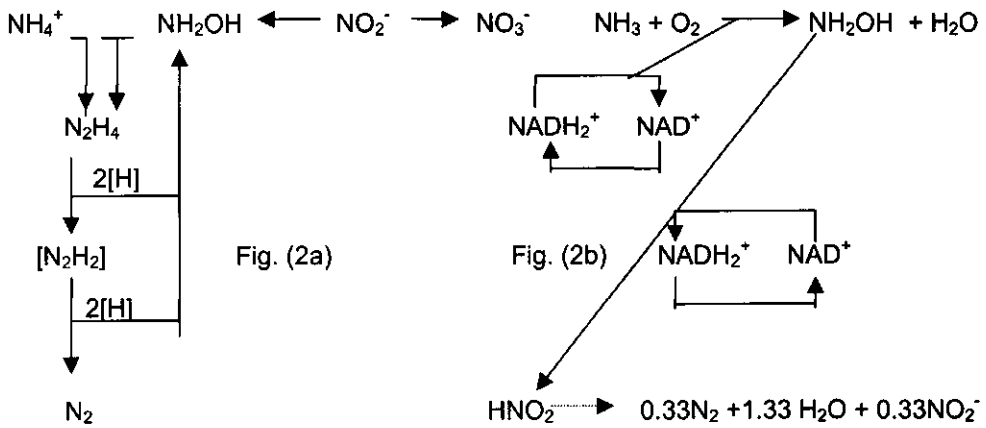

Fig. 2a Possible pathway for the ANAMMOX- process (after van de Graaf *et al.*, 1996). Fig. 2b *Possible degradation of ammonia to dinitrogen and nitrite (after* Binswanger *et al.*, 1997)

AMMONIFICATION

A third process, specific for highly nitrogenous wastewater's, has been described by the University of Hannover (Hippen *et al.*, 1996). The process involves the conversion of ammonia to nitrogen gas under conditions of non-stoichiometry with respect to the electron donor. The organisms involved in this special conversion process named the aerobic de- ammonification process are not yet known. The key feature of the process is the very careful supply of oxygen as demonstrated by Muller *et al.*, (1995), where autotrophic nitrifying sludge can produce nitrogen gas under very low oxygen pressures (1 kPa or ca. 0.2% O_2 in the gas phase). The maximum observed conversion was 58 % oxidation of the ammonia at 0.3 kPa-dissolved oxygen. However, a stable, practically useful process has not been achieved. Binswanger *et al.*, (1997) also reported about aerobic ammonia removal by means of nitrification-denitrification in wastewater's containing high NH_4^+ concentrations. In rotating contactors, removal rates of 90 - 250 $gN/m^3.d$ at surface loading rates of 2.5 $g\ N/m^2.d$ were achieved, without the supply of any biodegradable organic carbon. The researcher postulate that part of the nitrite is reduced by NAD^+, generated during the oxidation of ammonical nitrogen (Fig. 2a,b).

OLAND

In the laboratory of Microbial Ecology, Gent, an active enrichment culture of autotrophic bacteria was used as a biocatalyst to treat water rich in ammonia.

Table 6. Stoichiometry of oxygen limited autotrophic nitrification- denitrification reaction (OLAND) the conventional nitrification - denitrification reactors.

Nitrification followed by denitrification	G°(kj/molN)	G°(kj/reaction)
$NH_4^+ + 2O_2 \longrightarrow NO_3^- + H_2O + 2H^+$	-349.3	-349.3
$NO_3^- + H^+ + 0.83\ CH_3OH \rightarrow 0.5N_2 + 2.17\ H_2O + 0.83CO_2$	-546.1	-546.1
$NH_4^+ + 2O_2 + 0.83\ CH_3OH \rightarrow 0.5N_2 + 3.17\ H_2O + H^+ + 0.83CO_2$	-895.4	-895.4
OLAND		
$0.5NH_4^+ + 0.75O_2 \longrightarrow 0.5\ NO_2^- + 0.5H_2O + H^+$	-271	-135.5
$0.5NH_4^+ + 0.5NO_2^- \longrightarrow 0.5\ N_2 + H_2O$	-358.8	-179.4
$NH_4^+ + 0.75O_2 \longrightarrow 0.5\ N_2 + 1.5H_2O + H^+$	-314.9	-314.9
Savings of OLAND compared to conventional		
Overall savings in O_2		62.5%
Overall savings in alkali requirement		0%
Overall savings in e-donor		100%

The key feature is to provide an amount of oxygen such that the nitrification only proceeds to nitrite, and then subsequently, due to shortage of electron acceptor, the nitrite is used to oxidise ammonia. Abeliovich and Vonshack (1992) have reported the mechanism of nitrite dismutation by Nitrosomonas species. This process of oxidative-reductive N removal, brought about by autotrophic nitrifiers as biocatalysis is labelled as OLAND (Oxygen Limited Autotrophic Nitrification Denitrification) (Kauai and Verstraete, 1998). The reactions summarised in Table 6 show that the Nitrosomonas species can obtain sufficient energy for cell maintenance out of this combined action. Moreover, it indicates that the key parameter to control the process is the oxygen. However until now this appeared to be hard to achieve under mixed culture conditions. In this approach, the sludge is subjected to a pH – controlled aeration. Indeed, by carefully supplying oxygen, which stoichiometrically corresponds to the amount of protons generated, the sludge is forced to consume the nitrite produced. The fact that the autotrophic enriched sludge within a matter of one day shifts to this process is of direct practical interests because in this way, a reliable technical process can be set-up.

POST-TREATMENT SYSTEMS.

Table 7 provides an overview of the systems, which have been used, for post-treatment of anaerobic effluents and its efficiency at optimum operating conditions. An important conclusion from the data presented in Table 7 is that fixed film systems are feasible as a post-treatment option for anaerobically digested sewage compared to the frequently applied waste stabilisation ponds which needs retention time exceeding 20 days. Less than 5 h are only needed in the fixed film system to achieve a high quality effluent. If an effluent of a high quality is required for e.g. for unrestricted irrigation, a retention time of about 10 h should be implemented but this

values is still very low compared to the retention time in algal pond systems. Moreover, fixed film systems, have three important additional advantages as compared to algal pond systems: the very small area required no bad odour and contrary to conventional lagoon, evaporation of water is very limited. The absence of odour and the relatively small area makes the RBC system much easier applicable than pond systems near or even within rural and urban areas.

For all these reasons, we selected a Rotating Biological Contactor for our research as a post-treatment system of anaerobically pre-treated domestic sewage. In our research special emphasis will be given to the removal of pathogenic bacteria, COD colloidal, the conversion of ammonia nitrogen by nitrification and partially denitrification of nitrate effluent.

ROTATING BIOLOGICAL CONTACTOR (RBC).

The interest in the application of RBC's for the biological treatment of wastewater has been growing markedly since the first application in 1960 in Europe (Strom and Chung, 1985). The aerobic RBC has become increasingly popular as a small – scale wastewater treatment plant. Research conducted a RBC-systems was particularly directed for improving their performance. The effect of temperature on the performance of RBC systems was studied by Rittmann *et al.*, (1983) while Gilbert *et al.*, (1986) investigated the energy consumption and savings in order to optimise the RBC performance. (Kinli, 1999) studied the effect of disc rotating velocity on the nitrification performance of RBC system and biofilm kinetics. (Patrick, 1983) studied the effect of seasonal wastewater variation. Ware *et al.*, (1990) evaluated alternatives for conventional disc support media. Numerous investigations have been made in order to examine the relationship between hydraulic and organic loading, residence time, effluent concentration and the removal of nitrogen (Pike *et al.*, 1982) BOD removal and nitrification (Antonie *et al.*, 1974).

Rotating biological contactors have been tried out extensively in both single stage configuration and for sequential stage configurations for BOD removal and nitrification from municipal wastewater's (Huang, 1982). The effect of operational parameters like turbulence (Kugaprasatham *et al.*, 1992), disc rotation speed (Friedman *et al.*, 1979), hydraulic conditions, effluent recirculation (Figueroa and Silverstein, 1992), on organic particular matter removal (Klees and Silverstein, 1992) on nitrification have been studied in detail.

Recently El-Gohary, (1998) investigated the feasibility of the RBC system for the treatment of the anaerobic effluent, at a hydraulic loading rate of 0.063 m^{-3} $m^{-2}.d^{-1}$. The removal efficiencies obtained in terms of COD $_{total}$ ranged from 30 – 79 % while nitrification was complete and the Faecal coliform reduction was around 3 $\log_{10}$. Also Castillo *et al.*, (1997) used RBC units for treatment of anaerobic effluent. The removal

Table 7. Summary of results for recent post-treatment experiments with UASB effluents using different systems.

Process or configuration	HRT	COD $_{inf}$.mg/l	%R (COD) or COD eff.	%R (NH_4-N) or nitrification rate (g/m^2.d)	Reference
Integrated Pond system 10.0ponds (2 duckweed, 3 algae ponds and 5 duck weed ponds)	(4.2 days)	(132 ± 87)	(55.0 ± 26)	(53.0 ± 30.0)	Van der steen *et al.*, (1998)
Integrated Stabilisation Ponds	(2.0 days)	BOD inf. (83.0)	(48.0)	-	Dixo *et al.*,(1995)
Algal Pond	(10.0 days)	-	39.0	-	El-Gohary *et al.*, (1998)
Duck weed (lemna Pond)	(10.0 days)	-	66.0		
Aerobic filter	5.5h	-	BOD eff ≈10 mg/l	complete	Kim *et al.*, (1997)
DHS (Down-flow Hanging Sponge-Cubes)		COD inf (70 mg/l)		1.9 –3.5 gNH_4-N/m^2.d	Agrawal *et al.*, (1997)
DHS (Down-flow Hanging Sponge-Cubes)	(1.3 hr.)	144	70.0	(73 - 78.0)	Machdar *et al.*,(1997)
Fixed bed reactors Submerged bed Percolating bed, downstream of fluidised bed		 102 (104 - 119)	 30.0 (57 - 64)	 - (0 - 78)	Collivignarelli *et al.*,(1990)
Chemical treatment Dissolved air floatation			 (87 - 91)	 -	Penetra *et al.*,(1999)
Rotating Biological Contactor 2-stage 1-stage(aeration) +2-stage 1-stage (aeration)+2-stage 2-stage(aeration)	(45 min.) (2.0 h) (4.0 h) (2.0 h)	 (456 mg/l)	(47 - 56.0) (49 - 57.0) - -	- 43.0 67.0 86.0	Castillo *et al.*,(1997)
Submerged aerated biofilter		COD=112	56%	90%	Goncalves *et al.*, (1998)
Two anaerobic filter		1.5 - 24h	60 - 90 mg/l	-	Chernicharo and Machado (1998)
Sequencing batch aerobic reactors (SBR)		COD = 58	8.2%	90.4%	De Sousa and Foresti. (1996)
Aeration tank		COD = 258	82.7	96%	Garuti *et al.*, (1992)
Waste stabilisation ponds	20 days		30 - 35%	22.8%	Ghosh *et al.*, (1999)
DHS (Downflow hanging sponge-cubes)		COD = 144	72.2%	81.3%	Araki *et al.*, (1999)
Activated sludge	3.9 h	COD = 137	43-56%	-	Sperling *et al.*, (2001).
Activated sludge	-		42%	-	Silva *et al.*, (1995)

efficiency of COD $_{total}$ obtained at a hydraulic loading rate of 0.34 m^{-3} $m^{-2}.d^{-1}$ranged from 84 to 88 % and final BOD values from 7 to 35 mgl^{-1}. The RBCs were also found capable to nitrify the effluent at a hydraulic load below 0.13 $m^{-3}.m^{-2}.d^{-1}$.

SCOPE OF THIS DISSERTATION

This Ph-D thesis describes the results of a research dealing with the assessment of the feasibility of an Rotating Biological Contactor (RBC) for the post-treatment of different effluent qualities produced from UASB reactor fed with domestic sewage. i.e. the removal of E.Coli, BOD (COD $_{biod.}$), colloidal matter and nitrification. An important aspect in this context is to assess the ability of the RBC-system to produce a final effluent quality suitable for safe reuse for restricted and unrestricted irrigation purposes. Factors affecting the E.Coli removal in biofilm system treating UASB effluent was discussed in **Chapter 2** i.e. dissolved oxygen concentration, pH, cationic polymer addition and different carrier material. The experiments in **Chapter 3** deal with the performance of an anaerobic RBC versus aerobic for treatment of high quality UASB reactor effluent. **Chapter 4.** deals with experiments of the performance of a single and two stages RBC system operated at the same HRT and OLR. Emphasis was devoted to the removal efficiency of the various COD fractions (COD $_{suspended}$, COD $_{colloidal}$ and COD $_{soluble}$), as well as for ammonia and E.Coli removal. Furthermore the use of UASB effluents with a relatively low COD $_{biod.}$ content for pre-denitrification was evaluated.

Chapter 5 deals with experiments of the impact of biodegradable organic load applied on the nitrification rate, E.Coli and COD removal using a single and two stages RBC system for the treatment of different quality anaerobic effluents. The effect of a temporary high biodegradable COD loading rate on nitrification activity was investigated in the 2nd stage of RBC (nitrification stage). The experiments presented in **Chapter 6** deal with the COD removal, nitrification and E.Coli removal in a two stage RBC system at different organic loading rates. The performance of a two stage was compared with a single stage RBC operated at the same loading rate. The comparison covered COD, E.Coli removal, nitrification rate, and the stability of the RBC following a shock load. The experiments in **Chapter 7** deal with a three stage of RBC operated at different hydraulic retention time (HRTs). The measured parameters were E.Coli, COD removal and nitrification. In these experiments the effect of recirculation of the final effluent to the 1st stage has been studied. The final discussion and conclusions of the thesis are presented in **Chapter 8.**

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2

FACTORS AFFECTING THE E.COLI REMOVAL IN A ROTATING BIOLOGICAL CONTACTOR (RBC) SYSTEM TREATING UASB EFFLUENT.

This chapter has been submitted to Water Research as Tawfik A., Klapwijk A., Van Buuren J., El-Gohary F., Lettinga G.

ABSTRACT

The removal mechanism of E.Coli from UASB effluent using a Rotating Biological Contractor (RBC) has been investigated. Preliminary batch experiments indicated a first order removal kinetics between E.Coli removal and contact time. Variation in the dissolved oxygen concentration and E.Coli counts over the depth of the RBC has been recorded and indicated that, the RBC is not a completely mixed reactor. Consequently batch experiments were carried out, where the different operating conditions were put under control.

Factors affecting the removal of E.Coli via a biofilm system such as, stirring, sedimentation, dissolved oxygen concentration, pH, different carrier materials and addition of cationic polymer have been investigated. The results obtained indicated that the most important removal mechanisms of E.Coli in the biofilm are adsorption process, followed by sedimentation. Die-off is a relatively minor removal mechanism in RBC systems. Higher removal rates of E.Coli were observed in an aerobic system as compared to the anaerobic biofilm system. Variation of dissolved oxygen concentration from 3.3 to 8.7 mgl^{-1} and pH-values between 6.5 and 9.3 did not exert any significant effect on the removal rate of the E.Coli by the heterotrophic biofilm. The effect of the type of the biofilm carrier materials on E.Coli removal was found to be very important especially after full development of the biofilm. A rapid adsorption of E.Coli to the biofilm occurred during the first days after adding the cationic polymer, after which the adsorption slowed down.

INTRODUCTION

During recent years significant attention has been focused on post-treatment of effluent of Up-flow Anaerobic Sludge Blanket (UASB) reactors using a series of algal ponds. Special emphasis has been devoted to factors affecting the removal of pathogens. E.Coli is used as an indicator of these pathogens in the pond systems

(Van der Steen *et al.*, 1999). Much less attention has been given to the factors affecting the pathogen removal in other post-treatment technologies such as the RBC system. Tawfik *et al.*, (2001) found that the RBC is effective for the reduction of COD, ammonia and E.Coli from UASB-effluent. Since the removal of E.Coli is an important objective in post-treatment systems, optimisation of the RBC performance for this purpose is of prime importance. To optimise the removal of E.Coli from UASB effluent using a RBC system, adequate understanding of factors affecting the removal mechanism of E.Coli is essential.

The removal of pathogens in a RBC unit can be seen as the sum of the following mechanisms: die-off, sedimentation, and adsorption. According to Crane and Moore (1986) the die-off of E.Coli can be described by the following exponential equation:

$$N_t = N_0 exp^{-K_d T}$$

N_0 = influent E.Coli count (#/100ml).
N_t = effluent E.Coli count (#/100ml).
K_d = first order removal rate constant (d^{-1}).
T = time or detention time (d^{-1}).
In our research, we found that the total removal of E.Coli in a RBC can be also described by an exponential equation:

$$N_t = N_0 exp^{-K_r T}$$

In which K_r = first order removal constant.
Generally, the die-off rate of pathogens is influenced by factors such as, dissolved oxygen concentration (Parhad and Rao, 1974), depth (Mayo, 1989) and mixing in the treatment reactor (Moeller and Calkins, 1980). Polprasert *et al.*, (1983) found that increasing the retention time in an algal pond, not only led to reduction of bacterial concentrations, but also resulted in changes in the pond environment, such as biomass concentration, pH and nutrients availability, which influenced the die-off process. Mills *et al.*, (1992) also pointed out that the rate of bacterial die-off increases significantly at pH values exceeding 9.0. Saqqar and Pescod (1992) found that the faecal coliform die-off rate increases with increasing temperature, pH and by decreasing total BOD_5, soluble BOD_5 and surface organic loading rate.
Tawfik *et al.*, (2001) indicated that suspended E.Coli (> 4.4 µm) in the UASB effluent constitute 11 to 49 % of total suspended solids. Therefore, it was concluded that one of the removal mechanisms in the RBC is sedimentation.

Pathogenic bacteria can be considered as living colloidal particles. They usually have a net negative surface charge at the pH range of natural waters. Although the biofilm is negatively charged at this pH range, the Extra-cellular Polymeric Substances

(EPS) provide the possibilities for adsorption of pathogenic bacteria to the biofilm. When a pathogenic bacteria approaches a bio-film, one and the same polymer molecule may attach to both surfaces of the organism and the biofilm, there-by forming a "bridge". Banks and Bryers (1992) reported that a biofilm on different media such as glass, polycarbonate, and granular activated carbon surfaces enhanced removal of bacteria. A large variety of different heterotrophic bacteria (including potentially pathogenic bacteria) have been isolated from biofilms (LeChevallier *et al.*, 1987). According to Cunningham *et al.*, (1990) increasing the thickness of the biofilm, lead to an increase in pathogenic bacteria removal.

The aim of this research is to study the importance of the different removal mechanisms, namely, die-off, sedimentation and adsorption. Also, factors affecting the removal rate constant have been investigated.

MATERIAL AND METHODS

UASB effluent

The effluent of a pilot –scale UASB reactor as described by Grin *et al.*, (1985) was used for this study.

Batch experiments

Prior to start-up of the batch experiments, the RBC has been continuously fed with UASB effluent for a period of nine month.

RBC experiments

The RBC system (Fig.1) as described by Tawfik *et al.*, (2001) was used for the present study. It has a surface area of 6.5 m^2 and it was filled at (t = 0) with 60 l UASB effluent. The rotation speed was 5 rpm.

In the 1st series of the batch experiments, the effect of the reaction time on E.Coli removal has been investigated. Samples were taken at t = 0, 1, 2, 3, 4 and 5 h. All samples were taken at a depth of the 0.01 m after filling the RBC at a constant water level.

The 2nd series of the batch -RBC experiments were carried out at a constant reaction time of 2 h. The E.Coli and dissolved oxygen concentrations were measured at water depths of 0.01, 0.1, 0.2, 0.28, 0.35 and 0.45 m in the pilot-plant. Both experiments were repeated four times each.

Beaker experiments.

Based on the results of these preliminary experiments, it was clear that the RBC is not a homogeneously mixed reactor. Therefore, it was decided to conduct experiments in a 2-litre beaker, which enables a better control of the different operating variables.
Beakers of 2.0 litre capacity were used for this set of experiments (Fig.1). The beakers were filled with 1900 ml of UASB effluent, isolated from light and stirred at a constant speed of 5.0 rpm.

1. The mechanism of E.Coli removal in a bio- disc.

To identify the mechanism of the removal of E.Coli by the biofilm, the following experiments were carried out for a period of 7.0 days (Table 1).

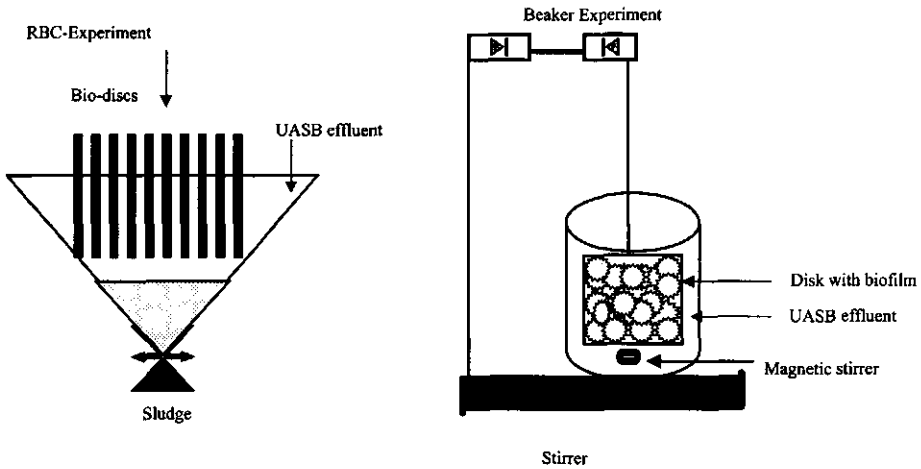

Fig.1 Schematic diagram for RBC and beaker batch experiment

Table 1. Batch experiments to elucidate the mechanism of E. Coli removal.

Exp. No.	Conditions in beaker	Main presumed removal mechanism
1a	Stirring, no bio-disc	Die-off
1b	No stirring , no bio-disc	Die-off + sedimentation
1c	Stirring , with bio-disc (0.13 x 0.13 m)	Die-off + adsorption

2. Factors affecting die-off and adsorption of E.Coli to a bio-disc.

Both the die-off and adsorption of E.Coli on the biofilm were investigated as a function of time. Other variables investigated included the effect of the dissolved oxygen, pH, type of carrier material for the biofilm and addition of cationic polymer.

Bio-disc segments of 0.13 x 0.13 m were placed in the beakers filled with UASB effluent. In some of the control experiments no bio-discs were used.

Analysis

Assessment of E.Coli was performed according to the method described by Havelaar *et al.*, (1988).

Table 2. Batch experiments for study of factors influencing die-off and adsorption.

Exp. No.	Experimental conditions	Expected removal mechanism
2a	*Effect of dissolved oxygen on E.Coli removal through adsorption and die-off at a constant pH of 7.0 ± 0.5.*	
	Four stirred beakers equipped with bio-discs and kept under controlled aeration at D.O of 3.3, 6.2, 7.3, and 8.7 mg/l.	Adsorption + die-off under aerobic conditions.
	Stirred beaker, without bio-disc under anaerobic conditions.	Die-off under anaerobic conditions
	Stirred beaker, with bio-disc under anaerobic conditions (the bio-disc was maintained under anaerobic conditions for two weeks before starting the experiment)	Die-off + adsorption under anaerobic conditions
2b	*Effect of pH on E.Coli removal through adsorption and die-off at constant dissolved oxygen of 7.3 mg/l.*	
	Four beakers equipped with bio-discs and stirring devices were aerated to control D.O. concentration at 7.3 mg/l throughout the experimental period of 5.0 days at different pH-values of 6.5, 7.5, 8.4 and 9.3 (using 10% conc. NaOH and 10% conc. HCl at intervals of 8.0 h).	Die-off + adsorption
2c	*Effect of different carrier materials without biofilm on E.Coli removal through adsorption and die-off at neutral pH and D.O of 3.3 mg/l.* *(All experiments were carried out under conditions of stirring).*	
	Smooth polyvinyl chloride (PVC)	Die-off + adsorption
	Rough polystyrene	Die-off + adsorption
	Rough polyurethane with a high specific surface area 1000 m^2/m^3.	Die-off + adsorption
2d	*Effect of the addition of different doses of cationic polymer (HMW 492).* *All beakers were equipped with bio-discs and stirred. D.O concentration and pH-value were kept constant at 3.3 mg/l and 6.9 respectively.*	
	Beaker without polymer addition (blank).	Die-off + adsorption
	Beaker with 1mg cationic polymer.	Die-off + adsorption + coagulation
	Beaker with 2 mg cationic polymer	Die-off + adsorption + coagulation
	Beaker with 3 mg cationic polymer.	Die-off + adsorption + coagulation

Increasing the dissolved concentration from 3.3 mgl^{-1} up to 7.3 mgl^{-1} did not exert any significant positive effect on the removal constant K_r. Hanes *et al.*, (1964) reported that the die-off of streptococci and coliforms was higher at oxygen concentrations corresponding to those of a normal non polluted water bodies (7.6 - 8.0 mg/ml) than at lower dissolved oxygen concentrations. According to Pearson *et al.*, (1987a) there is a negative correlation between dissolved oxygen and the number of faecal coliforms in treated wastewater.

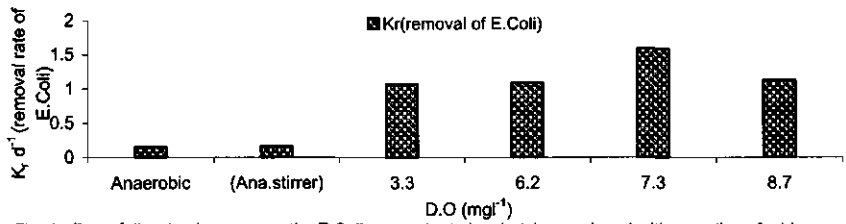

Fig. 4 effect of dissolved oxygen on the E.Coli removal rate in a batch experiment with a section of a bio disc.

- **Effect of pH on the E.Coli removal at a constant dissolved oxygen of 7.3 ±0.5mg/l.**

The effect of pH on the E.Coli removal is presented in Fig.5. Apparently, the optimum pH-value for the removal of E.Coli is around 7.4. Below and above this value the efficiency of the biofilm to adsorb E.Coli is slightly lower. This could be attributed to a reduction in the biofilm activity at these pH-values, leading to the retention of E.Coli in the water phase.

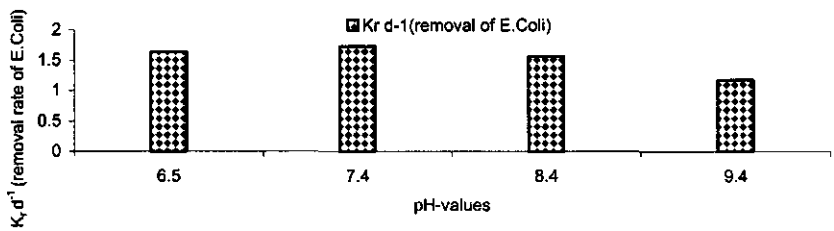

Fig. 5 effect of pH on the E.Coli removal in batch experiments with sections of bio-discs (measuring during 0 - 5 days)

- **Effect of different carrier materials on the E.Coli removal.**

The results of the investigation concerning the influence of the support carrier materials on the E.Coli removal are shown in Fig. 6. The removal rate constants K_r were calculated using the total experimental period (7 days), the period from the 1st to 4th day and that from 4th to 7th day. The results presented in Table 5 reveal that after

a 7.0 days contact time, polyurethane is the best support material in terms of E.Coli removal (K_r = -1.16 d^{-1}), followed by rough polystyrene (K_r = - 0.97 d^{-1}), where as smooth polyvinyl-chloride gave the lowest K_r value (- 0.74 d^{-1}). The three media polyvinyl – chloride (PVC), polystyrene and polyurethane have the same size but different specific surface areas. The specific surface area may affect the adsorption of bacteria because the area is correlated to the number of adsorption sites on the media (Weber, 1972).

Table 5. Effects of different carrier materials on the E.Coli removal rate at neutral pH and dissolved oxygen concentration of 3.3 mgl^{-1}.

Carrier Material	K_r (d^{-1}) (1 - 4 d)	K_r (d^{-1}) (4 - 7 d)	K_r (d^{-1}) (0 - 7 d)	Removal mechanism
Polyvinyl chloride (PVC) without roughness, (stirred).	-0.94	-0.66	-0.74	Die-off + adsorption
Polystyrene with roughness, (stirred).	-1.48	-0.39	-0.97	Die-off + adsorption
Polyurethane with roughness and high specific surface area (stirred).	-0.96	-1.15	-1.2	Die-off + adsorption

It is worth mentioning that a biofilm became visible few hours after the start up of the reactor and was already fully established within one day. The results in Fig. 6 show that the E.Coli removal begins after the first day. The time required for the full development of a biofilm depends on the nature of the supporting material and the surface roughness (Beachy, 1981).

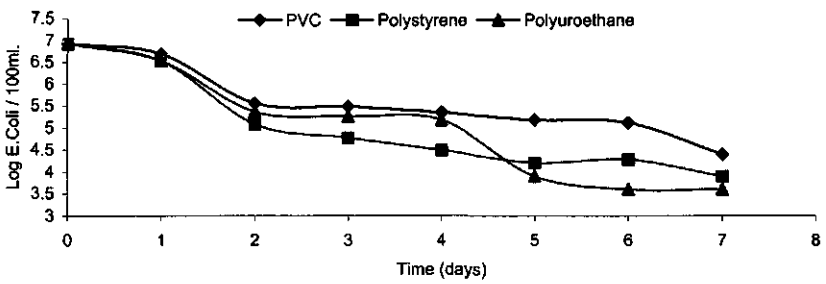

Fig. 6 effect of different carrier materials without biofilm on the E.Coli removal.

- **Effect of cationic polymer addition (HMW 492).**

The effect of adding a cationic polymer on E.Coli removal at concentrations ranging from 0 - 3 mgl^{-1} can be seen from the results presented in Table 6. From the available data it can be seen that the addition of cationic polymer indeed improves the removal rate during the period from 0 to 4 days presumably due to the adsorption of E.Coli onto the heterotrophic biofilm. Agglomerated particles, formed as a result of coagulation, can easily be adsorbed by the heterotrophic biofilm. However, the

results obtained during the period from 4 – 7 days indicate a clear decline in the removal rate at different doses of cationic polymer. Probably due to biodegradation of the cationic polymer.

Table 6. Effect of cationic polymer (492 HMW) addition on the E.Coli removal in a batch experiments with bio-disc sections at neutral pH and D.O of 3.3 mgl^{-1}.

Cationic polymer dose (492 HMW)	K_r (d^{-1}) (day 0 - 4)	K_r (d^{-1}) (day 0 - 7)
Bio-disc without polymer addition	-1.36	-1.06
1 mgl^{-1}	-1.46	-1.22
2mgl^{-1}	-1.73	-1.29
3mgl^{-1}	-2.41	-0.94

GENERAL DISCUSSION

Results of the present study indicate that the mechanism of E. Coli removal in a RBC system is a combination of physical and biological processes. Physical processes include adsorption, sedimentation and the biological removal mechanisms such as, antibiosis, predation, and attack by lytic bacteria and natural die-off. The results also reveal that increasing the reaction time has a clear positive impact on E.Coli removal (Fig. 2). Tawfik *et al.,* (2001), found that increasing the HRT from 2.5 to 10 h in a continuous RBC system treating UASB effluent increased the E.Coli removal from 89 to 99.5 %. It was also found that die-off in the aqueous phase of a RBC plays only a minor role.

The K_r values of the samples which, were allowed to settle were significantly higher than the stirred samples (Table 4). This indicates that sedimentation is one of the mechanisms responsible for the removal of E.Coli from anaerobically pre-treated sewage. According to Tawfik *et al.,* (2001) the major part of E.Coli is associated with suspended particles > 4.4 µm. These were removed by sedimentation or adsorption in the biofilm already in the 1st stage of a RBC (99.66%). The colloidal E.Coli present in the range of smaller particles (<4.4 - >0.45 µm) become adsorbed in the 2nd stage of RBC (99.78%). Also, Milne *et al.,* (1989) found that the survival of E.Coli is related to suspended solids. The higher removal rate of E.Coli in the presence of a heterotrophic biofilm can be attributed to enmeshment in, and/or adsorption of E.Coli to the biofilm. The adsorbed E.Coli cells in heterotrophic biofilm may become degraded by lytic processes and by predation through protozoa. The relatively long cell residence time and the aerobic conditions prevailing in the RBC could then cause further die-off of the attached E.Coli. According to Raman and Chakladar, (1972) this could take place even under anaerobic conditions. Ueda and Horan (2000) found that the use of a membrane without biofilm gave poor bacteriophage removal, but the removal efficiency increased after the development of a good biofilm on the membrane. These researchers proved that the biofilm accumulating on the surface of a membrane contributed significantly to phage removal. There are a number of explanations for the role of the biofilm in pathogenic bacteria removal. The physico-

chemical effect of the biofilm on pathogenic bacteria removal could be due to adsorption or entrapment to bacterial cells and extra-cellular polymeric substances. Subsequently, there will be biological predation of pathogenic bacteria by other microorganisms. Van der Drift *et al.,* (1977) demonstrated that the removal of E. Coli from wastewater treated with activated sludge is a bi-phasic process. First a rapid sorption of bacteria to the sludge flocs takes place, followed by a slower elimination of bacteria, which is presumably due to predation by ciliated protozoa. The results obtained by Omura *et al.,* (1989) indicate that the removal of coliform bacteria, enterococcus bacteria, and coliphages in the activated sludge process and trickling filter were due to adsorption on the activated sludge flocs and on the slime in the trickling filter.

Factors affecting E.Coli removal by a biofilm system have been assessed in this study; viz. dissolved oxygen, pH, cationic polymer addition and nature of the carrier material. As expected E.Coli removal under anaerobic conditions is significantly lower than that under aerobic conditions. This emphasizes the results of previous experiments carried out by Tawfik *et al.,* (2001) which indicated that E.Coli removal of 94.3% in the aerobic RBC system as compared to only 43 % in an anaerobic RBC treating UASB effluent. Barzily *et al.,* (1991) found that RBC achieved a Salmonella tym reduction of about six orders of magnitude in 6 days, where, oxidation ponds accomplished a similar level of Salmonella tym reduction in 14 days. The main difference between Salmonella tym behaviour in dialysis bags attached to the RBC drum and the in oxidation pond could be attributed to the changes in DO concentrations. Dissolved oxygen concentrations in the oxidation ponds are high at noon (rise to 20.0 mg/l) and low at night 0.0 mg/l. while the dissolved oxygen concentrations in RBC are constant during a day and night.

Influence of pH on the adsorption of bacteria depends on the nature of the bacterial surfaces and ionic strength of the solution (Harvey, 1991). pH affects bacterial surface zeta potential due to dissociation of carboxylic and amino groups located on the bacterial cell wall (Gannon *et al.,* 1991). The effect of pH depends also on the iso-electric point of the bacterial species. Since the pH of domestic wastewater often is close to 7.0 (Canter, 1985), the pH will probably have a minimal influence on the bacterial removal. In the present study, the effect of pH within the investigated range of 6.5 – 9.4 was found to be in significant.

The adsorption of E.Coli to the biofilm was found to become greatly enhanced for a short period after the addition of cationic polymer as shown in Table 6. This is because cationic polymers interact directly with specific ionizable groups on the protein surface coat of the E.Coli, thus achieving a surface charge redistribution favourable to the adsorption of E.Coli to negatively –charged biofilm. According to Gambrill *et al.,* (1989) chemical treatment can achieve Faecal coliforms and

Salmonella removal values of 99.999 and 99% respectively, by using of lime and Clari-floc as coagulant and coagulant aid.

CONCLUSIONS

From the above mentioned results and discussion, the following can be concluded:

- In a RBC system, adsorption is the main E.Coli removal mechanism followed by sedimentation. Die-off has a relatively minor role for removal of E.Coli in the RBC system.
- the removal rate of E.Coli under aerobic conditions is significantly higher than under anaerobic conditions.
- the adsorption of the E.Coli to heterotrophic biofilm slightly decreases at pH values higher than 8.
- the absorption effect of different support carrier materials on the E.Coli removal improves after the biofilm has been developed.
- a significant improvement in the removal rate of E.Coli can be achieved when cationic polymer is added for a short period. However, on the long run, polymer addition exerted almost no improvement in the removal rate.

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3

COMPARISON BETWEEN THE EFFICIENCY OF AN ANAEROBIC AND AN AEROBIC RBC TREATING UASB EFFLUENT

This chapter has been submitted to Bioresource & Technology journal as Tawfik A., Klapwijk A., El-Gohary F., Lettinga G.

ABSTRACT

The performance of an anaerobic versus aerobic Rotating Biological Contractor (RBC) for the treatment of UASB effluent fed with domestic sewage has been investigated. Both RBC units were operated at the same OLR of 14.5 g COD $m^2.d^{-1}$ and HRT of 2.5 h. The results obtained indicated that the efficiency of the aerobic RBC exceeds that of the anaerobic one. The achieved percentage removal values of COD $_{total}$, COD $_{colloidal}$ and COD $_{soluble}$ in the aerobic RBC unit were 56.0, 90.0 and 28.0 as compared to 23.0, -16.0 and 14.0 in the anaerobic one. In terms of E.Coli especially for the supra-colloidal fraction, higher removal values have been recorded for the aerobic RBC unit. Residual E.Coli $_{total}$, E.Coli $_{suspended}$ and E.Coli $_{supra-colloidal}$ counts in the effluent of the aerobic RBC were 1.06 x 10^5, 5.58 x 10^4 and 4.14 x 10^4/100 ml. Corresponding values for the anaerobic unit were 9.38 x 10^5, 2.3 x 10^5 and 6.1 x 10^5/100 ml respectively.

The introduction of an anaerobic stage of RBC prior to the aerobic RBC increased the overall removal efficiency of COD $_{total}$ by only 9.0 %. But it didn't improve the overall removal of E.Coli $_{total}$ and the nitrification rate as compared to the single stage aerobic RBC system. Therefore, this study strongly supports the use of an aerobic RBC as a post-treatment step after a UASB reactor.

INTRODUCTION

The use of the up-flow anaerobic sludge blanket reactor has been shown to be an attractive technology for the removal of organic contents of domestic wastewater. The performance of the UASB reactor depends on the waste strength, temperature, activity, weight of the biomass and the HRT (Lettinga *et al.,* 1993). However, the use of a UASB reactor is considered as a first step and a post treatment is usually required. The use of an aerobic RBC unit as a post treatment step has been successfully implemented by Tawfik *et al.,* (2001). Since the main removal

mechanism in the aerobic RBC system is adsorption on the biofilm, it is questionable whether an anaerobic RBC would behave similarly.

Since the anaerobic RBC is not subject to the same rate limiting factors as the aerobic process, namely transfer of oxygen into the liquid phase, much higher amounts of biomass per unit volume can be employed. However, as the aerobic biofilm growth is higher than the anaerobic biofilm growth, an aerobic biofilm will therefore develop on the surface more readily than an anaerobic film (Echaroj, 1986). Also, an anaerobic post-treatment so far is not regarded as really effective process for the treatment of a low biodegradable COD viz. less than 130 mgl^{-1}, such as anaerobic effluent, Sperling *et al.*, (2001).

According to Yoda *et al.*, (1985) $COD_{colloidal}$ is removed from domestic wastewater at a lower degree under anaerobic conditions than under aerobic or micro-aerobic conditions. The $COD_{colloidal}$ represents 60 - 80 % of the COD_{total} of an anaerobic reactor effluent, which clearly indicates the low removal rate of this fraction in an anaerobic reactor.

Polprasert and Hoang (1983) reported that faecal coliforms and bacteriophages present in septic tank effluents could be removed by using the anaerobic filter. The mode of removal seems to be primarily through the combined effects of filtration and die-off of the microbial cells within the anaerobic filter liquid phase, and to a lesser extent, adsorption of the microbial cells on the biofilms of the filter media. Since few studies have been published on the mechanism of $COD_{colloidal}$ and E.Coli fraction removal in an anaerobic RBC unit, it was decided to carry out this work to explore some aspects of the process.

The objectives of this study are:

- to compare the efficiency of an aerobic versus anaerobic RBC units for the removal of residual COD and E.Coli in a UASB effluent.
- to compare the performance of a two stage RBC system consisting of anaerobic followed by aerobic (HRT = 5 h) with a single stage aerobic RBC unit (HRT = 2.5h) for the treatment of a UASB effluent.

MATERIAL AND METHODS

The research work has been carried out in a pilot plant station situated in the village Bennekom, in the Netherlands, using the domestic sewage of the village (collected in a combined sewer system). The two RBC units (Fig. 1) were fed with the effluent of a 6 m^3 UASB pilot - plant, previously investigated by Grin *et al.*, (1985) for treatment of raw domestic sewage.

UASB effluent

The main characteristics of the effluent of the UASB reactor operated at a temperature of 30 °C during the experimental period are given in Table 1.

Table 1. Characteristics of the UASB.

pH	COD mgl^{-1}				NH4-N	TKN	E.Coli
	total	Sus.	Coll.	Sol.	mgl^{-1}	mgl^{-1}	/100ml
7.2	164 ± 20	50 ± 13	43 ± 11	72 ± 9	42 ± 6.8	56 ± 3.8	$1.6 \times 10^6 \pm 4.7 \times 10^5$

RBC system

Two pilot-scale identical RBC reactors were designed and manufactured to treat UASB effluent (t = 30 °C). One was operated under anaerobic conditions and the second under aerobic conditions (Fig.1). Each RBC unit had a working volume of 60 l, and was equipped with 10 polystyrene foam disks with a total effective surface area of 6.5 m^2 and rotating at 5 rpm. The disk diameter was 0.6 m with a thickness 0.02 m and they were spaced at 0.02-m distances. Submerged surface area was 40 % in both systems. The disks were mounted on a steel shaft. The anaerobic RBC reactor was covered and sealed by plexi glass material.

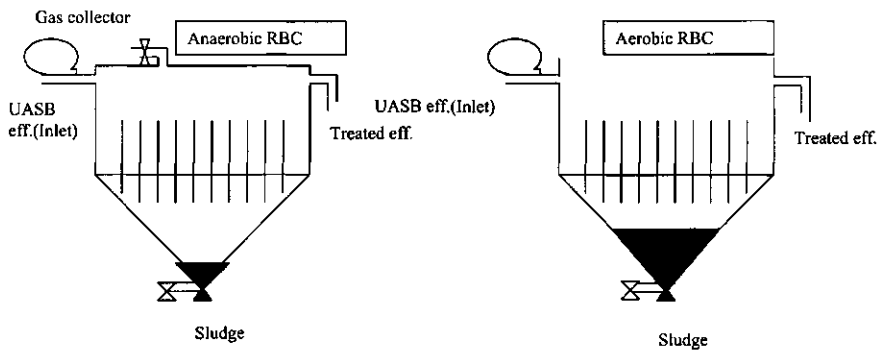

Fig.1 schematic diagrams for anaerobic and aerobic RBC system.

Sampling and Analysis:

Forty-eight hours composite samples of the influent and the effluent of each reactor were collected in containers stored in the fridge at 4 °C and analysed. Temperature, DO and pH were measured using grab samples. Samples for each run were analysed six times along the experiment periods.

COD was analysed using the micro-method as described by Jirka and Carter (1975). Raw samples were used to determine COD_{total}. The filtrate of 4.4 um folded filter

paper (Schleicher & Schuell 595 ½) was used to determine the $COD_{filtrate}$; and the filtrate of the 0.45 um membrane (Schleicher & Schuell ME 25) was used to determine the dissolved COD ($COD_{soluble}$). The $COD_{suspended}$ and $COD_{colloidal}$ were calculated by the difference between COD_{total} and $COD_{filtered}$, $COD_{filtered}$ and $COD_{soluble}$, respectively.

Ammonia, nitrite and nitrate were determined using auto-analyser (SKALAR SA-9000). Examination of E.Coli was performed according to the method described by Havelaar, *et al.*, and (1988).

Start-up of anaerobic RBC system.

The start-up of the anaerobic RBC system was carried out using UASB effluent. The UASB reactor was operated at a temperature of 30 °C. The anaerobic RBC system was run for 4 month at a temperature of 20 °C and operated at a HRT of 2.5 h. Microorganism's attachment to the rotating disks was clearly visible on the 29th day.

Both RBC's were continuously fed for 7.0 months following the start up period with the same effluent of the UASB reactor at an OLR of 14.5 gCOD $m^{-2}.d^{-1}$.

RESULTS AND DISCUSSION

Comparison between the efficiency of the anaerobic and aerobic RBC systems as post-treatment.

The results presented in Fig.2 show that the residual of COD_{total}; $COD_{colloidal}$ and $COD_{soluble}$ in the effluent of the aerobic RBC were lower than that of the anaerobic RBC. The percentage removal values of COD_{total}, $COD_{colloidal}$ and $COD_{soluble}$ were 56.0, 90.0 and 28.0 for the aerobic RBC as compared to 23.0, -16.0 and 14.0 in the anaerobic RBC unit respectively.

The behaviour of the anaerobic RBC is completely different from the aerobic one with regard to $COD_{colloidal}$ removal. Colloidal particles removal is not only the rate limiting step under anaerobic conditions but the $COD_{colloidal}$ concentration even increased from 43 to 48 mgl^{-1} in an anaerobic RBC. At the same time a significant reduction in the concentration of $COD_{suspended}$ was achieved, emphasising the findings reported by Karr and Kemath (1978).

The organic removal rate in the aerobic RBC was 8.0 g COD $m^{-2}.d^{-1}$. Corresponding value for the anaerobic RBC was 3.3 g COD $m^{-2}.d^{-1}$, which mainly resulted from the removal of $COD_{suspended}$. Apparently, adsorption and hydrolysis of suspended particles on the anaerobic biofilm was very efficient.

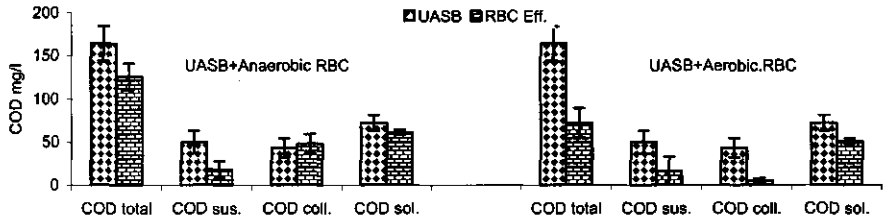

Fig.2 the COD $_{total}$, COD $_{suspended}$, COD $_{colloidal}$ and COD $_{soluble}$ concentrations in the effluent of an anaerobic RBC compared with the effluent of aerobic RBC at the same operating conditions.

The results in Fig 3 show that the E.Coli $_{total}$ removal efficiency in the anaerobic RBC was 43.0 % while the aerobic RBC system eliminated 94.0 %. The overall removal of E.Coli $_{suspended}$ in the anaerobic RBC amounted to 64.8 % as compared to 78 % in the aerobic RBC system. Removal values of E.Coli $_{supra\ colloidal}$ in anaerobic and aerobic RBC units were 6 % and 94 % respectively (Fig. 3).

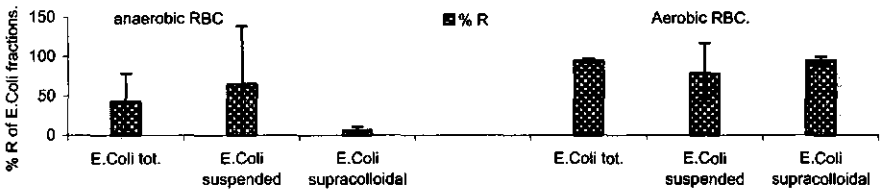

Fig. 3 the E.Coli $_{total}$, E.Coli $_{suspended}$ and E.Coli $_{supra\text{-}colloidal}$ removal efficiency in an anaerobic RBC compared with an aerobic RBC at the same operating conditions.

The effect of introducing an anaerobic RBC unit as a first step on the performance of the aerobic RBC.

In an attempt to improve the quality of the final effluent the use of a treatment scheme consisting of the UASB followed by two RBC system (anaerobic & aerobic) was investigated.

The HRT of the two RBC was 5.0 h and the overall OLR was 7.3 g COD $m^{-2}.d^{-1}$.

The results, presented in Fig.4 reveal that residual total COD concentration in the effluent of a combined anaerobic – aerobic was lower than the single stage aerobic RBC by 14 mgl^{-1}. This likely can be attributed to the higher loading rate of 14.5 g COD $m^{-2}.d^{-1}$ applied to the single stage aerobic RBC as compared to the two stage RBC system (anaerobic followed by aerobic), where the applied OLR was 7.2 g COD $m^{-2}.d^{-1}$.

As the anaerobic RBC system achieved a high removal of COD $_{suspended}$ (66%). The aerobic RBC received a relatively low COD $_{suspended}$ loading rate of 1.55 g $m^{-2}.d^{-1}$ as compared to 4.4 g COD $m^{-2}.d^{-1}$ applied to the single stage aerobic RBC system. This lower COD $_{suspended}$ loading rate resulted in a very low COD $_{suspended}$ in the effluent of the two stage RBC system (3 ± 1.8 mgl^{-1}) as compared to 16.0 mgl^{-1} in the effluent of the single stage aerobic RBC as shown in Fig. 4.

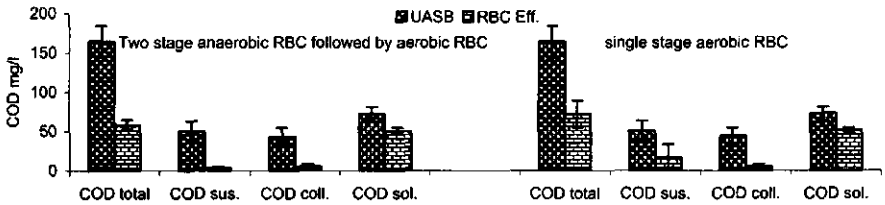

Fig.4 the COD $_{total}$, COD $_{suspended}$, COD $_{colloidal}$ and COD $_{soluble}$ concentrations in the effluent of two stage anaerobic-aerobic RBC compared with the effluent of single aerobic stage RBC system.

The results presented in Fig. 5 show that the overall ammonia removal rate in the combined anaerobic - aerobic RBC system (0.86 g $m^{-2}.d^{-1}$) was lower than found for the single stage aerobic RBC system i.e. 1.7 g $m^{-2}.d^{-1}$. However, the calculated ammonia removal rate in the 2nd stage of two stages RBC was the same 1.7 g $m^{-2}.d^{-1}$.

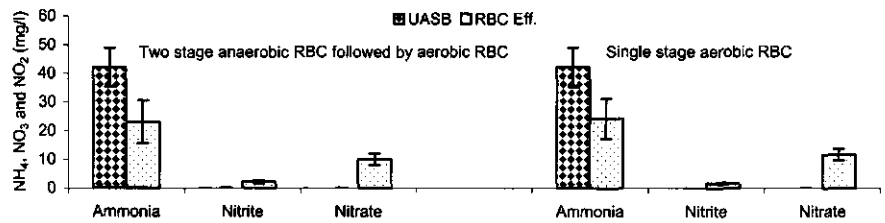

Fig. 5 ammonia, nitrite and nitrate concentrations in the final effluent of two stage anaerobic followed by aerobic RBC compared with the final effluent of single stage aerobic RBC.

The residual ammonia and the nitrate concentrations in the final effluent were 23 and 10.0 mgl^{-1} for the two stage RBC as compared to 24 and 11.5 mgl^{-1} for the single stage aerobic RBC unit, respectively.

The results in Fig. 6 show that the removal of E.Coli $_{total}$ and E.Coli $_{supra-colloidal}$ were very similar in the combined anaerobic-aerobic and the single stage aerobic RBC (95 & 94.3 % and 96 & 94 % respectively). The two stage RBC system was more efficient in removing suspended E.Coli (91.4 %) than the single stage aerobic RBC (78 %).

The single stage aerobic RBC achieved a higher E.Coli $_{colloidal}$ removal (96 %) than the two stage RBC system (92 %), which can be due to the release of colloidal particles to the effluent of the anaerobic RBC unit which created a competition between colloidal particles and E.Coli $_{colloidal}$ for the adsorption sites of the biofilm.

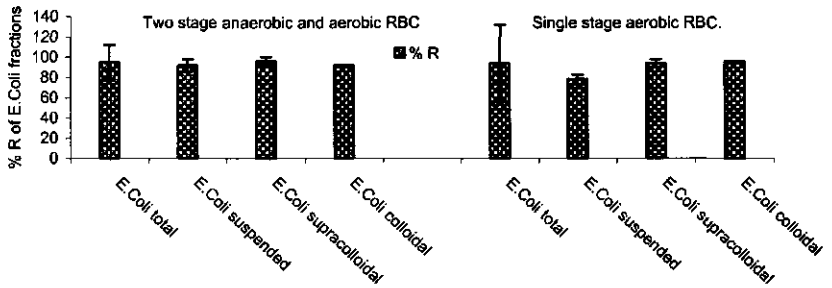

Fig. 6 the overall removal efficiency of E.Coli fractions in the two stage RBC anaerobic followed by aerobic compared with single stage aerobic RBC.

From the available results, it could be concluded that the advantages obtained by the addition of an anaerobic RBC unit prior to the aerobic one are not significant.

GENERAL DISCUSSION

It is conceivable that the COD removed by the UASB reactor is the more readily biodegradable fraction of the domestic sewage. This study shows that the COD and E.Coli in the UASB effluent are significantly easier to adsorb on an aerobically biofilm than by anaerobically biofilm. Barker *et al.,* (1999) studied the characterisation of residual of COD in anaerobic wastewater treatment effluents. They found that the anaerobic effluents are significantly easier to degrade aerobically than anaerobically which demonstrates the advantages of employing an aerobic polishing step after an anaerobic reactor.

A significantly high removal of COD $_{colloidal}$ matter was found in aerobic RBC as compared to COD $_{colloidal}$ particles in anaerobic RBC. This can be attributed to the difference in the characteristics of the biofilm. Aerobic biofilm has a high capacity for adsorbing colloidal particles, while the anaerobic biofilm apparently hydrolyse coarse suspended solids to dispersed colloidal particles.
The aerobic RBC system also achieved a higher removal of E.Coli $_{total}$ and E.Coli $_{suspended}$ as compared to anaerobic RBC system as which emphasis the results of previous experiments carried out by Tawfik *et al.,* (2001).

The anaerobic RBC doesn't comprise an efficient post –treatment step especially for effluents of an efficiently perform UASB reactor. This is due to the slow bacterial growth rates, the relatively low amount of active anaerobic biomass and the relatively low concentration of biodegradable COD in the UASB effluent. The aerobic RBC

system has a much better treatment efficiency as compared to anaerobic RBC system for removing both COD fractions and E.Coli fractions.

The introduction of an anaerobic stage of RBC in front of the aerobic RBC increased the overall removal values by only 9.0 % for COD_{total}, 31.0 % for $COD_{suspended}$, and 13.5 % for $E.Coli_{suspended}$. But it didn't improve the overall removal of $E.Coli_{total}$, $E.Coli_{colloidal}$ and the nitrification rate as compared to single stage aerobic RBC system. This can be due to the release of colloidal particles in an anaerobic RBC leading to an increase in the $COD_{colloidal}$ loading rate of the 2nd stage RBC from 3.7 to 4.3 g $m^{-2}.d^{-1}$. Also hydrogen sulphide concentration increased in the anaerobic RBC effluent, which negatively affected the nitrification rate.

CONCLUSIONS

Comparing the performance of the anaerobic and aerobic RBC operated at the same HRT of 2.5 h and OLR of 14.5 g COD $m^{-2}.d^{-1}$. The following can be concluded:

- the aerobic RBC performs significantly better than the anaerobic RBC for COD_{total}, $COD_{colloidal}$ and $COD_{soluble}$ removal.
- $COD_{colloidal}$ particles become highly entrapped in the biofilm of an aerobic RBC, while this $COD_{colloidal}$ fraction increased in the anaerobic RBC.
- the $E.Coli_{total}$ removal efficiency in an anaerobic RBC was only 43.0 %, whereas, the removal efficiency in an aerobic RBC system reached 94.0 %.
- the overall removal of suspended E.Coli in an anaerobic RBC was 64.8 % compared to 78% in the aerobic RBC system.
- the overall removal of E.Coli supra – colloidal in the anaerobic RBC was only 6.0 % as compared to 94 % in the aerobic RBC system.

The introduction of an anaerobic RBC stage prior to of the aerobic RBC stage reduced the total OLR from 14.5 to 7.3 g COD $m^{-2}.d^{-1}$ and increased the overall removal efficiency By 9.0 % for COD_{total}, 31.0 % for $COD_{suspended}$, and 13.5 % for $E.Coli_{suspended}$. However, didn't improve the overall removal of $E.Coli_{total}$, $E.Coli_{colloidal}$ and nitrification rate as compared to the single stage aerobic RBC unit. Therefore the combination of an aerobic RBC unit with a UASB reactor is a promising treatment scheme.

ACKNOWLDGEMENT

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4

POTENTIALS OF ROTATING BIOLOGICAL CONTACTOR SYSTEMS FOR POST-TREATMENT OF A GOOD QUALITY ANAEROBICALLY PRE-TREATED DOMESTIC SEWAGE.

This chapter has been submitted to Environmental Science & Technology journal as Tawfik A., Klapwijk A., El-Gohary F., Lettinga G.

ABSTRACT

The performance of a single and two-stage RBC system for post-treatment of the effluent of an UASB reactor operated at a temperature of 30 °C has been evaluated. The single and two stage RBC systems were operated at the same OLR of 14.5 $gCOD_{total}/m^2.d$ and at a HRT of 2.5 h. but at different flow rates and different temperatures, viz. of 0.576 & 1.152 m^3/d and 24 & 17 °C respectively. In both systems the same residual effluent values were found for COD_{total} (72 mg/l), for $COD_{suspended}$ (16 mg/l), for $COD_{colloidal}$ (5 mg/l) and for $COD_{soluble}$ (51 mg/l). The removal efficiency of E.Coli also was almost the same, viz. amounting to 94 %. However, the ammonia removal in the single stage RBC system amounted to 50 % of which 71% was nitrified compared to only 23 % in the two-stage RBC system. This better performance can be attributed to the higher temperature of the wastewater during the operation of the single stage RBC system.
In view of the results obtained we recommended to use a single stage RBC system for COD removal and for a partial removal of ammonia and E.Coli at OLR of 14.5 g COD_{total} $/m^2.d$ (10 g $COD_{biod.}$ $/m^2.d$) and at HRT of 2.5h for post-treatment of the effluent of UASB reactor operated at high temperature as generally prevails in tropical countries.

The nitrogen removal from the nitrified effluent was investigated using a biofilm system in a system consisting of three stages; viz. an anoxic up-flow submerged bio-filter followed by a segmental two stage aerobic RBC's. The nitrified effluent of the 2nd stage RBC was recycled to the anoxic up-flow submerged bio-filter reactor. The results obtained reveal that the introduction of an anoxic reactor as a 1st stage combined with recirculation of the nitrified effluent of the 2nd stage RBC is accompanied with a conversion of nitrate into ammonia, at least in case the content of $COD_{biod.}$ in the UASB effluent is low. In such a situation the ammonia needs to be nitrified two times, which obviously should be avoided. Therefore in such 'extreme' situations, i.e. a too 'high quality anaerobic effluent in terms of biodegradable COD

content, the introduction of a separate anoxic reactor for denitrification as final post-treatment step can not be recommended.

INTRODUCTION

In tropical and subtropical regions with ambient temperature ranges between respectively 20 -35 °C and 17 - 30 °C, high rate anaerobic reactors offer a good potential for the treatment of domestic sewage (Van Haandel and Lettinga, 1994; El-Gohary and Naser 1999). A large part of the organic matter present in these wastewater's can be eliminated with such systems (65 % to 80 % COD removal) with very low energy consumption using relatively very simple technologies. Moreover the excess sludge production of these systems is low, while they are much easier to operate as compared to conventional aerobic systems (Schelllinknout and Osario 1992). However, generally the anaerobic effluents need some additional treatment in order to meet the standards, e.g. applicable for reuse in restricted irrigation purposes. So the residual COD values need to be < 80 mg/l. The need for removal of ammonia and pathogenic bacteria depends on the type of crop, the growth season and the irrigation system applied.

Several systems have been investigated for post-treatment of anaerobic effluents. Bovendeur *et al.*, (1990) found a RBC system treating UASB effluent quite efficient in removing particulate organic matter, i.e. substantially more effective than a trickling filter. Moreover, they found higher $COD_{soluble}$ removal rates per unit area in a single stage RBC system than a trickling filter.

A single stage trickling filter followed by a settler was studied for the treatment of a 64 m^3 UASB reactor in Cali, Columbia (Haskoning, 1985). A poor quality effluent was found when the filter medium consisted of big stones (diam. 9 cm), mainly due to their very low specific surface area for attached biofilm growth. At an imposed surface loading rate of 20 $m^3.\ m^{-2}.d^{-1}$ the average COD_{total} and ammonia removal efficiencies found were 16 and 10 % respectively. Faecal coliform removal was poor.

Van Buuren (1991) compared the efficiency of a trickling filter, a submerged filter and a RBC system for post-treatment of UASB effluent. He found that at an HRT of 3.3 h the trickling filter and the submerged filter removed 50 % of COD_{total} while, the RBC removed about 70%. Moreover, at much lower HRT of 0.24 h the RBC still achieved 40 - 80% COD_{total} removal. He therefore concluded that RBC's are more effective in removing organic matter than trickling filters and submerged filters. This was attributed to a better contact between the wastewater and the biofilm and to the higher oxygen concentrations prevailing in the RBC. Moreover, he also found much lower surface ammonia removal rates in the two stage trickling filter and in the

submerged filter as compared to the two-stage RBC under the same operational conditions.
RBC-systems obviously offer big potential's for post-treatment, regarding their high efficiency for the removal of the different COD fractions ($COD_{suspended}$, $COD_{colloidal}$ and $COD_{soluble}$) and potentially for ammonia and E.Coli removal as well (Tawfik *et al.*, 2001).

These systems produce a relatively small amount of rather well stabilised sludge and they can accommodate relatively high hydraulic and organic shock loads. According to the our previous results (Tawfik *et al.*, 2001) a two stage RBC system can also be used successfully for post-treatment effluent's of UASB reactors operated at low temperature of 11 °C.

Therefore, the objectives of this study are, to evaluate the performance of the single and two- stage RBC system operated at the same HRT and OLR. Emphasis will be afforded to the removal efficiency of the various COD fractions ($COD_{suspended}$, $COD_{colloidal}$ and $COD_{soluble}$), and for ammonia and E.Coli removal. An important aspect in this context is to assess the ability of the RBC-system to produce a final effluent quality suitable for safe reuse for restricted irrigation purposes. Furthermore the use of UASB effluents with a relatively low $COD_{biod.}$ content for pre- denitrification will be evaluated.

MATERIAL AND METHODS

Continuous Experiment

Three experiments were conducted in this investigation 1) a single stage RBC system 2) two identical RBC systems operated in series 3) an anoxic up-flow submerged bio-filter reactor followed by two identical RBC's operated in series. All these installations (Fig. 1) were fed with the effluent of a 6 m^3 UASB pilot - plant, previously investigated by Grin *et al.*, (1985) for treatment of raw domestic sewage. The UASB reactor was fed with raw domestic sewage collected in the combined sewer system of the village Bennekom, the Netherlands. The operational temperature of the UASB reactor was controlled at 30 °C.

UASB effluent

The main characteristics of the UASB reactor effluent, i.e. the feed of the RBC systems on these experiments is given in Table 1a and b.

Table 1a. Mean characteristics of the UASB reactor at operational temperature of 30°C in the 1st and 2nd experiments.

Total	COD (mgO_2/l) suspended	colloidal	soluble	Ammonia mg/l	TKN mg/l	E.Coli /100ml
164 ± 21	57 ± 11	28 ± 11	82 ± 22	59 ± 11.5	64.4 ± 11	$1.2*10^6$

Since the nitrate removal primarily depends on the amount of biodegradable COD present in the feed, we estimated this important parameter according to the data obtained by Elmitwalli *et al.*, (2001). They found that the biodegradability of the COD $_{total}$ of domestic sewage of Bennekom village amounted to 71 %. And accordingly for the effluent of the UASB reactor: we can estimate the COD_{biod} as follows,
$COD_{biodegradable} = COD_{total}$ (UASB effluent)* Biodegradability of COD_{total}.

Table 1b. Mean characteristics of the UASB effluent at operational temperature of 30 °C and of the mixed influent of the system (inlet of the anoxic up-flow submerged bio-filter reactor) in the 3rd experiments.

Parameter Unit	COD_{biod} mgl^{-1}	COD_{total} mgl^{-1}	Ammonia mgl^{-1}	Nitrite mgl^{-1}	Nitrate mgl^{-1}	TKN mgl^{-1}	E.Coli/ 100ml
UASB eff	101	142	38.1	-	-	47.6	$1.8*10^6$
UASB & recir effl.	67.5	95 ± 2.5	24 ± 11.6	0.7 ± 0.3	22.9 ± 3.6	28.7 ± 1.8	$9.1*10^5$

Pilot –plants

The schematic diagram of the pilot- plants is shown in Fig. 1. In the 1st experiment a single stage RBC system with a working volume of 60 l and equipped with 10 polystyrene foam disks with a total effective surface area of 6.5 m^2 was used. The reactor was operated at wastewater temperature of 22 - 25 °C and at a flow rate of 0.576 m^3/d for a period of three month. The disk diameter was 0.6 m with a thickness of 0.02 m and the discs were spaced at 0.02 m distance and operated at 5 rpm. The submerged surface amounted to 40 %. The disks were mounted on a steel shaft. The experiment with the single stage RBC system was carried out during the summer months when COD concentration in the raw domestic sewage reached to 700 mg/l.

In the 2nd experiment with two stages RBC system, the operational temperature was 16-18 °C. The imposed flow rate was 1.152 m^3/d during the full experimental period of three months.

In the 3rd experiment we included as 2nd treatment step an anoxic up-flow completely submerged bio-filter with a working volume of 15 l and filled up to 80.0 % of its volume by polyethylene carrier material (specific surface area of 363 $m^{-2}.m^{-3}$). The anoxic unit was followed by two identical stage RBC system as described above. The operational wastewater temperature was 16 °C. Effluent from the final nitrifying RBC

stage was collected in a holding tank from which, it partially was recycled and partially discharged into the drain. In this study, the applied recirculation ratio defined as, the ratio of the returned flow rate to that of the inlet flow, was 1. A flow control pump was used to return 50 % of the effluent from the holding unit to an anoxic reactor. This system was operated at a flow rate of 0.324 m^3/d for a period of three month.

Sampling and analytical methods.

Analysing 48 hrs composite samples of the influent and the effluent of each step followed the performance of the reactors. The samples were collected in a fridge at 4 °C.

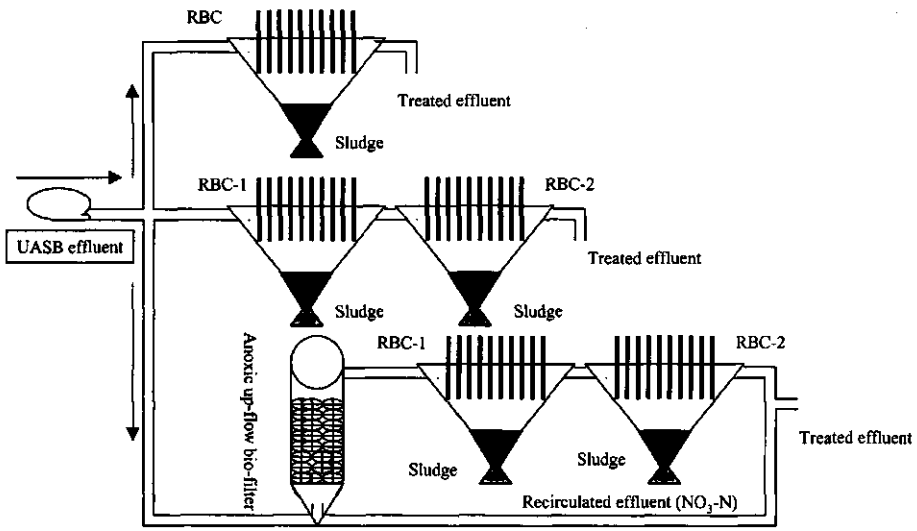

Fig.1 Schematic flow diagram of pilot plants.

Parameters like dissolved oxygen, pH and temperature were measured regularly in situ. The COD was analysed using the micro-method as described by Jirka and Carter (1975). Raw samples were used for COD $_{total}$, 4.4 um folded paper filtered (Schleicher & Schuell 595 1/2) samples for COD $_{filtrate}$ and 0.45 um membrane filtered (Schleicher & Schuell ME25) samples for dissolved COD (COD $_{soluble}$). The COD $_{suspended}$ and COD $_{colloidal}$ were calculated by the difference between COD $_{total}$ and COD $_{filtered}$, COD $_{filtered}$ and COD $_{soluble}$, respectively. Ammonia, nitrite and nitrate were determined on a stationary auto-analyser (SKALAR SA-9000), total Kjeldahl nitrogen according to the Dutch Standard Normalised Methods, (1969) and E.Coli according to the method described by Havelaar *et al.*, (1988).

Batch denitrification experiment.

For a number of complementary batch denitrification experiments, two six- litre cylinders were used. One of the cylinders was supplied with 2 litre UASB effluent of COD_{total} of 156 and the other with 2 litre of acetate solution with COD_{total} of 156 mg/l (cylinder 2). To each cylinder 2.0 litre denitrifying sludge (3.1 g MLSS/l and 2.3 g VSS/l) from a denitrifying activated sludge pilot-plant was added together with an aqueous solution of sodium nitrate. Samples were withdrawn from the batches every 15.0 minutes for a total period of 2.0 hrs for nitrate concentration measurement.

In order to assess the endogenic respiration of the denitrifiers a similar series of batch denitrification experiments were conducted. One cylinder contained denitrifying activated sludge (3.1 gMLSS & 2.3 gVSS/l) and an aqueous nitrate solution. The second cylinder contained denitrifying activated sludge, an aqueous nitrate solution and the UASB effluent. Also here samples were withdrawn from the reactors every 15.0 minutes for a total period of 2.0 hrs for measurement nitrate concentration.

RESULTS AND DISSCUSION

Results of 1st experiment with the single stage RBC system.

Removal of various COD-fractions: The COD_{total}, $COD_{suspended}$, $COD_{colloidal}$ and $COD_{soluble}$ removal data found in the single stage RBC system operated at OLR of 14.5 $gCOD_{total}/m^2.d$, and HRT of 2.5 h are depicted in Fig. 2. The results clearly reveal that the single stage RBC system achieved a substantial reduction of COD_{total} resulting in an average effluent concentration of only 72 mg/l. This indicates the high efficiency of a single stage RBC for removing of COD_{total} at a relatively short HRT and a relatively high OLR. The high removal efficiency of the UASB reactor for the various COD fractions at high temperature conditions clearly positively affects the performance of the single stage RBC system.

Based on the assessed removal efficiency of 56 % for COD_{total} in the RBC system and assuming a COD removal efficiency of the anaerobic pre-treatment step of 75 % under these conditions, an overall COD_{total} removal efficiency of about 90% can be achieved for the combined UASB - single stage RBC system.

The results presented in Fig.2 furthermore show that the single stage RBC system achieved an almost complete removal of $COD_{colloidal}$ i.e. only 5 ± 2.7 mg/l of this COD-fraction remained in the final effluent. This excellent performance towards the removal of colloidal matter can be attributed to entrapment or/and adsorption followed by hydrolysis and degradation.

The results also reveal that the system performs better with respect to the COD $_{colloidal}$ removal as compared to the COD $_{suspended}$ fraction. The COD $_{colloidal}$ decreased from 43 to 5 mg/l, whereas the COD $_{suspended}$ dropped from 50 to 16 mg/l.

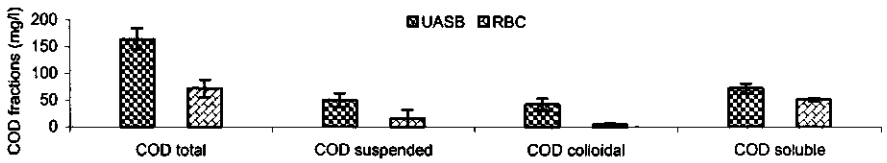

Fig.2 The COD fractions in the UASB effluent and effluent of the single stage RBC system.

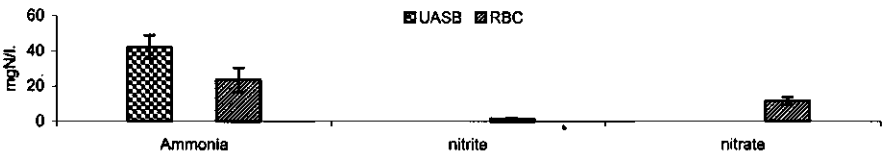

Fig.3 The ammonia in the UASB effluent and the effluent of ammonia, nitrite and nitrate of the single stage RBC system.

Ammonia-nitrogen removal: The single stage RBC system not only performed very satisfactory for the removal of COD fractions but also a considerable ammonia removal was accomplished at temperatures of 22 - 25 °C. The results presented in Fig.3 reveal that about 50 % ammonia was eliminated at an HRT of 2.5 h or OLR of 14.5 g COD $_{total}$ /m^2.d and temperatures ranging from 22 to 25 °C. Nitrate and nitrite data reveal that 71 % of the ammonia removed occurred through nitrification. The remaining portion of ammonia removed (5.2 mg/l) probably occurred as a result of adsorption. According to Temmink *et al.,* (2001) ammonia can be adsorbed in the biofilm. It is known that the biofilm consists mainly of bacterial cells and extra-cellular polymeric substances (EPS) which all have a negative surface charge. Consequently various cations, of mono-, di-, and trivalent can be bound, including ammonium. Another explanation for the gap in the ammonia balances cannot be provided at this moment.

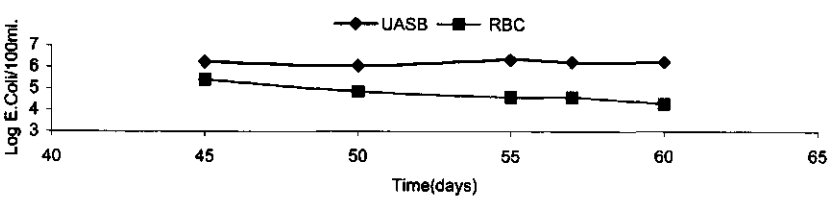

Fig.4 The course of E.Coli in the final effluent of the single stage RBC system.

E.Coli removal: The results presented in Fig. 4 show that the single stage RBC system achieved a substantial removal of E.Coli, with values ranging from 89.4 to 96.4 % (average value of 93.4 ± 2.6 %). The E.Coli count in the final effluent

amounted to 1.1 x 10^5 /100ml, which means that according to prevailing (too severe) WHO-standards the effluent only can be reused for restricted irrigation purposes.

Results of 2nd experiment with the two stages RBC system.

Removal of the various COD fractions: The measured effluent COD $_{total}$, COD $_{suspended}$, COD $_{colloidal}$ and COD $_{soluble}$ concentrations obtained from the two stage RBC system operating at total HRT of 2.5 h., and total OLR of 14.5 g COD /m^2.d are presented in Figs. 5a, b, c, and d. The COD $_{total}$ concentration measured in the effluent of the two-stage RBC effluent ranged from 49 to 87 mg/l, with an average value of 72 mg/l (Fig.5a). Moreover, the result also clearly demonstrate that the two stages RBC system can produce an effluent containing very low concentrations of COD $_{suspended}$ and COD $_{colloidal}$ (Fig. 5b and c).

Ammonia-nitrogen removal: The results Table 2 indicate that a small amount of ammonium was eliminated in the 1st stage of two stage RBC system with an average value of 5 % of the influent, i.e. an imposed HRT of 1.25 h and OLR of 29 g COD/m^2.d. The 1st stage of the system, which is operated at high OLR unable to develop a sufficient 'enrichment' of slowly growing nitrifiers. Therefore, a substantial nitrification can not be accomplished in this 1st stage.
It is clear from the results presented in Table 2 indicate as in fact could be expected that the nitrification mainly proceeded in the 2nd stage of two stage RBC system at OLR of 20 g COD/m^2.d. The residence time of nitrifiers is relatively high here as a result of the relatively low growth in of heterotrophic organisms. The calculated nitrification rate of the 2nd stage according to the nitrate production amounted to 1.56 g NO_3-N /m^2.d.

Table 2. The nitrogen removal in the 1st and 2nd stage of two stage RBC system operated at total OLR of 14.5 g /m^2.d and total HRT of 2.5 h.

Parameter	Unit	UASB eff.	1st stage	%R	2nd stage	%R	Overall eff.
Ammonia	mg/l	58.5 ± 11.5	55.8 ± 11.8	4.9 ± 2.6	45.9 ± 12.5	19 ± 10	23 ± 10.5
Nitrite	mg/l	-	1 ± 0.2	-	1.9 ± 0.7	-	-
Nitrate	mg/l	-	0.4 ± 0.6	-	9.2 ± 2.5	-	-
TKN	mg/l	64.4 ± 11	61.4 ± 11.8	5.0 ± 3.5	57 ± 10.5	7.2 ± 3	12.1 ± 4.5

E.Coli removal: The removal of E.Coli in the 1st stage of two stage RBC system amounted to 78 (± 10.4)% and 66.4 (± 10.2) in the 2nd stage of the system, resulting in an overall removal value of 93 (± 4.1)% as shown in Fig.6. Although the 1st and 2nd stage of the two stages has been operated at the same HRT of 1.25 h, the removal in the 1st stage was higher. This indicates that: 1) a rapid removal of attached E.Coli with suspended solids took place in the 1st stage of the system 2) a slow elimination

of free suspended E.Coli (E.Coli colloidal) has been achieved in the 2nd stage (Tawfik *et al.*, 2001, Gannon *et al.*, 1983).

Results of 3rd experiment with the two stage RBC + anoxic submerged biofilter.

COD $_{total}$ removal: From the results in Fig.7 it is clear that the anoxic unit operated at an HRT of 1.1h. only provides a poor COD $_{total}$ removal, i.e. amounting to 16.7 %. The combined system (viz. the anoxic unit + 1st RBC-stage) clearly is quite effective in removing COD, because a very low concentration of COD $_{total}$ was obtained, i.e. amounting to 62 mg/l at total HRT of 5 h. It is obvious that only a minor amount of COD can be removed in the 2nd stage of two -stage RBC system. As follows from the results in Fig. 7 only 13 mg/l of COD $_{total}$ was removed in the 2nd stage, resulting in an average residual value of 48 mg/l for total COD, which is exceptionally low indeed.

Nitrate removal: Due to a low COD removal in the anoxic unit, it can be expected that the denitrification will be limited. The results in Fig. 7 even reveal average ammonia

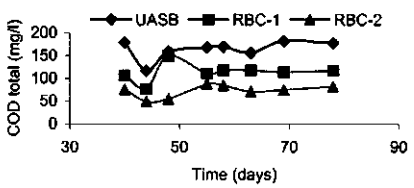

Fig.5a the course of COD total effluent in the two stage RBC system.

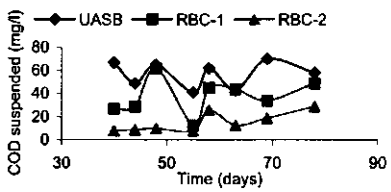

Fig.5b the course of COD suspended effluent in the two stage RBC system.

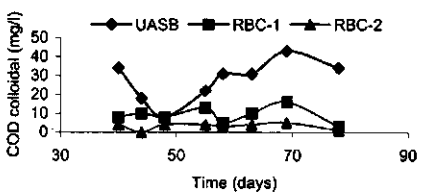

Fig.5c the course of COD colloidal effluent in the two stage RBC system.

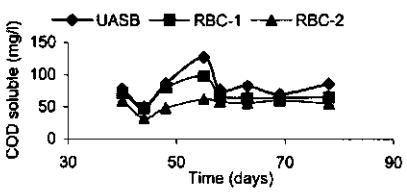

Fig.5d the course of COD soluble effluent in the two stage RBC system.

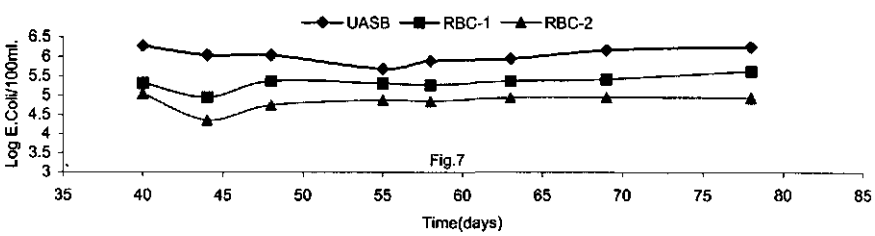

Fig.6 the course of E.Coli effluent in the two stage RBC system.

production of 6.3 mgl^{-1} while the nitrate reduction only amounted to 5.3 mgl^{-1} in this unit. Apparently a dissimilatory nitrate reduction to ammonia-nitrogen of 5.3 $mgNO_3$-

$N.l^{-1}$ occurs when the concentration of COD $_{biod.}$ is low. This would mean that under these specific prevailing conditions ammonia formers develop more favourably than the denitrifiers.

Nitrification: despite the increase of ammonia concentration in the anoxic unit, a nitrification rate of 0.72 $gNH_4\text{-}Nm^{-2}.d^{-1}$ (75.6 %) was achieved in the 1st stage of the RBC system, when operated at OLR of 4.2 g COD $_{total}$ $m^{-2}.d^{-1}$ (Fig.7). This obviously can be attributed to the low OLR applied to the 1st stage of the RBC system. The concentrations of ammonia and nitrate in the effluent of 1st aerobic stage amounted to 15 and 28 mg/l respectively. Only 6 mg/l ammonia removal was achieved in the 2nd stage of the system (see Fig.7) resulting in an overall average residual value of 9 mg/l for ammonia and 9.8 mg/l for TKN.

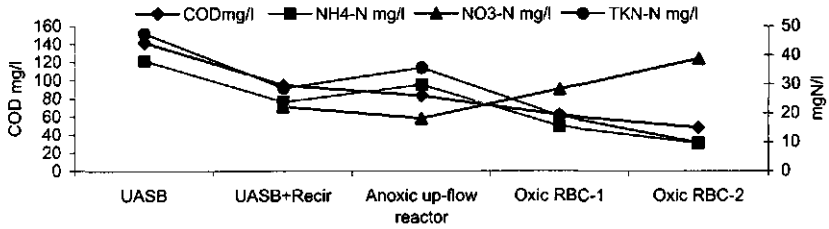

Fig.7 The ammonia, COD, TKN and nitrate concentrations along the three systems conducted with a feed of low COD biod .

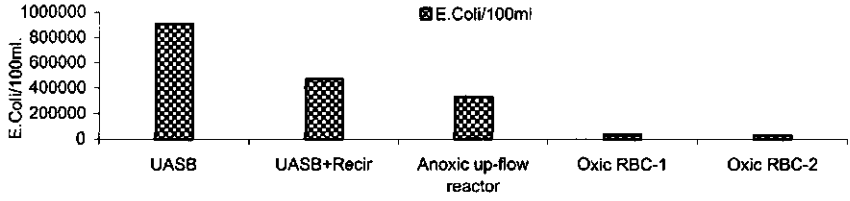

Fig. 8 The E.Coli concentrations along total system conducted with a feed of a low COD biod.

E.Coli removal: The results of the E.Coli examinations (Fig. 8) reveal that the removal of E.Coli in the anoxic unit only amounted to 0.2 log_{10}. This low efficiency likely mainly can be due to a very low concentration of dissolved oxygen prevailing here, the low removal efficiency for COD and the applied short HRT. On the other hand the E.Coli concentration dropped substantially, i.e. by 2.9 x 10^5 /100ml, in the 1st stage of the RBC system. The results clearly demonstrate the high efficiency of the aerobic RBC system for removing of E.Coli. The results in Fig. 8 also reveal that

$_{total}$ with a poor quality UASB-effluent, viz. at the same HRT of 2.5 h but at a significantly higher OLR of 25.4 gCOD/m^2.d. Apparently the two stage RBC system in the present experiments with a high quality UASB-effluent has been operated under organic substrate limiting conditions. Therefore in that case the two stage RBC system certainly can accommodate OLR 's twice as high (i.e. 14.5 gCOD/m^2.d in the present experiments) without any risk of deterioration in the final effluent quality, at least as far as COD removal concerned. Moreover, since the UASB-effluent quality was found not to affect the removal efficiency of COD fractions in the two stage RBC system, it is clear that this reactor type can accommodate OLR-shock loads up to 52 gCOD $_{total}$ /m^2.d (Tawfik *et al.*, 2001). Accordingly, we can conclude that in case for any reason the UASB reactor doesn't perform satisfactory, it is recommendable to apply a two-stage RBC system as post-treatment.

The next question is whether or not it would be beneficial to use a high quality UASB-effluent in case nitrification is needed and high E.Coli removal efficiency needs to be accomplished. In the present investigation, a high nitrification rate of 0.97 gNO_3-N/m^2.d and a low residual value of E.Coli of 1.1 x 10^5 / 100ml was found for the single stage RBC system when treating a high UASB-effluent quality at an imposed OLR of 14.5 gCOD/m^2.d and HRT of 2.5 h. This nitrification rate is higher by 0.8 gNO_3-N/m^2.d and the residual count of E.Coli lower by 0.8 $\log_{10}$ than found earlier for a poor UASB-effluent quality with a single stage RBC at HRT of 5 h and OLR of 13 gCOD/m^2.d (Tawfik *et al.*, 2001). The poorer performance of the system in latter case was due to a higher COD particulate (COD $_{suspended}$ and COD $_{colloidal}$) concentrations by 41 % in the UASB effluent. This negatively affects the nitrification rate due to its entrapment in the biofilm, diluting the fraction of nitrifying organisms in the biofilm and consuming part of the oxygen which otherwise would have been available for the nitrifiers (Temmink *et al.*, 2001). Moreover the particulate matter in the UASB effluent (t=14 °C) also protects the E.Coli against environmental factors like light and biological processes like predation. Once again the results illustrate that an efficient anaerobic pre-treatment with respect to the removal of COD particulate in its effluent will substantially increase the nitrification rate and improve the E.Coli removal in a single stage RBC system by decreasing the COD particulate.

The denitrification rate in a post-treatment system obviously mainly will depend on a) denitrifying bacterial activity of the retained sludge and b) the amount of biodegradable COD available in the wastewater. Therefore, in case an efficient pre-treatment is achieved in the UASB reactor, consequently the feed to post-treatment facilities is very low in COD $_{biod.}$ content, the pre-denitrification process will be quite detrimentally affected. The results obtained with the anoxic reactor under conditions of a low COD_{biod} content in the feed indicate even an increase of the ammonia concentration upon effluent recycling to the anoxic reactor, while at the same time a drop is found in the nitrate concentration. Regarding the conversion of nitrate to

ammonia, recycling of nitrified effluent in order to achieve a pre-denitrification can not be recommended under these conditions.

The reason for the occurrence of nitrate reduction to ammonia in the anoxic reactor is unknown and further research is needed to study these phenomena. According to Barber *et al.,* (2000) who investigated nitrogen removal in a modified anaerobic baffled reactor using a synthetic wastewater, some nitrate can be reduced to ammonia in case hydrogen is available, viz. according to equation 1. The source of hydrogen gas for dissmilatory nitrate might be propionate.

$$NO_3 + 2H^- + 4H_2 \rightarrow NH_4^+ + 3H_2O \quad -600\ kj/mol\ N \qquad (1)$$

Krul (1975) investigated the oxygen uptake and the dissimilatory nitrate reduction by anaerobically grown cells of a denitrifying *Alcaligenes* strain, prevailing in floc or in suspension at different oxygen concentrations in the medium. It was observed that at oxygen concentrations in the medium below 1.5 mg/l, the nitrate reduction to ammonia by the organisms present in flocs increased considerably. The dispersed cells only gave an increased nitrate reduction to ammonia at oxygen concentrations below 0.1 mg/l.

The results of the batch experiment presented in Table 3 clearly reveal that the COD present in high quality anaerobic effluent is unsuitable for denitrification, because it only allows 3 mg/l-nitrate removal. Obviously the extent of pre-denitrification can be improved by allowing a high biodegradable COD content in the UASB effluent, consequently applying higher loading rates to the UASB- reactor (e.g. lower operational temperatures). Collivignarelli *et al.,* (1990) found a nitrate removal of 9.5 mgNO_3-N/l in a 1st anoxic biological fluidised bed reactor when using an UASB-effluent containing 185 mgCOD_{tot}/l, which corresponds to a COD_{biod} of 132 mg/l at the minimum. Likewise the results obtained by Garuti *et al.,* (1992) indicated that 63.5% nitrate removal could be achieved in an anoxic baffled reactor, when using an effluent of the anaerobic baffled reactor with a COD-content amounting to 258 mg/l.

CONCLUSIONS

- A single stage RBC system of the type investigated in the present study represents an efficient post-treatment process for a high quality anaerobically pre-treated domestic sewage (e.g. UASB-reactors applied at tropical ambient temperatures). Average residual effluent COD-values as low as 72 mg/l for COD_{total} can be obtained with ammonia concentration up to 24 mg/l at an HRT of 2.5 h and OLR of 14.5 gCOD/m^2.d. Considering these results we recommended to apply a single stage RBC system for post-treatment of high quality anaerobic effluent's.

- A combined UASB and the single stage RBC system represent a very promising option for the treatment of domestic sewage in tropical and subtropical countries. The UASB reactor under these conditions can be operated at a very short hydraulic retention time of 4-6 h (Chernicharo and Machado 1998) and a single stage RBC post-treatment system at 2.5 h, resulting in a very compact and low cost treatment unit, which provides a final effluent quality that complies for reuse in restricted irrigation purposes.
- The results with two stages RBC system investigated here and in our previous studies indicate that the COD concentration in the effluent of a two stage RBC system treating high and poor quality UASB effluent is the same. Therefore if for any reason the UASB reactor doesn't perform satisfactory, a two stage RBC system is recommended.

- Application of an anoxic reactor for treating recycled final nitrified effluent from the RBC is not recommended, because the system suffers from the drawback that part of nitrate will be converted to ammonia in case of the content of $COD_{biod.}$ in the UASB-effluent is very low

ACKNOWLDGMENTS

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5

EFFECT OF COD $_{BIODEGRADABLE}$ LOADING RATE OF UASB EFFLUENT ON COD, E. COLI REMOVAL AND NITRIFICATION IN ROTATING BIOLOGICAL CONTACTOR (RBC).

This chapter has been submitted to Water Research Journal as: Tawfik A., Klapwijk A., E.Gohary F. and Lettinga G.

ABSTRACT

This study has been carried out in order to assess the impact of COD $_{biod.}$ loading rate applied to the single and two stage RBC system on the removal efficiency of distinguished COD fractions (COD $_{suspended}$, COD $_{colloidal}$ and COD $_{soluble}$), E.Coli and the nitrification efficiency when using different anaerobic effluent qualities as influent. The results clearly show a significant improvement of the residual values of COD $_{total}$ and E.Coli of the effluent of the single stage RBC system at a low COD $_{biod.}$ loading rate. Moreover, the calculated sludge stability (VSS/MLSS) amounted to 0.63 in the single stage RBC system, fed with low COD $_{biod.}$ content compared to 0.74 when fed with high COD $_{biod.}$ content.

The efficiency of two-stage RBC was compared at COD $_{biod.}$ loading rate of 9 and 18 g $m^{-2}.d^{-1}$ and at constant HRT of 2.5 h. The results reveal that the residual values of distinguished COD fractions in the final effluent remained unaffected, when changing the imposed COD $_{biod.}$ loading rate from 9 to 18 g $m^{-2}.d^{-1}$. This indicates that robust system that is resilient to organic load variation and therefore, if two stage RBC system designed properly for post-treatment of anaerobic effluent in the tropical countries, it will perform similarly in subtropical and moderate climate countries. Moreover, the RBC-system is quite effective in eliminating the COD $_{coll.}$, COD $_{sus.}$ and COD $_{sol.}$ at both applied COD $_{biod.}$ loading rates. Despite the residual value of E.Coli in the final effluent of two stage system decreases from 3.4×10^5 at the higher COD $_{biod.}$ loading rate to 7.6×10^4 /100ml at the lower one, the removal of E.Coli is limited. The 1st stage RBC system achieved extremely low ammonia removal efficiency, therefore the nitrification only proceeded in the 2nd stage of two stage RBC at both COD $_{biod.}$ loading rates. The calculated nitrification rate in the 2nd stage of two stage RBC system was decreased from 1.56 to 1.1 g NO_3-N.$m^{-2}.d^{-1}$ with increase the COD $_{biod.}$ loading rate from 11.3 to 16 g $m^{-2}.d^{-1}$.

INTRODUCTION

The Up-flow Anaerobic Sludge Blanket (UASB) effluent will contain both residual and non-or slowly biodegradable COD dependent on the UASB reactor efficiency. Wang (1994) carried out experiments to assess the maximum achievable amount of degradation for an effluent of the Up-flow Hydrolysis Sludge Blanket (HUSB) reactor under anaerobic conditions. He concluded that for this specific HUSB effluent the non-biodegradable COD concentration is around 130 mg l^{-1}.

The $COD_{biod.}$ concentration in the effluent of an UASB reactor will change with the imposed temperature in the reactor and with process conditions as well. Lettinga and his workers have studied since 1976 the application of UASB reactors for sewage treatment under low temperature conditions in the Netherlands. Grin *et al.,* (1985) operated a 6 m^3 UASB seeded with digested sewage sludge at an HRT of 14 - 17. The. COD reduction reached 85 – 65 % and 70 – 55 % at 20 and 13 - 17 °C. De Man *et al.,* (1986) concluded that anaerobic treatment of raw domestic sewage (COD = 500 - 700 mg l^{-1}) can be accomplished at 12 - 18 °C applying HRT's of 7 - 12 h with total COD and BOD removal efficiencies of 40 – 60 % and 50 – 70 % respectively.

In tropical countries where high temperatures prevail, anaerobic treatment of sewage has found wider acceptance, and there are several full-scale plants already in operation. The demonstration/pilot plant built in Cali is claimed to be the first of its kind in the world (Louwe Kooijmans *et al.,* 1985). A 64 m^3 reactor was operated at an HRT of 4 - 6 h and average sewage temperature of 25 °C. COD and BOD removal efficiencies higher than 75 % were obtained. In Brazil Goncalves *et al.,* (1999) investigated the UASB performance in terms of SS and BOD removal at HRT of 8 h and high temperature. They found that the anaerobic effluent presented the following mean characteristics of SS = 37 mg l^{-1}, COD = 112 mg l^{-1} and BOD_5 = 36 mg l^{-1}.
The characteristics of an anaerobic effluent at high operational temperatures are quite different from those at low temperature e.g. they generally have a low COD biodegradable ($COD_{biod.}$) content. This obviously is an important factor for the post-treatment process. The performance of a post-treatment system will depend on the characteristics of the anaerobic effluent, and therefore its design will depend on both the influent characteristics and the effluent standards for discharge or reuse. For instance, the nitrification capacity is expected to improve significantly in the post - treatment system as compared to the situation in which raw sewage would be treated directly, since a substantial part of the biodegradable organic matter is removed in anaerobic pre-treatment at high operational temperatures. Moreover it also can be expected that a lower $COD_{biod.}$ concentration in the UASB effluent such as will be the case for a well designed UASB-system in tropical countries that, the excess sludge production will decrease while the sludge stability will increase. Moreover, also a better E.Coli removal efficiency can be expected. It is known that particulate organic

matter normally present in anaerobically pre-treated effluents adversely will affect the removal of E.Coli. A higher removal of suspended and colloidal COD in the UASB reactor therefore would positively affect the pathogenic bacteria removal in the post-treatment system. However, anaerobic pre-treatment systems like UASB reactors are not very efficient in removing colloidal matter (El-Mitwalli 2001).

The experiments reported in this paper have been carried out in order to assess the potential increase of the COD removal efficiencies, the nitrification capacity and E.Coli removal efficiency in the single and in a two stage RBC system when treating different quality of anaerobic effluent. We investigated the effect of the $COD_{biod.}$ loading rate using an effluent with a low $COD_{biod.}$ content (i.e. pre-treated in a UASB at temperature of 30 °C) and high $COD_{biod.}$ content (i.e. pre-treated by UASB at temperature of 11°C). Additionally, we attempted to use the 2nd stage of two stages RBC system for removal of COD instead of ammonia by introducing the UASB effluent directly to the 2nd stage of two-stage RBC system. During this experimental phase the 1st stage of two-stage RBC system was put out the operation. At the same time we assessed the change in nitrifying bacteria activity of the 2nd stage RBC system during a period of 9.0 days, when fed at a high $COD_{biod.}$ loading rate of 13.2 g $COD_{biod.}$ $m^{-2}.d^{-1}$, following a feed less period of 7.0 days.

The objectives of this study therefore are,

1. to compare the effect of the presence of higher and lower concentrations of $COD_{biod.}$ in the UASB effluent on the performance of a single and two-stage RBC system at imposed different $COD_{biod.}$ loading rate but the same HRT.
2. to examine the effect of a temporarily imposed high $COD_{biod.}$ loading rate on the activity of nitrifying sludge in the 2nd stage of two-stage RBC system.

MATERIAL AND METHODS

The research was carried out in the experimental pilot plant station, situated in the village Bennekom, The Netherlands, using the domestic sewage of the village (collected in a combined sewer system). A small part of the raw domestic sewage is treated at two different operational temperatures of 11 and 30 °C in a 6 m^3 Up-flow Anaerobic Sludge Blanket (UASB) reactor previously extensively investigated by Grin *et al.*, (1985).

According to recent results obtained by Elmitwalli *et al.*, (2001) the anaerobic biodegradability of $COD_{suspended,}$ and $COD_{colloidal\ in}$ in domestic sewage of Bennekom village amounts to 86 and 77 % respectively. According to our previous results (Tawfik *et al.*, 2001) the average $COD_{soluble}$ in the final effluent of two stage system operated at a long HRT of 10 h always amounted to 50 mg l^{-1}, which can be

considered as inert organic matter. Accordingly, we estimated the values for biodegradable of $COD_{suspended}$, $COD_{colloidal}$ and $COD_{soluble}$ in the UASB effluent at operational temperature of 30 and 11 °C as follows,

$COD_{biod.\ (Sus\ and\ coll.)} = COD_{sus.\ and\ coll.}$ (UASB eff.)* Biodegradability of $COD_{sus.\ and\ coll.}$

$COD_{soluble\ biod.} = COD_{sol.}$ (UASB eff)- inert $COD_{sol.}$

UASB effluent quality at treatment temperatures of 30 and 11°C.

The main characteristics of the UASB effluent under operational temperature conditions of 30 and 11°C are given in Table 1.

Table 1. Mean COD effluent characteristics of the UASB reactor.

Parameter	$COD_{biod.}$ (mgl^{-1})				$COD_{biod.+non\text{-}biod.}$ (mgl^{-1})			
Samples	total	Sus.	Coll.	solubl e	total	Sus.	Coll.	Sol.
UASB eff. (30°C)	100	44	24	32	164	57	28	82
UASB eff. (11°C)	208	71	58	79	287	91	67	129

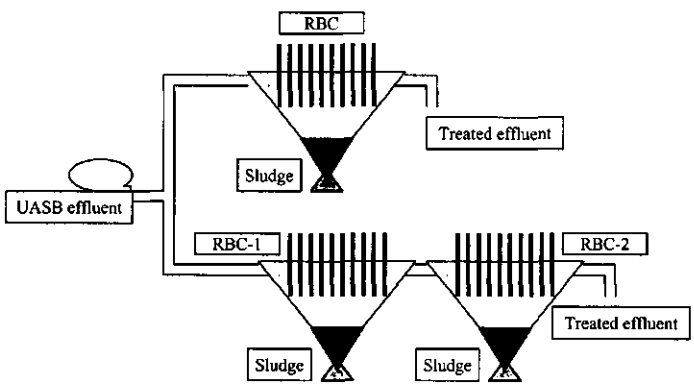

Fig.1 Schematic representation of the single stage and two stage RBC system treating different qualities of UASB effluent.

Rotating Biological Contactor (RBC) system.

Two experiments was investigated here (Fig.1), by using 1) single stage RBC system 2) two identical stage RBC system operated in series. The all systems have the same design criteria. Each RBC reactor equipped with 10 polystyrene foam disks with a working volume of 60 l and with a total effective surface area of 6.5 m^2. The reactors were operated at wastewater temperature of 18 and 16 °C in the experimental hall. The disk diameter was 0.6 m with a thickness of 0.02 m and the discs were spaced at 0.02 m distance and operated at 5 rpm. The submerged surface amounted to 40 %. The disks were mounted on a steel shaft.

Sampling and Analysis:

Composite 48 hrs samples of the influent and the effluent of each reactor were collected in containers placed in a fridge at 4 °C. Grab samples were used for measuring the temperature, DO, H_2S and pH.

The COD was analyzed using the micro-method as described by Jirka and Carter (1975). Raw samples were used for COD $_{total}$, 4.4 um folded paper filtered (Schleicher&Schuell 595 ½) samples for COD $_{filtrate}$ and 0.45 um membrane filtered (Schleicher&Schuell ME25) samples for dissolved COD (COD $_{soluble}$). The COD $_{suspended}$ and COD $_{colloidal}$ were calculated by the difference between COD $_{total}$ and COD $_{filtered}$, COD $_{filtered}$ and COD $_{soluble}$, respectively. Ammonia, nitrite and nitrate were determined using an auto-analyser (SKALAR SA-9000). Total Kjeldahl nitrogen and sludge analyses were carried according to the Dutch Standard Normalized Methods (1969). E.Coli was analysed according to the method described by Havelaar *et al.*, and (1988).

Duration of the experiments.

The investigations in the single and two stages RBC-system were conducted over a period of almost 7.0 months.

Complementary experiment with the 2nd stage of two-stage RBC system loaded with a temporary high COD $_{biod.}$ loading rate.

In a complementary experiment, the 2nd (nitrifying) stage of two-stage RBC system was temporarily fed at different COD $_{biod.}$ loading rate, in order to 1) assess the effect of a high COD $_{boid.}$ loading rate over its nitrifying activity. 2) use the 2nd stage of two stages RBC system for removal of COD directly from the UASB effluent. Emphasis was put not only on the preservation of ammonia in the final effluent of 2nd stage for reuse purposes as fertilizer along with application of the effluent for irrigation purposes but also on the removal of COD and the rate of recovery of nitrification when feeding of the 2nd stage with the effluent of 1st stage was renewed. For this purpose the effluent of the UASB reactor was introduced to the 2nd stage RBC system (nitrifying stage) for 9 days, after it had been exposed to a feed-less period of 7 days. A flow diagram of the operational steps is shown in Fig 2.

RESULTS AND DISCUSSION

During the RBC experiments presented here the UASB reactor was operated at a more or less constant OLR but at two different operational temperatures viz. 30 and 11°C. As a result of this quite different operational temperatures the COD $_{biod.}$ concentration in the UASB effluent is quite different, and consequently also the COD

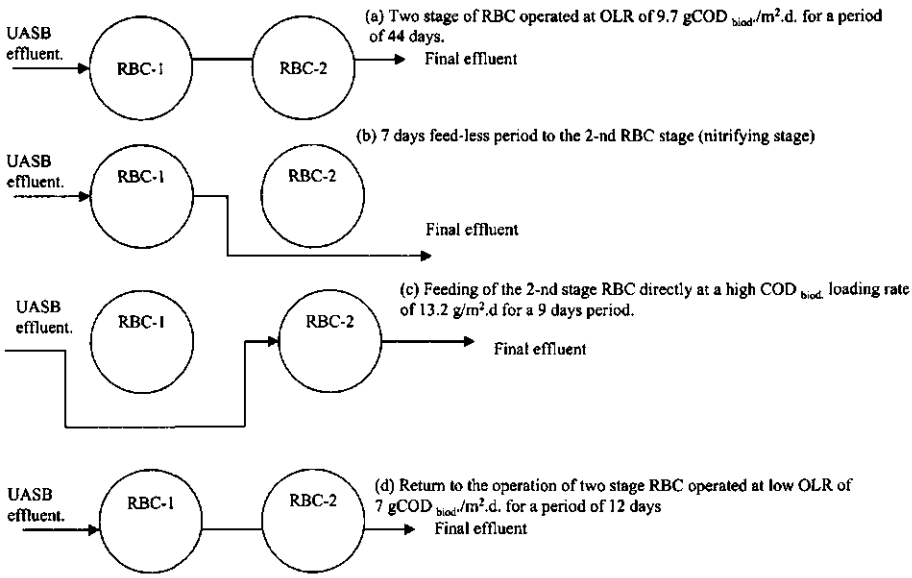

Fig.2 Schematic diagram shows the experimental procedure applied in the complementary nitrifying RBC - reactor under conditions of a temporary high COD $_{biod.}$ loading rate.

$_{biod.}$ loading rate imposed to the RBC-system. This enabled us to 1) assess the effect of the COD $_{biod.}$ loading rate on the performance of the single and two stage RBC system as post-treatment 2) evaluate the potentials of the single and two stage RBC system to be used as a post-treatment in tropical, subtropical and cold climate countries.

Results of the 1st experiment.

Effect of COD $_{biod.}$ loading rate on the performance of the single stage RBC system.

Removal of various distinguished COD fractions: The performance results of the single stage RBC system operated at a constant HRT of 1.25 h and COD $_{biod.}$ loading rates of 17.7 and 36.8 g $m^{-2}.d^{-1}$ are summarised in Table 2. It is clear that, the quality of the single stage RBC effluent distinctly improves at the lower imposed COD $_{biod.}$ loading rate, viz. 17.7 g $m^{-2}.d^{-1}$ in this case i.e. the final effluent COD $_{total}$ became lower by a value of 27 mgl^{-1}. This indicates that the effluent quality of the single-stage system depends on the applied organic loading rate. The COD $_{total}$ removal efficiency decreases from 51 to 31 % with decreasing both the influent COD $_{total}$ concentrations from 287 to 164 mg/l and COD $_{biod.}$ loading rate from 36.8 to 17.7 g $m^{-2}.d^{-1}$. This indicates that the single stage RBC system at a low COD $_{biod.}$ loading rate of 17.7 g $m^{-2}.d^{-1}$ has been operated under organic substrate limiting conditions.
The results presented in Table 2 reveal that the residual values of COD $_{suspended}$ and COD $_{colloidal}$ become lower at COD $_{sus.\ biod.}$ and COD $_{coll.\ biod.}$ loading rates (7.8 and 4.3

g $m^{-2}.d^{-1}$ in our experiments), than those found at the higher $COD_{sus.\ biod.}$ and $COD_{coll.\ biod.}$ loading rates, viz. 12.6 and 10.3 g $m^{-2}.d^{-1}$. This indicates that the remaining part of $COD_{suspended}$ and $COD_{colloidal}$ depend on its applied loading rates. On the other hand the residual value of $COD_{soluble}$ remained unaffected at both $COD_{sol.\ biod.}$ loading rates of 5.7 and 14 g $m^{-2}.d^{-1}$, consequently independent on the $COD_{sol.\ biod.}$ loading rates in the range considered here. The results indicate that 1) uptake of $COD_{soluble}$ is a relatively quick process, probably it results from a rapid sorption onto the surface of the biofilm 2) the single stage RBC system at low $COD_{sol.\ biod.}$ loading rate of 5.7 g $m^{-2}.d^{-1}$ has been operated under soluble organic substrate limiting conditions 3) the system has a high capacity for removal of $COD_{soluble}$.

Table 2. Treatment results of the single stage RBC system with different UASB effluent qualities at operational temperature of 16 and 18 °C.

Parameter / Sample	OLR $gCOD_{biod}.m^2.d^{-1}$	$COD_{(biod.+non\text{-}biod.)}$ (mgl^{-1}) Total	Sus.	Coll.	Sol.	NH_4-N mgl^{-1}	TKN-N mgl^{-1}	E.Coli /100 ml
UASB eff.		164 ±	57 ±	28 ±	82 ±	59 ±	64 ±	$1.2*10^6$
at 30 °C		21	11	11	22	11	11	
One stage	17.7	114 ±	38 ±	9 ± 4	70 ±	56 ±	61 ±	$2.4*10^5$
RBC eff.		20	15		15	12	11	
UASB eff.		287 ±	91 ±	67 ±	129 ±	34 ±	54 ±	$3.4*10^6$
at 11°C		61	33	15	43	14	11	
One stage	36.8	141 ±	52 ±	17 ±	72 ±	32 ±	46 ±	$2.2*10^6$
RBC eff.		32	20	12	23	15	12	

E.Coli removal: The overall E.Coli removal in the single stage RBC system amounted to 80% at a low $COD_{biod.}$ loading rate of 17.7 g $m^{-2}.d^{-1}$ as compared to only 35 % at a high $COD_{biod.}$ loading rate of 36.8 g $m^{-2}.d^{-1}$ (see Table 2). This can be attributed to the lower $COD_{colloidal\ biod.}$ and $COD_{suspended\ biod.}$ loading rate of 4.3 and 7.8 g $m^{-2}.d^{-1}$ imposed to the single stage RBC when fed with low $COD_{biod.}$ UASB effluent. It is known that the removal of E.Coli in the presence of high concentrations of COD_{sus} and $COD_{col.}$ occurs mainly due to a physical process of settling of E.Coli attached to or entrapped in suspended solids and to entrapment of freely dispersed E.Coli by biofilm especially at short HRT. So it can be expected that a high removal of suspended and colloidal particles from the UASB effluent will improve the removal of E.Coli in the post-treatment system as a result of a combined physical and biological processes. Freely suspended E.Coli cells are easily predated by protozoa or ciliates present in the RBC system, as has been established by Gude (1979) in experiments with the activated sludge process.

Ammonia- nitrogen removal: The results in Table 2 reveal that approximately the same amount (5.0 %) of ammonia removal is achieved in the single stage system at both $COD_{biod.}$ loading rates applied (17.7 and 36.8 g $m^{-2}.d^{-1}$.), viz. corresponding to about 3.0 mg NH_4-N. l^{-1}. Apparently the heterotrophic bacteria assimilate about the same part of the influent ammonia for the elimination of various amounts of $COD_{biod.}$

On the other hand the removal of TKN amounted to 15.4 % at high COD $_{biod.}$ loading rate of 36.8 g $m^{-2}.d^{-1}$ compared to only 4.7 % at low COD $_{biod.}$ loading rate of 17.7 g $m^{-2}.d^{-1}$. The nitrification in the single stage RBC system remains insignificant at both COD $_{biod.}$ loading rates i.e. the nitrate concentration amounted to 1 mg/l in both situations.

Sludge production and sludge characteristics: The excess sludge production is strongly affected by the imposed COD $_{biod.}$ loading rate, because the results presented in Table 3 reveal that the sludge production in the single stage RBC system operated at COD $_{biod.}$ loading rate of 36.8 g $m^{-2}.d^{-1}$ is six times higher than at COD $_{biod.}$ loading rate of 17.7 g $m^{-2}.d^{-1}$.

Table 3. Sludge characteristics of the single stage RBC system operated with different UASB effluent qualities at operational temperature between 16 and 18 °C.

Characters	Unit	COD $_{biod.}$ loading rate (17.7 g $m^{-2}.d^{-1}$)	COD $_{biod.}$ loading rate (36.8 g $m^{-2}.d^{-1}$)
MLSS	g l^{-1}	3 (± 0.8)	18.8 (± 7.1)
VSS	g l^{-1}	1.9 (± 0.06)	13.96 (± 5.3)
Sludge stability		0.63	0.74
Sludge volume index	ml g^{-1}	53.3 (± 8.1)	56.8 (± 20.5)
Sludge production	g d^{-1}	5.5	28.6

According to Loy, (1988) the sludge produced will be stabilised, when the fractional amount of total volatile solids to total solids in the sludge produced is equal 0.6. The calculated sludge stability (VSS/MLSS) amounted to 0.63 at low COD $_{biod.}$ loading rate as compared to 0.74 at high COD $_{biod.}$ loading rate. This can be due to a higher sludge retention time at low COD $_{biod.}$ loading rate. Based on these results the discharged sludge from the single stage RBC system treating high quality UASB effluent 1) is rather well stabilised at this operational conditions 2) the single stage RBC system then can simultaneously treat UASB effluent and stabilise sludge produced 3) a conventional digestion tank then can be eliminated from the process, especially in tropical and sub-tropical countries where the temperature exceeding 20 °C in the UASB reactor treating domestic sewage followed by single stage RBC system as post-treatment 4) the investment cost of the digestion system generally amount to 30 to 40 % of the total cost of the whole sewage treatment plant, a large portion of the investment can be saved by using both efficient UASB and RBC system as post-treatment instead of a system consisting of a conventional treatment system and sludge digester.

The mean value of the net sludge yield found amounted to 0.06 g sludge COD $_{total}$ per g COD $_{total}$ removed for the single stage, when loaded at low COD $_{biod.}$ loading rate of 17.7 g $m^{-2}.d^{-1}$ as compared to 0.128 g sludge COD $_{total}$ per g COD $_{total}$ removed, when

the single stage loaded at high COD $_{biod.}$ loading rate of 36.8 g $m^{-2}.d^{-1}$. The excess sludge fraction corresponds to only approximately 6 % of the total influent COD at lower COD $_{biod.}$ loading rate and to 12.8 % at the higher one. This is a very important feature of the system, since it is significantly smaller than normally found in conventional aerobic systems. Moreover, the settling properties at both high and low COD $_{biod.}$ loading rate were extremely good (SVI = 53 & 57 ml g^{-1}).

Results of the 2nd experiment.

Effect of COD $_{biod.}$ loading rate on the performance of a two stage RBC system.

COD fractions removal: The two stages RBC was operated at an HRT of 2.5 h and at COD $_{biod.}$ loading rate of 9 and 18 g $m^{-2}.d^{-1}$ respectively. From the results, presented in Figs.3a, b, c and d, it can be seen that, the residual values of COD $_{total}$ in the final effluent remained unaffected when changing the imposed COD $_{biod.}$ loading rate from 9 to 18 g $m^{-2}.d^{-1}$. At both COD $_{biod.}$ loading rates a final COD concentration of 70 mg l^{-1} was achieved, which is quite low indeed for domestic sewage. Altogether, this indicates that when the RBC system is designed properly it will perform similarly in tropical, subtropical and moderate climate regions.

The results also reveal that the increase of COD $_{biod.}$ loading rate from 9 (14.5 g COD $_{total}$ $m^{-2}.d^{-1}$) to 18 g $m^{-2}.d^{-1}$ (25.4 g COD $_{total}$ $m^{-2}.d^{-1}$) leads to an increased COD $_{total}$ removal rate from 8.3 to 19.2 g COD $_{total}$ $m^{-2}.d^{-1}$, corresponding to increased removal efficiency from 57 to 76 % respectively. This result is not surprising because the biofilm formation balances the excess of the high load and the RBC system is known to be capable to handle high COD $_{biod}$ loading rates successfully, viz. at good removal efficiencies (Bovendure *et al.*, 1990). The higher removal efficiency of two stages RBC system for removal of COD $_{total}$ at higher COD $_{biod.}$ loading rate of 18 g $m^{-2}.d^{-1}$ means that 1) the organic load of 9 gCOD $_{biod.}$ $m^{-2}.d^{-1}$ to the two-stage RBC system was low when feeding with a high effluent quality of UASB reactor and a higher load than 9 gCOD $_{biod.}$ $m^{-2}.d^{-1}$ can be applied without the danger of a serious drop of the removal efficiency because at this OLR the two stages RBC system has been operated under organic substrate limiting conditions. 2) the two-stage RBC system can be applied for post treatment of UASB effluent more or less independent of the effluent of the UASB reactor (at least for efficiencies exceeding 40 - 50 % COD removal however, at higher efficiency in the UASB e.g. when applied at higher temperature can substantially reduce the volume of two stage RBC system.
From the data presented in Fig. 3c, it is also clear that the RBC-system is quite effective in eliminating the colloidal COD fraction at both applied COD $_{biod.}$ loading rates. Moreover, a combined UASB-RBC system apparently offers quite interesting potentials for sewage treatment, the more so because the system is effective in lowering the COD $_{soluble}$ and COD $_{suspended}$ substantially as shown in Figs 3 b and 3 d.

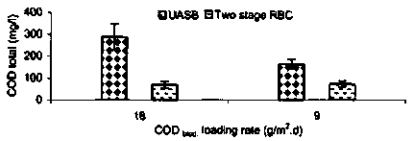

Fig. 3a COD total effluent concentration in the two stage RBC at different COD biod. loading rate.

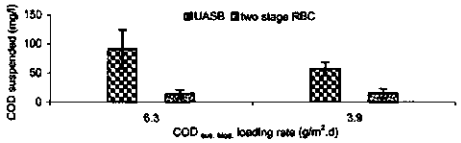

Fig. 3b COD Suspended effluent concentration in the two stage RBC at different COD sus. biod. loading rate.

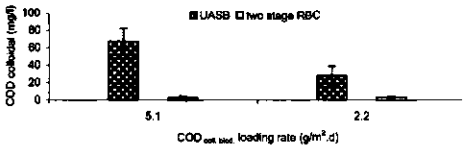

Fig. 3b COD colloidal effluent concentration in the two stage RBC at different COD col. biod. loading rate.

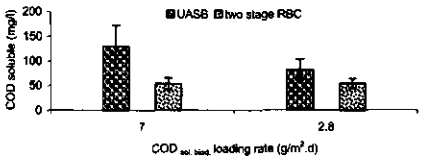

Fig. 3d COD soluble effluent concentration in the two stage RBC at different COD sol. biod. loading rate.

Nitrification: As mentioned already above, in the 1st stage of two stages RBC system only could achieve extremely low ammonia removal efficiency. The nitrification therefore only proceeded in the 2nd stage of two stages RBC system (Fig.4). The results presented in Table 4 reveal that the calculated nitrification rate according to nitrate production in the 2nd stage of two stage RBC decreased from 1.56 to 1.1 gNO_3-N $m^{-2}.d^{-1}$ at an increase of the COD_{total} removal rate from 7.8 to 13 g $m^{-2}.d^{-1}$. This can be attributed to the low content of $COD_{biod.}$ in the 30 °C UASB effluent together with the low residual value of COD in the effluent of 1st stage RBC system. This provides 1) a better situation for nitrification in the 2nd stage 2) a longer residence time of the nitrifying bacteria in the aerobic zone of the biofilm (Harremoes, 1982) 3) a lower sludge growth rate and the relatively higher fraction of nitrifiers contained in it. This obviously results in a considerable increasing of the nitrification capacity of the 2nd stage of two stages RBC system. . Moreover, a high $COD_{biod.}$ content in the UASB effluent as obtained when the operational temperature was 11 °C, leads to a distinctly higher COD_{biod} in the effluent of 1st stage which give 1) a higher production of non-nitrifying biofilm material in the 2nd stage, i.e. consisting of adsorbed organic matter and heterotrophic bacteria (Tawfik *et al.*, 2001) 2) shorter residence time of the nitrifying bacteria in the aerobic zone of the biofilm. This indicates that the part of biodegradable COD in the effluent of 1st stage ultimately resulted in a definite drop of the nitrification capacity due to competition for dissolved oxygen and for space in the biofilm. Most likely the difference can be attributed to attachment of the suspended solids on the surface of the nitrifying biofilm where they take away oxygen which otherwise would have been available for the nitrifiers. The impact of the temperature on the nitrification process in the 2nd stage of two-stage RBC system was neglected because the temperature was almost the same (16 - 18 °C) at both applied loading rates.

E.Coli removal: The results in Fig. 5 show that the residual value of E.Coli in the final effluent of two stage system decreases from 3.4 x 10^5 at the higher $COD_{biod.}$

loading rate of 18 g $m^{-2}.d^{-1}$ to 7.6 x 10^4 $_{at}$ the lower COD $_{biod.}$ loading rate of 9 g $m^{-2}.d^{-1}$, i.e. corresponding to a removal value of 90 and 94 % $_{respectively.}$ Apparently, an increasing COD $_{biod.}$ loading rate clearly leads to a decrease in the E.Coli removal but the remaining residual part of E.Coli at both loading rates still high indicates that the removal of E.Coli is limiting factor under these conditions.

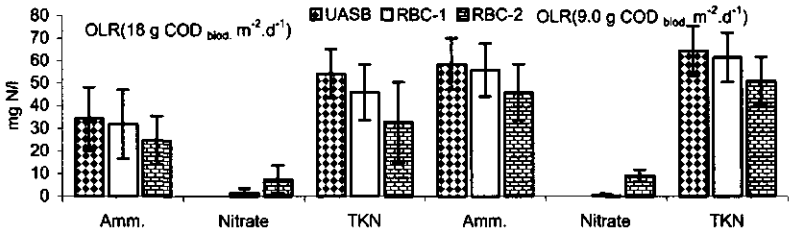

Fig. 4 Ammonia, Nitrate and TKN concentrations in the effluents of the various stages of a combined UASB- two stage RBC system at different COD $_{biod.}$ loading rate.

Table 4. Effect of COD $_{biod.}$ loading rate on the nitrification rate in the 2nd stage of a two stage RBC system.

Temperature °C	OLR (g COD $_{biod.}$/$m^{-2}.d^{-1}$)	Organic removal rate (g COD $_{total}$ $m^{-2}.d^{-1}$)	Nitrification rate (gNO_3-N $m^{-2}.d^{-1}$)
18	11.3	7.8	1.56
16	16	13	1.1

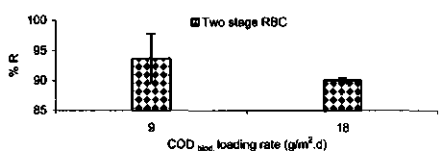

Fig. 5 the overall E.Coli removal efficiency in the two stage RBC system at different COD $_{biod.}$ loading rate.

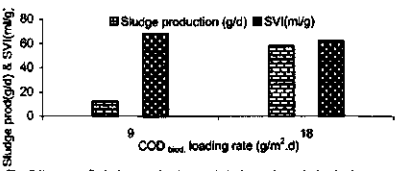

Fig. 6 the overall sludge production and sludge volume index in the two stage RBC at different COD $_{biod.}$ loading rate.

Sludge production: It is quite clear from Fig. 6 that the sludge production varies with the COD $_{biod.}$ loading rate imposed to the system. The calculated average sludge production for the two stages RBC-system operated at a low COD $_{biod.}$ loading rate of 9 g $m^{-2}.d^{-1}$ amounted to 12.0 g MLSS d^{-1}, corresponding to 0.11 g MLSS/g COD $_{total}$ removed, while it was around 57.8 g MLSS d^{-1} (corresponding to 0.23 MLSS/g COD $_{total}$ removed), for the higher COD $_{biod.}$ loading rate of 18 g $m^{-2}.d^{-1}$. This can be due to the high solids retention time in the two stages RBC system when fed with low COD $_{biod.}$ loading rate. The average calculated sludge stability (VSS/MLSS) of two-stage system amounted to 0.68 at a low COD $_{biod.}$ loading rate of 9 g $m^{-2}.d^{-1}$ as compared to 0.78 at a high COD $_{biod.}$ loading rate of 18 g $m^{-2}.d^{-1}$. The sludge produced in both cases needs more or less far stabilisation for instance by using anaerobic digestion.

The results demonstrate that the characteristics of the sludge produced in the post - treatment system depends on the COD $_{biod.}$ content of the UASB reactor.

Effect of imposing temporary a high COD $_{biod.}$ loading rate on the activity of nitrifying bacteria and COD removal in the 2nd stage of two-stage RBC system.

A feed-less (ammonia limitation) period of 7 days followed by 9 days feeding with high COD $_{biod.}$ loading rate (UASB effluent) was investigated to elaborate, if the nitrifiers are capable to convert ammonia to nitrate after totally 16 days when returning back to the normal operating conditions. The results of the experiments are depicted in Fig 7a,b and c.

Normal operating conditions: During the first period of 44 days, the two stages RBC - system was operated at total COD $_{biod.}$ loading rate of 9.7 g $m^{-2}.d^{-1}$ and HRT of 5 h. Its nitrification efficiency and COD concentration in the final effluent are shown in Fig. 7a. The average calculated nitrification rate according to nitrate production in the 2nd stage of two stage RBC system amounted to 2.18 g NO_3-N $m^{-2}.d^{-1}$ and the average remaining COD in the final effluent amounted to 65 mg l^{-1}.

Feeding period: Before feeding was resumed to the 2nd stage of two stages RBC system at a COD $_{biod.}$ loading rate of 13.2 g $m^{-2}.d^{-1}$. We observed an increase of suspended solids and turbidity in the bulk liquid. This is due to the detachment of biofilm during feed-less period. Unfortunately, we didn't follow up the changing of ammonia, nitrate, COD and biofilm in the nitrifying stage during feed-less period. The results in Fig. 7b reveal an immediate deterioration of the nitrification process after exposing the 2nd stage system to a COD $_{biod.}$ loading rate of 13.2 g $m^{-2}.d^{-1}$ and also the COD concentrations in the final effluent was surprisingly higher than in the period 0 - 44, bit it gradually decreased to 88 mg l^{-1} after 6 days feeding and to 77 mg l^{-1} after 9 days feeding. The results in the Fig. 7b show that 3 hrs after resuming the feeding there still prevail a high nitrate concentration in the bulk liquid of the effluent of 2nd stage, while at the same time the ammonia concentration also was higher. The high nitrate concentration in the bulk liquid presumably results from the oxidation of ammonia which already was present in the bulk liquid after resuming the feeding and from detachment of the biofilm during the feed-less period.

Normal operating conditions: After returning to the original total COD $_{biod.}$ loading rate of 7 g $m^{-2}.d^{-1}$, the nitrification process in the 2nd stage of two-stage RBC system recovered within 2 - 3 days as shown in Fig. 7c. This indicates that the nitrifiers were presented in the system but the activity was diminished at high loading rate and during ammonia limitation period. Therefore, the recovering of the nitrification capacity is not a result of growth. The growth of nitrifiers takes at least one month before enough nitrification capacity is produced in the biofilm systems without nitrifiers (Temmink *et al.,* (2001). Thus nitrifiers can recover after a total period of 16

days without activity. The calculated nitrification rate after 3 days amounted to 1.5 g NO_3-N $m^{-2}.d^{-1}$ and ammonia concentration amounted to about 10 mg l^{-1}. The COD concentration in the final effluent remained around 70 mg l^{-1}.

The results of the experiment clearly show a strong and immediate detrimental effect of imposing a high $COD_{biod.}$ loading rate of 13.2 g $m^{-2}.d^{-1}$ on the nitrification process in the 2nd stage of two stages RBC system. This can be attributed to the rapid growth of heterotrophs, these organisms likely will cover (entrap) the autotrophic bacteria resulting in serious restrictions of the supply of dissolved oxygen to the nitrifiers. Moreover also the sludge age might drop, resulting in a lower fraction nitrifying sludge in the sludge solids. Additionally, the results show that it is possible to use the 2nd stage of two stages RBC system for removal of COD and kept high concentrations of ammonia in the final effluent enabling its as fertiliser when the effluent is applied for irrigation purposes. However, the nitrification activity can be recovered after a total period of 16 days working under stress conditions.

FINAL DISCUSSION

The results of the present investigation reveal that the single stage RBC system treating high and poor UASB reactor effluent represents a proper choice for removing of COD fractions ($COD_{suspended}$, $COD_{colloidal}$ and $COD_{soluble}$) at very short HRT of 1.25 h and at $COD_{biod.}$ loading rate of 17.7 and 36.8 g $m^{-2}.d^{-1}$. Unfortunately the effluent quality of COD_{total} at both $COD_{biod.}$ loading rates still exceeded the standards (COD > 80 mg l^{-1}) for reuse in restricted irrigation purposes. The earlier experiments (Tawfik *et al.*, 2001) we increased the HRT to 2.5 h and decreased the $COD_{biod.}$ loading rate to 10.3 g $m^{-2}.d^{-1}$ imposed to the single stage RBC system treating high quality UASB reactor effluent. And we found for the single stage RBC system a residual COD_{total} value of 72 mg l^{-1}. In another study Tawfik *et al.*, (2001) we found that increasing the HRT to 5 h and decreasing the $COD_{biod.}$ loading rate to 9.2 g $m^{-2}.d^{-1}$a residual COD_{total} value of 76 mg l^{-1} in the single stage RBC system treating poor quality UASB effluent (t = 14 °C). Also in another non-published data, we found that the single stage RBC system treating UASB effluent (t = 20 °C) achieved a COD residual value of 85 mg l^{-1} at HRT of 2.5 h and $COD_{biod.}$ loading rate of 14.2 g $m^{-2}.d^{-1}$ (20 g COD_{total} $m^{-2}.d^{-1}$). Based on the results presented here and the previous results for single stage RBC system with a high quality UASB effluent, it can be concluded that the $COD_{biod.}$ loading rate imposed to the single stage RBC system should not exceed 14 g $m^{-2}.d^{-1}$ and HRT should remain at 2.5 h especially for removal of COD_{total}. On the other hand the HRT imposed to the single stage RBC system treating poor quality UASB effluent should be prolonged to 5 h in order to reduce the COD_{total} to the minimum value. The use of the single stage RBC system for post-treatment of high quality UASB reactor effluent can substantially reduce the volume of the system. The single stage RBC system clearly represents a very attractive post-treatment of

anaerobic effluent for COD removal, particularly for developing countries, but

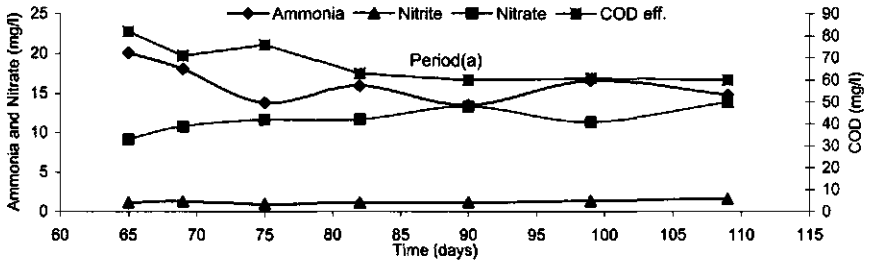

Fig.7a The course of ammonia, nitrate and nitrite in the effluent of 2-nd stage of a two stage RBC system at total OLR of 9.7 gCOD $_{biod.}$/m^2.d.

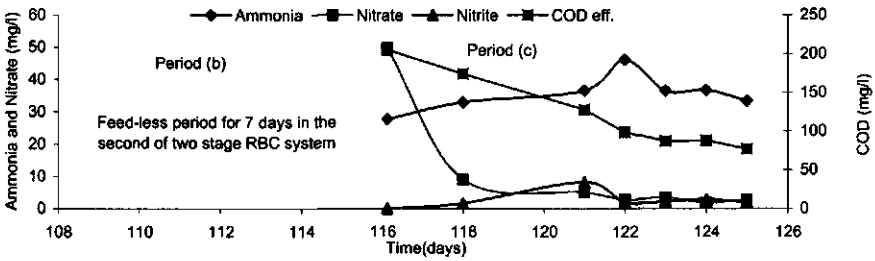

Fig.7b The course of ammonia, nitrate and nitrite in the effluent of 2-nd stage RBC system fed directly with UASB effluent at OLR of 13.2 gCOD biod./m2.d.

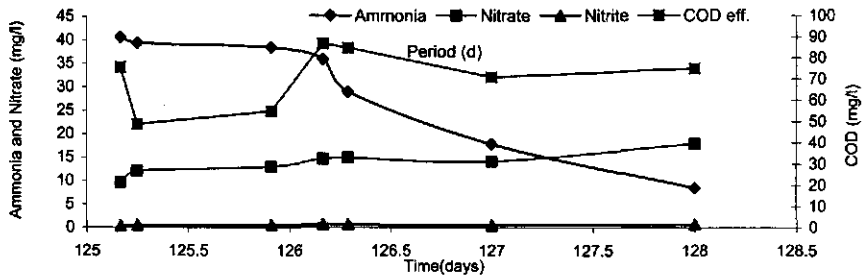

Fig.7c The course of ammonia, nitrate and nitrite in the effluent of 2-nd stage of two stage RBC system at total OLR of 6.6 gCOD $_{biod.}$/m^2.d.

certainly also for the prosperous industrialised world.

If we compared the results obtained by us Tawfik *et al.*, (2001) for single stage RBC system treating high quality UASB reactor effluent at COD $_{biod.}$ loading rate of 9.3 g m^{-2}.d^{-1} with the results obtained in the present investigation for two stages RBC system at the same HRT and the same COD $_{biod.}$ loading rate. Under these conditions the single stage RBC system achieved similar residual of COD $_{total}$ in the final effluent to that found in the two-stage RBC system. Based on these results it is preferable to use single stage system for COD fraction removal from high quality UASB reactor effluent. For a more removal of ammonia and E.Coli, which in main cases undoubtedly is required, a 2nd stage RBC system will be needed.

Despite of receiving fluctuating effluent from the proceeding UASB reactor at operational temperature of 30 and 11 °C and consequently different COD $_{biod.}$ loading rate, the two-stage RBC system achieved the same low level of 70 mg l^{-1} for COD $_{total.}$ These results are consistent with the results obtained by Sperling *et al.*, (2001) for an activated sludge system treating different UASB effluent qualities at a constant HRT of 2.8 h and 1.1h final clarifier.

Goncalves *et al.*, (1999) used a submerged aerobic filter as post-treatment. The applied space organic loads imposed to the submerged aerated bio-filter ranged from 5.0 to 9.0 kg COD $m^{-3}.d^{-1}$. Residual COD value obtained in the submerged aerobic filter at COD influent of 112 mg l^{-1} at HRT in the UASB reactor of 4.0 h, were similar to those obtained at COD influent of 88 mg l^{-1} when a HRT of 6.0 h was applied in the UASB reactor, but they only found a high nitrification capacity in the submerged aerobic filter with low COD influent. They then achieved an ammonia removal efficiency exceeding 90 %. A significant drop in nitrification rate was observed in a submerged aerobic filter at high-applied OLR conform our results obtained for nitrification rate presented in Table 4.

Unfortunately, we don't have data at COD $_{biod.}$ loading rate higher than 18 g $m^{-2}.d^{-1}$ for two stage RBC system but in accordance to the results obtained by Bovendrue *et al.*, (1990) who found that at COD loading rates of 80 and 10 g $m^{-2}.d^{-1}$ imposed to a two stage RBC system treating UASB effluent, results in about 70 % COD removal. However, higher loading rates up to 400 g $m^{-2}.d^{-1}$ resulted in either removal rates directly proportional to the loading rates or a constant removal rate of approximately 40 g COD $_{total}$ $m^{-2}.d^{-1}$. Also in previous experiments depicting with hydraulic shock loads (Tawfik *et al.*, 2001) we showed that a two stage RBC system can be operated at higher organic loading rate without problems with the final effluent quality of the two stages RBC for COD removal. The imposed hydraulic shocks to the two stage RBC system corresponding to an HRT of 1.66 h and organic loading rate of 52 g COD $_{total}/m^2.d$. The results obtained show that the COD levels in the final effluent remain at the same level as compared to the reference conditions (86.0 mg l^{-1}).

CONCLUSIONS

- The results for COD and E.Coli clearly show a significant improvement of the quality of the effluent of the single stage system treating high quality UASB reactor effluent than those in the single stage RBC system treating poor quality UASB reactor effluent at the same HRT of 1.25 h.
- The calculated sludge stability (VSS/ MLSS) of 0.63 in the single stage RBC system fed with a feed of high quality UASB effluent is substantially higher than the value found for the feed with poor quality UASB effluent i.e. being 0.74 where the COD $_{biod.}$ loading rate in the feed is higher.
- The results of two stage RBC system reveal that the final effluent COD $_{total}$ remained at a value of 70 mg/l, independent on the imposed OLR and the

efficiency of UASB reactor. Therefore, if two stages RBC system designed properly for post-treatment of anaerobic effluent in the tropical countries, it will perform similarly in subtropical and moderate climate countries.

- The calculated nitrification rate in the 2nd stage of two stage RBC system fed with high quality UASB reactor effluent was decreased from 1.56 to 1.1 g NO_3-N /m^2.d when feeding with poor quality of UASB reactor.

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6

TREATMENT OF A POOR QUALITY ANAEROBICALLY PRE-TREATED DOMESTIC SEWAGE BY A ROTATING BIOLOGICAL CONTACTOR.

The modified version of this chapter has been published in Water Research, Vol.36, No.1, pp. 147-155 as Tawfik A., Klapwijk A., El-Gohary F., Lettinga G.

ABSTRACT

The performance of a Rotating Biological Contactor (RBC) for the treatment of an Up-flow Anaerobic Sludge Blanket (UASB) effluent was the subject of this study. Different hydraulic and organic loading rates have been investigated. The removal efficiencies of COD_{total}, $COD_{suspended}$, $COD_{colloidal}$ and $COD_{soluble}$ increased slightly at higher HRT and lower influent OLR. The results obtained indicated that a two-stage RBC reactor at HRT of 10 h and an organic loading rate of 6.4 g COD m^{-2}. d^{-1} represents an effective post-treatment process. Most $COD_{suspended}$ and $COD_{colloidal}$ were removed in the 1st stage while nitrification proceeded in the 2nd stage.

The overall nitrification efficiency was 92 % at OLR of 6.45g m^{-2}. d^{-1}. Total E.Coli removal at HRT's of 10, 5 and 2.5 h were 99.5, 99.0 and 89.0 % respectively. The major part of suspended E.Coli (> 4.4 µm) was removed by sedimentation and/or adsorption in the biofilm of the 1st stage of RBC (99.66 %). However, E.Coli in the colloidal fraction (< 4.4 - > 0.45 µm) was eliminated in the 2nd stage of RBC (99.78 %).

Comparison of the performance of a single stage versus two-stage RBC system, operated at the same total OLR, revealed an improvement in the quality of the two-stage effluent as compared to the single-stage effluent.

The two stages RBC were used to examine the effect of hydraulic shock loads on reactor performance in terms of COD, nitrification and E.Coli removal.

INTRODUCTION

Several studies carried out at pilot plant and full-scale demonstrated that an Up-flow Anaerobic Sludge Blanket (UASB) reactor represents a reliable and simple technology for pre-treatment of domestic sewage (Lettinga *et al.*, 1997). Therefore, this technology could be successfully applied for wastewater treatment and reuse in arid and semi-arid regions, particularly in developing countries. However, UASB

effluents still will contain pathogens, COD, sulphides and ammonia. Therefore, the anaerobic treatment has to be complemented by an adequate post-treatment unit in order to meet the standards set for irrigation purposes. It will be necessary to reduce the concentrations of pathogens and retain as much as possible of the nutrients in the effluent before it is used for irrigation and fertilisation. However in case, the effluent needs to be obviously nutrients should be removed as complete needs to be as possible.
Various aerobic systems have been proposed as post- treatment, such as activated sludge, trickling filters, submerged aerated biofilters and duckweed and stabilisation pond systems (Sperling *et al.*, 2000; Augusto *et al.*, 2000 and Goncalves *et al.*, 1999; Van der steen *et al.*, 1999).

In this study we investigated the feasibility of a Rotating Biological Contactor for post-treatment of the effluent from UASB reactor.

RBC plants have found already widespread application in the wastewater treatment, because this system offers several advantages:

- It is compact,
- it has little negative impact on the landscape,
- doesn't give rise to noise / or odour nuisance,
- its operation is simple and operational costs are relatively low.
- it can be operated by using the electric power generates a solar Electric Generation Panel under enough sunlight,
- head losses are low,
- it produces access sludge with excellent settleability and higher specific removal rates.

RBC has been used extensively for the treatment of municipal wastewater for single stage carbon removal and nitrification and multistage for requirements of BOD removal and nitrification (Haung, 1982). Effects of various operating parameters like turbulence (Kugaprasatham *et al.*, 1992) disk rotation speed (Friedman *et al.*, 1979) and hydraulic conditions (Kugaprasatham *et al.*, 1991) have been studied in detail. The occurrence simultaneous nitrification and denitrification have been studied in micro-aerobic films (Watanabe *et al.*, 1992).

The objectives of the present investigation are:

1. To examine the COD, and E.Coli removal and the nitrification rate at different OLR in a two stage RBC system.
2. To compare the performance of a two-with a single stage RBC operated at the same total loading rate with regard to COD, E.Coli removal and nitrification rate.

3. To evaluate the stability of the RBC reactor under hydraulic shock loads and its performance following a shock load.

MATERIALS AND METHODS

The RBC systems were fed with the effluent of a 6 m^3 Up-flow Anaerobic Sludge Blanket (UASB) reactor previously investigated by Grin *et al.*, (1985) and fed with raw sewage of Bennekom, The Netherlands.

UASB effluent

The main characteristics of the effluent of the UASB reactor, which did not perform satisfactorily during most of the experimental period, are given in Table 1.

Table 1. Mean effluent characteristics of the UASB fed to the two stage of RBC.

pH	Temp	COD mgl^{-1}				Nitrogen mgl^{-1}		Sol. P	H_2S	E.Coli /100ml
	°C	total	sus.	coll.	sol.	NH_4-N	TKN	mgl^{-1}	mgl^{-1}	
6.5 -8	12.3	288	77 ±	72 ±	139	43	47	4.9	9.4	$0.3*10^6$
		± 49	23.8	16	± 39	± 14.3	± 11	± 2	± 3.7	$\pm 0.3*10^6$

Rotating Biological Contactor (RBC).

The RBC system (Fig.1) consisted of two identical RBC reactors connected in series. Each reactor had a working volume of 60 l and was equipped with 10 polystyrene foam disks with a total effective surface of 6.5 m^2 and rotating at 5 rpm. The disk diameter was 0.6 m with a thickness of 0.02 m and they were spaced at 0.02 m intervals. The submerged surface amounted to 40 %. The disks were mounted on a steel shaft.
Throughout the experimental study, the reactors were operated at the ambient temperatures of the raw sewage, i.e. 12 - 15°C.

Analysis

48 hrs, composite samples of the influent and the effluent of each reactor were collected in containers stored in a fridge at 4 °C and analysed. Temperature, dissolved oxygen, sulphide content and pH values were measured using grab samples.

COD was analysed using the micro-method as described by Jirka and Carter (1975). Raw samples were used for COD_{total}, 4.4 µm folded paper filtered (Schleicher&Schuell 595 ½) samples for $COD_{filtrate}$ and 0.45 µm membrane filtered (Schleicher&Schuell ME25) samples for dissolved COD ($COD_{soluble}$). The

$COD_{suspended}$ and $COD_{colloidal}$ were calculated by the difference between COD_{total} and $COD_{filtered}$, $COD_{filtered}$ and $COD_{soluble}$, respectively.

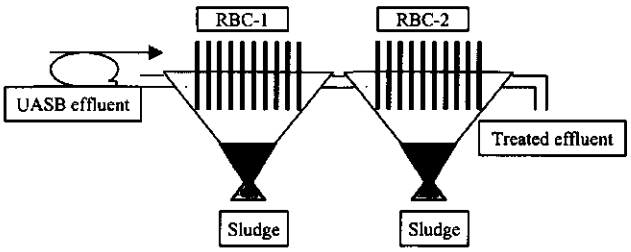

Fig.1 Schematic representation of a two stage RBC system treating UASB effluent.

Ammonia, nitrite and nitrate were determined on auto-analyser (SKALAR SA-9000). Total Kjeldahl nitrogen and sludge analysis were measured according to the Dutch Standard Normalised Methods, (1969).

E.Coli, analyse was performed according to the method described by Havelaar *et al.*, (1988). Suitable volumes of a sample (or a dilution in 0.1 % peptone –saline) were filtered through membrane filters. The membranes were then placed on tryptone soya agar and incubated at 37 ± 1°C for 4 ± 1 h. After that they were transferred to tryptone bile agar and incubated at 44 ± 0.5 °C until the total incubation period was 24 ± 1 h. The membranes were then placed on a piece of filter paper saturated with indole reagent according to Sharpe *et al.*, (1981) (0.5 g 4-dimethylaminobenzaldehyde in 100 ml 1 mol / 1 HCL). The colour was allowed to develop under a U.V source (250 – 400 nm) for 10 - 30 min. depending on wave length and lamp intensity, after which colonies were scored as indole positive, negative or doubtful.

Experimental set-up

This study describes results of three experimental runs conducted at the following different flow rates:

- 0.288 $m^3 d^{-1}$ (HRT = 10 h) during 2.0 month.
- 0.576 $m^3 d^{-1}$ (HRT = 5.0 h) during 2.5 month.
- 1.152 $m^3 d^{-1}$ (HRT = 2.5 h) during 2.0 month.
-

During the 2nd experiment the effect of the application of a shock load, at a HRT of 1.66 h. and flow rate of 1.74 $m^3 d^{-1}$ was examined.

RESULTS

Performance of a two stage RBC.

To study the effect of the organic load applied on the performance of the system, the organic loading rate of the two stage RBC (fed with the anaerobically pre-treated domestic sewage) was increased from 6.45 to 23.5 g COD m^{-2}. d^{-1} by decreasing HRT from 10.0 to 2.5 h. The results obtained are presented in Fig. 2a, b, and c and d. Available data shows that by increasing the OLR, the overall removal efficiency was reduced as follows:

- COD_{total} from 83.0 (± 3.2) to 73.0 (± 4.2)%.
- $COD_{suspended}$ from 92 (± 11.3) to 83 (± 10.9)%.
- $COD_{colloidal}$ from 95.3 (± 3.4) to 95.1(± 3.6)%.
- $COD_{soluble}$ from 73 (± 5.8) to 56 (± 13.5)%.

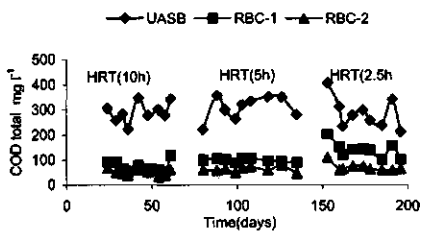

Fig.2a Variation of COD total at different HRT.

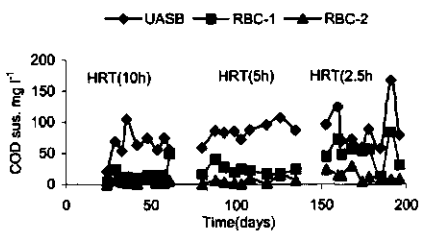

Fig.2b Variation of COD suspended at different HRT.

The results in Fig. 2b and c show that most of the $COD_{colloidal}$ and $COD_{suspended}$ was removed in the 1st stage. and that little additional removal was achieved in the 2nd stage. This can be explained by the fact that the most COD particles were adsorbed in the 1st stage.

Similar results were observed for the removal of ammonia and E.Coli (Figs. 3a and 4a), viz. the efficiencies dropped for

- ammonia from 92 (± 3.6) to 18 (± 4.5)% and
- E. Coli from 99.5 (± 0.5) to 89.0 (± 8.6)%.

The overall ammonia removal (Fig. 3b) dropped at higher OLR, which can be attributed to the domination of heterotrophic bacteria at high OLR, which exerted a negative effect on the rate of nitrification.

The results in Fig. 4b show that the E.Coli count in the RBC effluent is proportional to OLR applied to the system. The results in Fig. 4a indicate that E.Coli removal proceeds in the 2nd stage.

Bacteriological examination showed that the mean residual counts of E.Coli were 3.5 x 10^4 at HRT of 10 h, 0.5 x 10^5 at HRT of 5 h and 3.4 x 10^5 at HRT of 2.5 h. Although efficient in reducing the E.Coli concentration from the UASB effluent, a two stage RBC produced an effluent not complying with WHO guidelines (1989) for unrestricted irrigation (1000 E.Coli/100ml). Further treatment is therefore required prior to reuse.
According to Gannon *et al.*, (1983) the major part of E.Coli in wastewater is associated with, suspended solids therefore, the prevalence of E.Coli was analysed in more detail especially in the fraction of 4.4 – 0.45 µm. The results obtained for both the 1st and 2nd stage RBC are presented in Fig. 5. Available data reveals that the major part of E.Coli, associated with the suspended fraction of > 4.4 µm was removed by sedimentation and/or adsorption in the biofilm of the 1st stage of RBC (99.66 %). The E.Coli present in the colloidal form (< 4.4 - > 0.45 µm) were adsorbed in the 2nd stage of RBC (99.78 %).

Furthermore the experimental results show that the majority of residual E.Coli in the final effluent was associated with particles having a size ranging between (4.4 - 0.45 µm). Apparently the adsorption (removal) of colloidal dispersed E.Coli is the limiting step in the RBC system.

The Sludge Biomass Index (SBI), defined as the ratio of total volatile solids to total solids of the settled sludge in the tank (Loy, 1988).
The results in Table 2 show that the Sludge Biomass Index of the sludge in the two stage RBC modules exceeded the value 0.6 for all organic loading rates. This means that the sludge in the RBC settling tank system was not sufficiently stabilised and requires further treatment prior to disposal.

Table 2. Sludge characteristics in the two stages RBC system at different HRT (10, 5 and 2.5 h).

Run NO.	Unit	Run NO. 1		Run NO. 2		Run NO. 3	
Parameter		RBC-1	RBC-2	RBC-1	RBC-2	RBC-1	RBC-2
Sludge volume	ml/l	775	117	870	197	945	882
MLSS	g/l	5.3	4.8	8.0	1.98	19.0	14.7
VLSS	g/l	4.0	3.6	6.5	1.5	12.7	10.7
SBI	-	0.75	0.75	0.8	0.76	0.66	0.73
SVI		199	24.0	115	71.0	56.0	69.0

Comparison of the performance of a single and two stage RBC system.

There are reasons to expect a better performance of a two-stage RBC compared to a single stage RBC. When both carbon removal and nitrification are necessary, the best solution to use a modulled, two stage RBC system. In order to limit competition between heterotrophic and autotrophic bacteria. Even if nitrification already would

proceed in the 1st stage, a 2nd stage will guarantee full nitrification regardless of OLR variations. Therefore, we compared the performance of a single-stage with a two-stage RBC for COD, E.Coli removal and nitrification at the same total loading rate.

The advantages of the use of two stages were clearly demonstrated in this study. The results obtained for a single and a two stage RBC operated at the same HRT of 5 and 2.5 h and the same OLR of 26.6 and 52.8 g COD. m^{-2}. d^{-1} is presented in Table 3.

The results in Table 3 clearly show that the difference values of COD_{total},

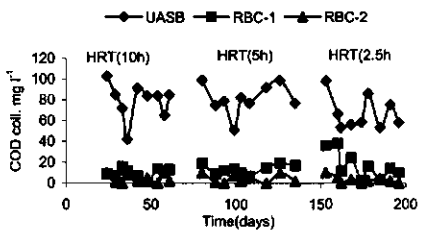

Fig.2c Variation of COD colloidal at different HRT.

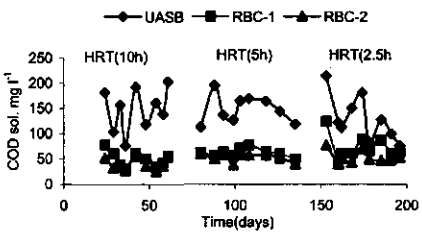

Fig.2d Variation of COD soluble at different HRT.

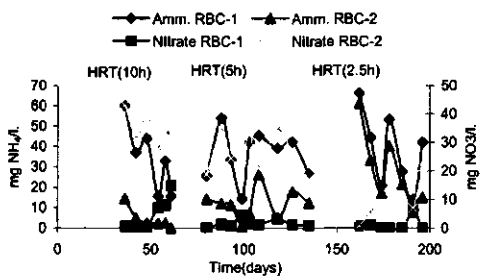

Fig. 3a Variation of the ammonia and nitrate at different HRT.

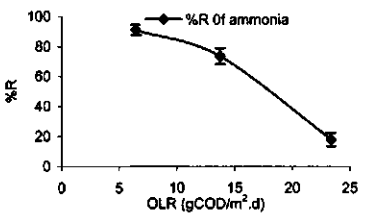

Fig. 3b Variation of the overall ammonia removal at different OLR.

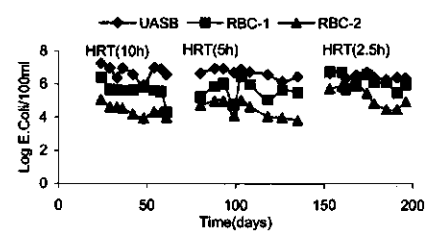

Fig. 4a Variation of the E.Coli concentrations at different HRT.

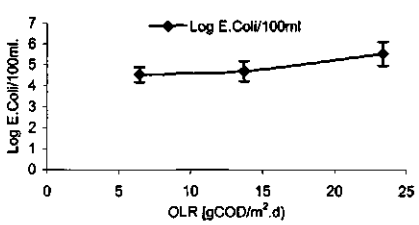

Fig. 4b Variation of the overall E.Coli removal at different OLR.

$COD_{suspended}$ and $COD_{colloidal}$ in the final effluent of a two and single stage are respectively 15, 11 and 7 mg l^{-1} at loading rate of 26.6 gCOD. m^{-2}. d^{-1} and 30, 9 and 11 mg l^{-1} at loading rate of 52.8 gCOD. m^{-2}. d^{-1}.

Table 3 shows the ammonia and nitrate concentrations in the effluent of a single and a two stage RBC when they were operated under the same ammonia-loading rate of 4.2 and 7.49 g m^{-2}. d^{-1}. The calculated nitrification rate in the single stage system under the investigated ammonia loading rates was around 1.152 and 1.03 g. m^{-2}. d^{-1} respectively. The results obtained in the two stages revealed a higher overall nitrification rate (3.1 and 1.152 gNH_4-N. m^{-2}. d^{-1}) compared to the single stage, consequently lower ammonia concentration in the effluent. It is obvious that the two stage RBC provides higher nitrate concentrations in the effluent (26 and 7.0 mg l^{-1})

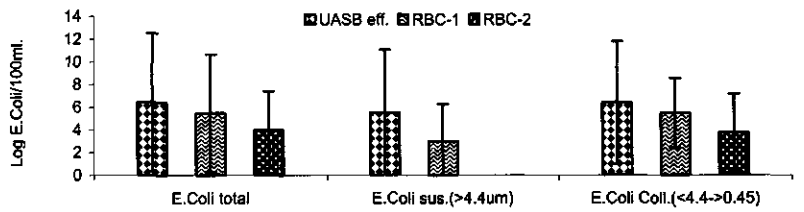

Fig.5 E.Coli $_{total}$, E.Coli $_{suspended}$ and E.Coli $_{colloidal}$ removal by 1st and 2-nd stage RBC system treating UASB effluent.

as compared to the single stage RBC (5.3 and 1.7 mg l^{-1}).
The high overall nitrification rate in the two-stage system compared with single stage system could be attributed to the staging of the biological conversions in the two-stage system.

Table 3. Comparison between the overall efficiency of a single versus two stage RBC systems.

Parameter	Unit	UASB eff	HRT=5 h		UASB eff	HRT=2.5 h	
			One stage	two stage		One stage	two stage
COD $_{total}$	mg l^{-1}	300±43	76±21	61±10	289±54	100±7	70±17
COD $_{suspended}$	mg l^{-1}	75±21	16±14	5±5	88±26	23±8	14±8
COD $_{colloidal}$	mg l^{-1}	77±17	10±5	3±3	71±16	14±5	3±3
COD $_{soluble}$	mg l^{-1}	148±35	50±15	52±8	139±38	62±8	53±12
Ammonia	mg l^{-1}	47±12	34±17	12±7	42±16	36±12	29±16
Nitrate	mg l^{-1}	-	5.3±5	26±7	-	1.7±1.4	7±6
E.Coli	/100ml	$6.5*10^6$ ±$5.2*10^6$	$6.5*10^5$ ±$7.5*10^4$	$0.5*10^5$ ±$4.3*10^3$	$4.3*10^6$ ±$2.6*10^6$	$7.9*10^5$ ±$9.8*10^5$	$2.1*10^5$ ±$0.3*10^6$

Most of the influent COD is removed in the 1st stage at all the applied organic loading rates investigated. Therefore heterotrophs dominates in the 1st stage and autotrophs in the 2nd stage. In this study, we found that the nitrification efficiency was improved by decreasing the concentration of dispersed COD particles in the 2nd stage. Apparently the removal of COD particles (COD $_{suspended}$ and COD $_{colloidal}$) are very important to achieve a high nitrification rate. The COD $_{particles}$ fraction in the UASB

effluent was around 51 % of the COD_{total}, while it was 34 % in the effluent of the single stage, and only 13 % in the effluent of the two stage. A rather limited nitrification was achieved in the single stage at the high influent $COD_{particles}$/N ratio of 2.9 and 3.1, the nitrification efficiency was 27.4 % and 14.0 % as compared to $COD_{particles}$/N ratio of 0.7 and 0.97 (Inlet of 2nd stage) the nitrification efficiency was 74.7 % and 31.0 %. The nitrification efficiency increased by 43.7 % when $COD_{particles}$/N ratio passes from 0.97 to 0.7.

The results in Table 3 indicate that the E.Coli reduction was around 1.0 and 0.73 log_{10} in the single stage as compared to 2.1 and 1.33 log_{10} in the two stages RBC. The efficiency of E.Coli removal is directly related with the $COD_{particles}$.

The effect of shock loads on the performance of the treatment system.

The two stage RBC was used to examine the effect of hydraulic and organic shock loads on the reactor performance in terms of COD, E.Coli removal and nitrification efficiency. The two stages RBC was operated at a HRT of 5.0 h and OLR of 13.7 g COD $m^{-2}.d^{-1}$. The imposed shock was at a HRT of 1.66 h. and an OLR of 52 g COD $m^{-2}.d^{-1}$. From the results presented in Figs. 6a, b and c it appears that, in general, decreasing the HRT from 5.0 to 1.66 h and increasing the OLR from 13.7 to 52 g COD $m^{-2}.d^{-1}$ resulted in:

- the COD levels in the effluent remained at the same level as compared to the reference conditions (86.0 mg l^{-1})
- the ammonia concentration in the effluent increased from 32.1 to 51.3 mg l^{-1}, accordingly the nitrate concentration decreased from 19.7 to 5.9 mg l^{-1}.
- The E.Coli percentage removal declined from 95.5 to 87.1%.

After returning to the reference HRT, the following observations have been recorded:

- the E.Coli percentage removal returned to their original values within 3.0h reaching 88.9 % and 95.6 %. after 24.6 h.
- the ammonia and nitrate concentrations in the effluent returned to their original values of 39.6 mg l^{-1} and 8.8 mg l^{-1} within 3.0 h and to 14.8 mg l^{-1} and 22.8 mg l^{-1} after 24.6 h.

The highest COD removal was found during the shock load condition in the 1st stage. The biofilm growth is directly related to the loading rate. At high organic loading rates, the biodegradable organic matter flux in the biofilm is also high, leading to a production of more microorganisms. A high biofilm growth rate occurs at the side-walls of RBC and at the disks. It is obvious that staging of RBC decreases the detrimental effect of shock load on the performance of the system.

The E.Coli concentration in the effluent of the two stages RBC increased at a lower HRT. Also the nitrification process deteriorated. This could be attributed to the lower contact time between the microorganisms and the substrate at low HRT in the 1st and 2nd stage.

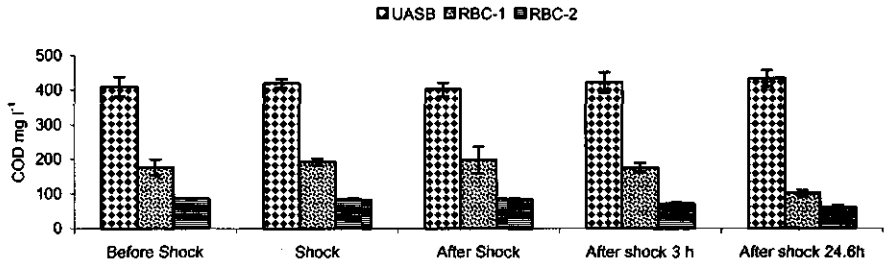

Fig. 6a Effect of the shock load on the COD removal in a two stage of a RBC system.

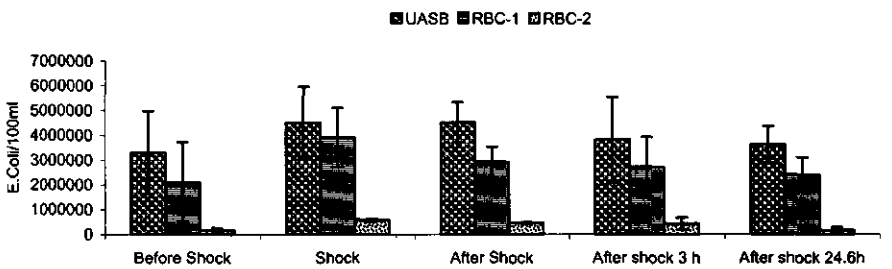

Fig. 6b Effect of the shock load on the E.Coli removal in a two stage of a RBC system.

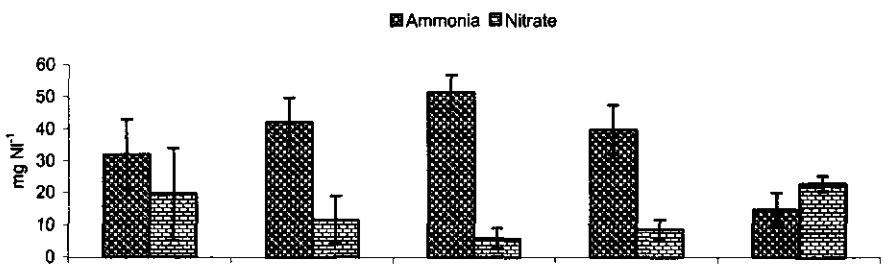

Fig. 6c Effect of the shock load on the ammonia removal in the second stage of a RBC

FINAL DISCUSSION

The benefits of anaerobic wastewater pre-treatment in many cases can only fully be realised if a proper (efficient and reliable) post-treatment system is available. This system should be simple in construction, operation and maintenance, stable under shock loads and its energy requirements should be low. For this reasons we selected a Rotating Biological Contactor as post-treatment system for the anaerobically pre-

treated domestic sewage. Emphasis in our investigations was given to the removal of COD, pathogenic bacteria and the conversion of ammonia- nitrogen by nitrification. The results of the investigation revealed that the major part of the suspended and colloidal COD is removed in the 1st stage of a two stage RBC. Nitrification mainly proceeds in the 2nd step due to the high COD and hydrogen sulphide loads prevailing in the 1st stage. The heterotrophs then displace the autotrophs, due to their much higher specific growth rates compared to the autotrophs.
According to observations of Boongorsrang *et al.*, (1982) the COD loading rate should not exceed 2.54 g m^{-2}. d^{-1} in order to enable nitrification to occur in a RBC-contactor. In the present investigation a high nitrification rate of 0.97 g NH_4. m^{-2}.d^{-1} (92 %) was found for two stages RBC at ammonia loading rate of 1.07 g m^{-2}. d^{-1} and COD loading rate of 6.45 g m^{-2}. d^{-1}. This nitrification efficiency is slightly higher than that obtained by Cheung, (1981), for a RBC system treating diluted domestic wastewater at an organic loading rate of 8.0 g COD m^{-2}. d^{-1}. Results of the present study show that increasing the organic loading rate from 13.7 to 21.0 g COD. m^{-2}. d^{-1} decreased the nitrification rate from 1.45 to 0.6 gNH_4. m^{-2}. d^{-1} for ammonia loading rates of 1.98 and 3.1 g. m^{-2}. d^{-1}, respectively. This nitrification rate is much lower than that obtained by Cheung, (1986) where 90% removal of BOD as well as NH_4^+-N removal could be achieved by using RBC study treating septic tank effluent at an organic loading rate of 8 - 10 g BOD. m^{-2}. d^{-1} and hydraulic retention time of 3 hrs.

In the RBC system described by Gonenc and Harremoes (1985) which was designed for carbon removal and nitrification, the nitrogen balance revealed that around of 8 % of the total influent nitrogen remained unaccounted for. Our results of the nitrogen balance made across the RBC indicate that 15.5 - 21.2 % nitrogen remained unaccountable in the single stage and 21.2 - 22.3 % in the two-stage system. We can expect that this amount of nitrogen be incorporated into the biomass produced.

Our results obtained with the two stages RBC, operated at a HRT of 10 h show a high percentage removal of E.Coli 99.5 % at a low organic loading rate of 6.4 g COD. m^{-2}. d^{-1}. These results are similar to the values obtained by Sagy and Kott (1990) who found efficiencies for E.Coli removal in a RBC of 99 - 99.9%, however at longer retention time. At higher organic loading rate of 13.7 and 20.96 g COD. m^{-2}. d^{-1} E.Coli removal was reduced from 99.0 to 89.0 %. According to Polprasert *et at.* (1983) a lower organic loading rate leads to faster pathogen removal as a consequence of limited availability of nutrients. Two Rotating Biological Contactor (RBC), one bench scale and the other at pilot-plant scale, treating domestic sewage were investigated by El-Zanfaly and El- Abagy (1987) using bacterial indices of pollution. The removal efficiency for total coliform (97.82 %), faecal coliform (99.74 %) and faecal streptococci (97.93 %) in the pilot RBC were much better than that obtained using laboratory scale unit (total coliform 92.76 %, faecal coliform 90.97 % faecal streptococci (87.69 %). However, the residual bacterial densities were high enough to

represent a potential health hazard such as 10^9 for total coliform, 10^6 for faecal coliform and 10^6 MPN-index/100ml for faecal streptococci compared to our results where residual of E.Coli in final effluent was 3.5 x 10^4/100ml. Therefore, a treatment scheme consisting of an anaerobic pre-treatment step in which a large proportion of the settable organic matter is removed, generally seem favourable for pathogen removal in post-treatment systems compared to conventional wastewater treatment systems.

CONCLUSIONS

The following conclusions can be drawn from the results presented in this study:

1. Increasing the organic loading rate applied to the two stages RBC from 6.45 to 21.95 g COD. m^{-2}. d^{-1} or decreasing the hydraulic retention time (HRT) from 10.0 to 2.5 h treating anaerobically treated sewage affected negatively the overall removal efficiency of the treatment scheme.
2. Most of the COD is removed in the 1st stage especially at lower OLR and that little additional removal occurs in the 2nd stage.
3. The experimental results reveal that the majority of residual E.Coli in the final effluent is associated with particle classes ranging in size between (0.45 - 4.4 µm) likely due to adsorption of E.Coli colloidal is however still limiting step in the RBC system.
4. Comparison of the performance of a single stage versus two stages RBC system, under conditions of the same total organic loading rate of 26.6 and 52.8 g COD. m^{-2}. d^{-1} reveal a clear improvement in the two-stage system.
5. A hydraulic shock load of three times the reference HRT of 5.0 h applied to the two stage system did not affect the COD levels in the 1st and 2nd stage of RBC, but E.Coli and nitrification efficiency declined. However the system recovered to its original performance within 3.0 and 24.6 h after the end of the shock.

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7

EFFICIENT AND COST EFFECTIVE E.COLI, COD AND AMMONIA REMOVAL VIA UP-FLOW ANAEROBIC SLUDGE BLANKET (UASB) IN COMBINATION WITH A SERIES OF ROTATING BIOLOGICAL CONTACTOR.

This chapter has been presented in *Proc. IAWQ Anaerobic Digestion Conference* 2 - 6 Sept. 2001 Antwerpen, Belgium as: Tawfik A., Klapwijk A., E.Gohary F. and Lettinga G.

ABSTRACT

A small-scale pilot plant consisting of three stage RBC system has been investigated for the removal of E.Coli, COD fractions and ammonia from anaerobically pre-treated domestic sewage. The results obtained indicate that a three stage RBC system operated at an HRT of 3.0 h. represents an effective and alternative post-treatment process. The remaining COD in the final effluent only amounted to 51 (± 6.5) mgl^{-1}. The overall removal of ammonia was 67 (± 7.6) %. The overall E.Coli reduction was 1.39 log_{10} at influent 6.5 log_{10} corresponding an overall removal efficiency of 95.8 (± 4.7) %. However, according to prevailing standards, E.Coli counts were still high for reuse for unrestricted irrigation purposes. The system was operated at an HRT of 10 h. This resulted in an overall E.Coli and ammonia removal of 99.9 (± 0.05) % and 92 (± 6.5) % respectively.

The efficiency of a two stage RBC-system and the effect of an additional 3rd stage with a high specific surface area and shallow depth with respect to E.Coli, COD and ammonia removal were investigated. The addition of a 3rd stage operated at an OLR of 23.6 g COD $m^{-2}.d^{-1}$ slightly improved the overall removal of COD_{total}, $COD_{suspended}$, $COD_{colloidal}$, $COD_{soluble}$. and E.Coli by 8.0, 13, 2, 6 and 7.0 % respectively. On the other hand the overall removal of ammonia was 52 %.

It was also shown that the recirculation of the final effluent from the 3rd stage to the 1st stage RBC system improved E.Coli removal which, so much an oriental that the effluent can be reused in unrestricted irrigation purposes.

INTRODUCTION

The Up-flow Anaerobic Sludge Blanket (UASB) reactor represents a good system for pre-treatment of domestic sewage, especially under Mediterranean climatic conditions (Castillo *et al.*, 1997). Unfortunately, the reductive environment in

anaerobic reactors doesn't allow a sufficient pathogen and ammonia removal which therefore, needs to be accomplished in a further aerobic phase. One option for post-treatment represents a Rotating Biological Contactor (RBC).

RBC is the most commonly used name for mechanical rotating disks operated as a wastewater treatment plant for various types of wastewater. It is claimed that their relative low cost, simple operation and maintenance, make them attractive for wastewater treatment (Lin *et al.*, 1986).

A combined anaerobic- aerobic system has promising characteristics for further development due to the positive energy balance and reduced sludge production. Therefore, such an integrated anaerobic reactor bio-process can be regarded as a "Cleaner Technology" (Castillo *et al.*, 1997).

In most publications dealing with RBC performance, emphasis has been devoted to physico- chemical characteristics of the effluent. Recently, Tawfik *et al.*, (2001) studied not only the COD removal, nitrification but also the reduction of E.Coli as well in a RBC system. The study showed that, the application of a two stage RBC system for post-treatment of UASB effluent can substantially reduce the concentration of E.Coli, but not sufficient to meet the limits of 1000 E.Coli/100ml set by WHO, (1989) for unrestricted irrigation. Apparently pathogens are not completely removed in a two stage RBC system and therefore, further treatment prior to reuse would be required. In this paper the use of a three stage RBC system for the treatment of an effluent of a rather poorly performing UASB reactor was investigated.

Traditionally faecal coliform (FC) has been used as an indicator of pollution. However, Dufour (1984) suggested that E.Coli is a more useful faecal indicator in fresh waters and presumptive E.Coli is also recommended by the WHO guidelines (1993) as the main indicator of faecal pollution. This supports the use of E.Coli assessment as the main indicator of faecal pollution. Therefore, E. Coli was used for this study.

The objectives of this study therefore are,

1. to evaluate the efficiency of a three stage RBC system operated at different HRT or OLR for E.Coli, COD fractions removal and nitrification rate.

2. to evaluate the effect of recirculation of the final effluent on the efficiency of the three stage RBC.

MATERIAL AND METHODS

The experimental arrangements (Fig. 1) consisted of a three stage RBC fed with anaerobic effluent from a 6.0 m^3 UASB pilot plant, which had been previously investigated by Grin *et al.*, (1985). The UASB was fed with raw domestic sewage collected in a combined sewer system of the village Bennkom, The Netherlands.

UASB effluent

The main characteristics effluent of the UASB reactor effluent which, in fact didn't perform satisfactory in this experimental period are summarised in Table 1.

Table 1. Characteristics of the Up-flow Anaerobic Sludge Blanket (UASB) effluent.

pH	Temp	COD mgl^{-1}				NH_4-N	TKN	H_2S	E.Coli/
	°C	total	Sus.	Coll.	Sol.	mgl^{-1}	mgl^{-1}	mgl^{-1}	100ml
6.5-	14.0	278	104	64.0	113	43.0	55.0	17.0	$3.5*10^6$
7.8		± 39.0	± 30.6	± 21.0	± 48.0	± 14.0	± 8.0	± 8.0	$\pm 0.2*10^6$

RBC characteristics

The RBC system consisted of three stages. The first two stages are provided with an internal settling tank and the disks are made of polystyrene foam (diameter 0.6 m & thickness 0.02 m). The last stage is provided with polyurethane rotating disks (specific surface area 1000 m^2/m^3) the diameter of which is 0.6 m and the thickness 0.01m. The surface areas of 1st, 2nd and 3rd stage of the RBC are 6.5, 6.5 and 45.0 m^2. In the 1st and 2nd stages of the RBC about 40 % of the rotating discs are submerged. In the 3rd stage 33 % of the discs are submerged. The rotational speed of the disks was fixed at 5 rpm throughout the study in the system. A variable speed motor device drives all stages of RBC. The net volume of the 1st, 2nd and 3rd stage are each 60 l, 60 l and 35 l, making up a total net volume of 155 l.

Recirculation set up

In one of the experiments, effluent from the 3rd stage was recirculated to the 1st stage. In this study, recirculation ratio was defined as the ratio of the returned flow rate to that of the inlet.

Analysis

48 hrs composite samples of the influent and the effluent of each reactor were collected in containers stored in the fridge at 4 °C and analysed. Parameters as dissolved oxygen, pH and temperature were measured in situ regularly.
COD was analysed using the micro-method as described by Jirka and Carter (1975). Raw samples were used for COD_{total}, 4.4 µm folded paper filtered (Schleicher & Schuell 595 ½) samples for $COD_{filtrate}$ and 0.45 um membrane filtered (Schleicher & Schuell ME25) samples for dissolved COD ($COD_{soluble}$). The $COD_{suspended}$ and COD

$_{colloidal}$ were calculated by the difference between COD $_{total}$ and COD $_{filtered}$, COD $_{filtered}$ and COD $_{soluble}$, respectively. Ammonia, nitrite and nitrate were determined on auto-analyser (SKALAR-SA 9000). Total Kjeldahl nitrogen and sludge analysis were measured according to the Dutch Standard Normalised Methods, (1969). E.Coli examination was performed according to the method described by Havelaar *et al.*, (1988).

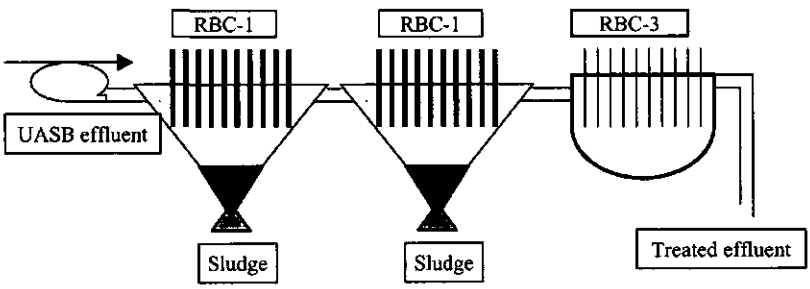

Fig.1 schematic description of the RBC system

Experimental set up.

The whole experiment was run for 7.0 month and can be divided into 2.0 periods according to the operational conditions,

Period (I): - The three stages RBC were operated for 2.5 month at a total OLR of 5.3 g COD. $m^{-2}.d^{-1}$, flow rate of 1.152 $m^3.d^{-1}$ and a HRT of 3.0 h.

Period (Π): - This period which took 4.5 month. This period was divided into two experimental parts referred to as period (Πa), and (Πb)

Period (Πa): - The three stage RBC was operated for 2.0 month at a total OLR of 1.6 g. $m^{-2}.d^{-1}$, a flow rate of 0.36$m^{-3}.d^{-1}$ and a HRT of 10.0 h.

Period (Πb): - The three stages RBC were operated for 2.5 month at the same conditions as in period (Πa) with effluent of the 3rd stage recirculated to the 1st stage (recirculation ratio 1:1).

RESULTS AND DISCUSSION

Efficiency of three stages RBC system for E.Coli and COD fractions removal; and the nitrification rate.

The performance of a three stage RBC treating UASB effluent operated under ambient temperature ranging from 11.0 to 17.0 °C was evaluated. E.Coli, and COD fractions removal and as well as the nitrification rate at HRT of 3 and 10 h were determined.

Increasing the overall HRT of the three stage system from 3.0 to 10.0 h achieved a minor improvement of COD and ammonia removal but a significant reduction of E.Coli counts. The average residual value of COD decreased from 51 to 43 mgl^{-1}, ammonia from 10.6 to 3.3 mgl^{-1} and E.Coli from 1.3×10^5 to 2.0×10^3 /100ml .

The results presented in Fig.2_a show the 1st stage is the most effective, particularly with respect to COD_{total} and $COD_{colloidal}$ removal which can be attributed to the fact that, the most of them becomes adsorbed and will be partially hydrolysed.

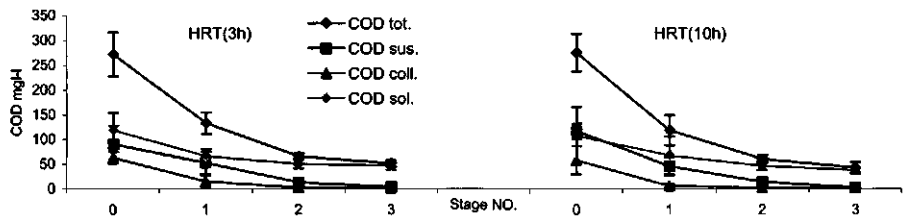

Fig.2a The effect of staging RBC system treating UASB effluent on the COD fractions removal.

Increasing the $COD_{colloidal}$ and $COD_{suspended}$ loading rates applied to the 1st stage from 3.3 to 10.8 $g.m^{-2}.d^{-1}$ and from 6.7 to 14.5 $g.m^{-2}.d^{-1}$ lead to increase of the $COD_{colloidal}$ and $COD_{suspended}$ removal rates from 3.0 to 7.8 $g.m^{-2}.d^{-1}$ and from 4.1 to 5.3 $g.m^{-2}.d^{-1}$ respectively. This indicates that entrapment of $COD_{suspended}$ by heterotrophic biofilm and sedimentation of big particles increased as the $COD_{suspended}$ loading rate increased. Also the removal rates of $COD_{colloidal}$ appear to be proportional to the corresponding $COD_{colloidal}$ applied loading rates and that $COD_{colloidal}$ removal is the net effect of adsorption onto and release from the biofilm surface.

The results in Fig. 2b show that nitrification was very limited in the 1st stage at HRT of 1.25 and 4.0 h. consequently OLR of 46.7 and 15.3 g COD_{total}. $m^{-2}.d^{-1}$. This was due to the presence of an insufficient NH_4-N oxidiser population at high OLR, as they can't compete with heterotrophs for space and oxygen. The produced NO_2-N and NO_3-N were comparable with the NH_4-N removed at imposed HRT of 1.25 and 4.0 h in the 1st stage. Apparently NH_4-N (1.5 and 6.3 g m^{-3}) was not mainly removed as a result of nitrification but also due to heterotrophic bacterial assimilation during removal of COD. In the 2nd and 3rd stage of RBC a high nitrification rate and additional amount of COD removal was achieved. Fig. 2_b shows that the nitrate concentration increased in the 2nd stage and 3rd stage at total HRT of 3 and 10 h.
Available data indicates that the most important parameter affecting the nitrification rate is the OLR because, it implies a competition between different bacterial groups. The influence of this parameter is clearly reflected by the results depicted in Fig. 3. These results demonstrate that at an OLR exceeding 23.6 g $COD.m^{-2}.d^{-1}$ heterotrophic bacteria still prevail in the 2nd stage of the RBC. But the nitrifying

bacteria clearly promoted in the 3rd stage of the RBC when the OLR drops to 1.6 g COD.m^{-2}.d^{-1} the ammonia removal was virtually approximately complete.

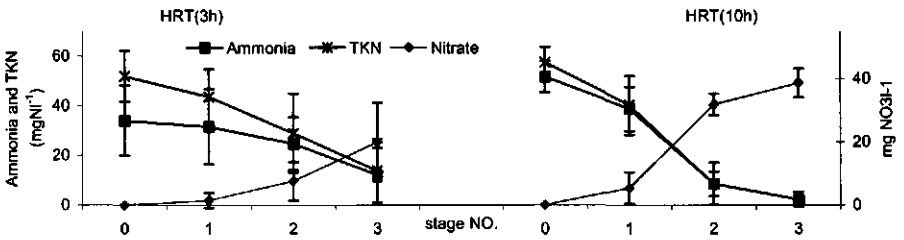

Fig. 2b The effect of staging RBC system treating UASB effluent on the ammonia and TKN removal.

The results in Fig. 4_a show an increase in the E.Coli removal from one stage to the other. The overall percentage removal was 95.8 ± 4.7 and 99.9 ± 0.05 at a HRT of 3 and 10 h. The RBC effluent median E.Coli counts were 1.3 x10^5 and 2.0 x10^3/100ml at HRT of 3.0 and 10.0 h respectively, which means that these effluents can be used for restricted irrigation but not for unrestricted irrigation purposes according to WHO (1989).

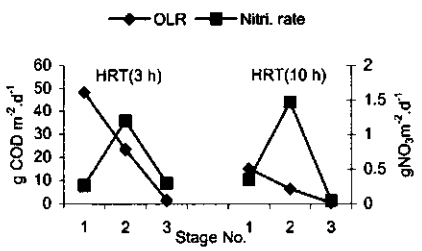

Fig.3 influence of the inlet OLR on the nitrification rate.

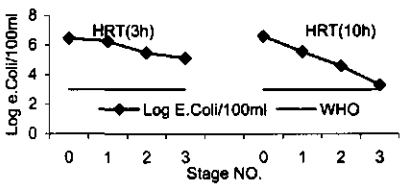

Fig.4a the effect of staging RBC system treating UASB effluent on the E.Coli removal.

The results presented in Figs.4_b and $_c$ show that the removal of E.Coli only significantly improved once the concentration of the dispersed COD $_{suspended}$ and COD $_{colloidal}$ has become very low, and the HRT has increased from 3.0 to 10 h. Dispersed COD removal leads to be very important to achieve a satisfactory E.Coli removal. Part of E.Coli will be removed in the 1st stage as a result of adsorption and sedimentation i.e. the fraction of pathogenic bacteria attached on the suspended solid. The free dispersed E.Coli were found to be adsorbed in the subsequent stages.

The efficiency of a two stage RBC-system and the effect of an additional 3rd stage with a high specific surface area and shallow depth with respect to E.Coli, COD and ammonia removal can be seen from the results summarised in Table 2. The addition of a 3rd stage operated at an OLR of 23.6 g COD m^{-2}.d^{-1} slightly improved the overall removal of COD $_{total}$, COD $_{suspended}$, COD $_{colloidal}$, COD $_{soluble}$. and E.Coli by 8.0, 13, 2, 6 and 7.0 % respectively. On the other hand the overall removal of ammonia was 52 %.

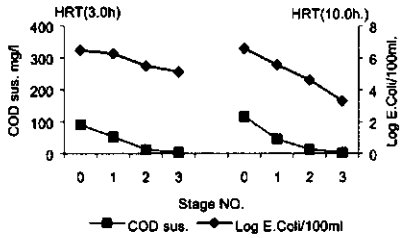

Fig.4b. The COD suspended and E.Coli removal.

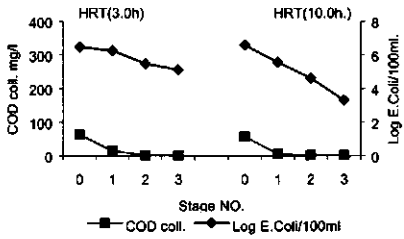

Fig.4c. The COD colloidal and E.Coli removal.

Table 2. Effect of addition of 3rd stage of RBC.

Parameter		COD mg l^{-1}			Total nitrogen mg l^{-1}				H_2S	E.Coli/
Sample	total	sus.	Coll.	Sol.	NH_4-N	NO_2	NO_3	TKN	mg l^{-1}	100ml
UASB eff.	272	90.0	63.0	119	35.0	-	-	52.0	11.0	$3.1*10^6$
	± 44	± 38	± 12	± 35	± 14			± 10		$\pm 1.7*10^6$
Two stage	70.0	14.0	03	53.0	29.0	0.7	7.0	29.0	0.0	$3.4*10^5$
	± 6.8	± 7.7	± 1.9	± 9.0	± 10.7	± 0.2	± 6	± 16		$\pm 3.2*10^5$
%R	73.0	83.0	95.0	56.0	17.0	-	-	22.0	100	89.0
Three	51.0	3.3	02	46.0	11	3.0	20	14	0.0	$1.3*10^5$
stage	± 6.6	± 5.3	± 2.6	± 8.0	± 10.9	± 1.5	± 12	± 13		$\pm 2.4*10^5$
%R	27.0	76.0	67.0	13.0	62.0	-	-	59.0	100	62.0
Overall eff.	81.0	96.0	97.0	62.0	69.0	-	-	68.0	100	96.0

Sludge characteristics.

Table 3. Sludge characteristics of the 1st and 2nd stage at different OLR's.

Parameter	Total OLR = 23.6 g.COD $m^{-2}.d^{-1}$		Total OLR = 6.9 gCOD $m^{-2}.d^{-1}$	
	RBC-1	RBC-2	RBC-1	RBC-2
Total Solids (%)	33	27	29	26
Volatile Solids (%)	67	73	71	74
Sludge Biomass Index (%)	67	73	71	74
Sludge Volume Index	55.7	69.0	65.9	88.7
Water Content (%)	98.2	98.6	98.6	98.9
Solid Content (%)	1.8	1.4	1.4	1.1
Capillary Suction Time (Sec)	90.6	14.4	61.9	38.0
Filterability (g^2/s^2m^4)	180	900	200	300

In view of its practical importance also relevant sludge characteristics was evaluated (Table 3). The filterability of sludge from the 2nd stage was better than the sludge from the 1st stage. The overall sludge production in the 1st and 2nd stage of the RBC were 57.8 and 37.1g d^{-1}, corresponding to 0.26 g sludge/g COD removed and 0.48g sludge/g COD removed respectively. These values are comparable to those found by Hack and Klapwijk (1984) 0.57 g sludge produced /g COD removed in RBC treating UASB effluent. i.e. the excess sludge produced in the UASB (which was not measured) should be added to those amounts. The excess sludge produced

obviously needs further treatment, for instance using anaerobic digestion. Along the experimental period, no sludge was produced in the 3rd stage of RBC system.

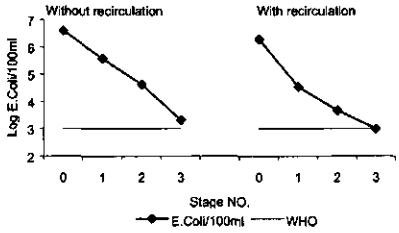

Fig. 5a Effect of recirculation on the E.Coli removal.

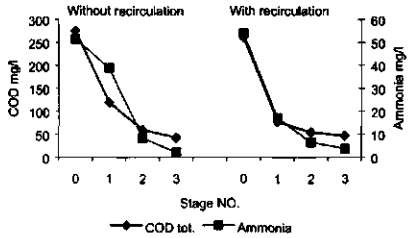

Fig.5b Effect of recirculation on the ammonia and COD removal.

Effect of recirculation of nitrified effluent on the performance of RBC system.

The effect of effluent recirculation on the overall removal of E.Coli, COD fractions and the nitrification rate was examined in a three stage RBC system. The results are presented in Fig.5_a it is clear that recirculation improves the E.Coli removal in the 1st stage RBC system. The reduction increased from 1.1without recirculation to 1.8 $\log_{10}$ with recirculation. Average residual E.Coli values in the final effluent decreased from 0.2×10^4 to 9.8×10^2 /100ml. And so the median counts of E.Coli in the final effluent satisfies WHO criteria for unrestricted irrigation (1989). This could be attributed to the fact that recirculation of the final effluent which is rich in ciliates, nematodes and other E.Coli predators like *Vorticella, Carchesium, Trachelophylum Pusillum, Paramecium Candatum, Rotifers, Nematoda* allows their presence in all stages of RBC system. Where freely colloidal E.Coli are easily predated by protozoa or ciliates (Gersberg *et al.,* 1987; Kinner and Curds, 1987; Green *et al.,* 1997 and Curds, 1992).

The results in Fig.5b show that recirculation didn't improve the overall removal efficiency of ammonia. This can be attributed to the grazing of nitrifiers by protozoa and metazoa (Natuscka and Welander, 1994). The results also in Fig. 5b show that the COD values of the final effluent were almost the same with and without recirculation. This can be due to the fact that most of COD biodegradable was already removed and the residual part of the COD in the nitrified effluent was almost non-biodegradable. This emphasise the findings of Rusten *et al.,* (1995) who reported that the effluent COD filtered from a fully nitrifying biofilm reactor is almost inert.

GENERAL DISCUSSION

It is well known that, the benefits of using an RBC system consisting of a series of stages is to provide improved environmental conditions for the removal of E.Coli, COD and ammonia. Our previous studies (Tawfik *et al;* 2001) indicated that a two stage RBC system attains higher performance efficiency than a single stage RBC

operated under the same OLR and HRT. This is probably due to the fact that a multi-stage scheme provides different environmental conditions for the removal of substrate constituents, namely, E.Coli, COD and ammonia. Accordingly, the mechanism of the removal of the different pollutants can be explained as follows:

In the 1st stage of an RBC system (hetertrophic stage) an efficient reduction of sulphide, COD_{total}, $COD_{ssuspended}$, $COD_{colloidal}$ and $COD_{soluble}$ are achieved. The partially treated effluent of the 1st stage will be the influent of the 2nd stage which contains more autotrophic bacteria usually has the ability a) to nitrify b) to reduce COD close to a minimum value c) to allow for free E.Coli removal (> 4.4 - < 0.45 μm) by adsorption on the biofilm as described by Tawfik *et al.*, (2001). In the 3rd stage autotrophic bacteria dominates and other microorganisms like protozoa and ciliates. When the residual COD in the 2nd stage effluent is low, the 3rd stage is capable to reduce ammonia close to a minimum value and allow for free E.Coli removal with a mechanism of adsorption and predation by abundant ciliates present here.

The overall removal of COD, ammonia and E.Coli in this study comparable to these obtained by other post-treatment systems such as wastewater stabilisation pond and integrated pond systems (Dixo *et al.*, 1995, Van der steen *et al.*, 1999). RBC reactors clearly provides the same removal efficiencies for COD, ammonia and E.Coli but at significantly lower HRT and higher OLR.

When a high removal is preceded for COD particles ($COD_{suspended}$ and $COD_{colloidal}$) and E.Coli the best solution is to use three independent stages in order to limit competition between COD particles ($COD_{suspended}$ and $COD_{colloidal}$) and E.Coli adsorption. The big part of $COD_{suspended}$ and $COD_{colloidal}$ is already adsorbed in the 1st stage and it block the sites onto the biofilm for E.Coli adsorption at an HRT of 1.25 and 4.0 h. Therefore, E.Coli removal increases in the 2nd and 3rd stage where, the abundance of available bacterial adsorption sites are available. The higher reduction of E.Coli achieved at total HRT of 10 h can be explained by presumed strong effect of detention time on the E.Coli reduction (Polprasert and Hoang 1983).
The results of the present study indicate that the removal of E.Coli from UASB effluent in a RBC system comprises a) sedimentation of coarse particles b) adsorption of E.Coli to the biofilm c) predation by ciliated protozoa. The same mechanism was proposed for activated sludge systems by Drift *et al.*, (1977). According to the observations of Ueda and Horan (2000) a membrane bio-reactor alone gives a poor removal of faecal coliforms and faecal streptococci but once a biofilm was developed, the removal efficiency increased to 7.0 $\log_{10}$. This indicates that the presence of a biofilm on the surface of the membrane is of major importance for bacteria removal.

CONCLUSIONS

- The addition of a 3rd stage RBC system operated at OLR of 24 $g.m^{-2}.d^{-1}$ to the two-stage RBC improved the overall removal efficiency of COD_{total}, $COD_{suspended}$, $COD_{colloidal}$ and $COD_{soluble}$ by only 8.0, 13, 2 and 6 % respectively. Therefore, If the aim is only COD removal & partial removal of ammonia and E.Coli, a two stage RBC system operated at HRT of 2.5 h and OLR of 24 g $COD_{total}/m^2.d$ is recommended.
- The additional 3rd stage achieved significantly better results for the overall removal of ammonia (52 %). Therefore, if nitrification is the main objective, three stage RBC system at HRT of 3 h is recommended. Our previous studied shows also that the two stage RBC system can achieve almost complete ammonia removal but at longer HRT of 5 h (Tawfik *et al.*, 2001).
- If E.Coli removal is required to use the treated wastewater for unrestricted irrigation purposes, a three stage RBC at HRT of 10 h is recommended.
- The recirculation (100 %) of the final effluent at a HRT of 10 h. reduced residual E.Coli in the final effluent from 2.0×10^3 to 9.8×10^2/100ml but didn't improve the overall removal efficiency of ammonia and COD.
- The sludge analysis revealed a good settleability and better filterability of that produced 2nd stage as compared to that produced in the 1st stage. The overall sludge production measured in 1st and 2nd stage of RBC were 57.8 and 37.1g d^{-1} depending on the OLR rates.

ACKNWOLEDMENTS

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8

SUMMARY, FINAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The anaerobic treatment process is increasingly recognised as the core method of an advanced environmental protection and resource preservation technology (Lettinga, 1997). Combined with other proper methods it represents a sustainable, robust and appropriate wastewater treatment system not only for developing countries but also for technologically advanced countries. It is already successfully applied for domestic sewage treatment in tropical countries, but also encouraging results have been obtained in subtropical and temperate regions.

It should be emphasised here that, Anaerobic Wastewater Treatment (AWT) mainly is effective in removing organic matter, soluble and dispersed. For the removal of remaining COD, ammonia-N, S^{-2} and pathogens a proper post-treatment is required. For this purpose several researchers (Van der Steen *et al.,* 1998 &1999; Augusto *et al.,* 2000; Frassinetti *et al.,* 2000, El-Gohary, 1998) investigated the applicability of algal pond. The main disadvantage of using algal pond systems comprises the large land area requirements, the high accumulation of excess sludge in the ponds, the frequently high loses of water due to evaporation, the required long liquid retention times and the rather low removal efficiencies achieved during winter time.

There exists a growing interest for using biofilm processes for post-treatment, such as trickling filters (Bouvenduer *et al.,* 1990), fixed media submerged biofilters (Goncalves *et al.,* 1998, 1999), granular media biofilters, fluidised bed reactors (Collivignarelli *et al.,* 1990) etc. They have all their specific advantages and disadvantages. So the trickling filter is not volume-effective, mainly due to difficulties to get an even distribution of the load over the whole carrier surfaces. The static granular media bio-filters need to be operated discontinuously because of the need for back washing and fluidised bed reactors suffer from hydraulic instability. According to Bovenduer *et al.,* (1990) a Rotating Biological Contactor (RBC) system represents an excellent option for post-treatment of UASB effluents at low operational temperature. Therefore we decided to investigate the RBC system in this PhD-study. RBC-systems are quite compact enjoying a sufficiently long biomass retention time, allowing the application of a higher

volumetric loading rate at low energy cost. Moreover the RBC system is easy to operate under conditions of high process stability.

This thesis deals with the applicability of RBC system for post-treatment (polishing) of different effluent qualities of UASB reactor with emphasis on the COD, ammonia and E.Coli removal.

Investigations dealing with the assessment of the removal mechanism of E.Coli from UASB effluent using a RBC are described in **Chapter 2**. Results of preliminary batch experiments indicated a first order removal kinetics of E.Coli. Factors affecting the removal of E.Coli using a biofilm system were investigated, viz. stirring intensity, occurrence of sedimentation, dissolved oxygen concentrations, pH, use of different carrier materials and cationic polymer addition. The results obtained reveal that, the most important removal mechanism of E.Coli in the biofilm comprises an adsorption process, followed by sedimentation. According to Omura *et al.*, (1989) bacterial die-off mechanism is a relatively minor importance in a biofilm system. Compared to the anaerobic biofilm system higher removal rates of E.Coli were observed in an aerobic biofilm system (D.O ranged from 3.3 to 8.7 mgl^{-1}). In the pH -range between 6.5 and 9.3 any significant effect of the pH on the removal rate of the E.Coli by the heterotrophic biofilm was not observed. The effect of the type of support carrier material on E.Coli removal probably mainly manifests once the biofilm has developed, which is consistent with the results obtained by Ueda and Horan (2000). We found a faster adsorption and a better removal rate of E.Coli to the biofilm after adding the cationic polymer compared to the biofilm without polymer addition.

Chapter 3 deals with a comparison of the performance of an anaerobic versus aerobic RBC system for the treatment of good quality UASB reactor effluent. Both RBC's units were operated at the same HRT of 2.5 h and OLR of 14.5 $gCOD/m^2.d$. The results obtained clearly demonstrated a better efficiency of the aerobic RBC, i.e. the achieved removal efficiency of COD_{total} and $COD_{soluble}$ in the aerobic RBC unit was 56 and 28 % respectively, compared to 23.0 and 14 % in the anaerobic one. Moreover, a significantly better removal of $COD_{colloidal}$ was found in aerobic RBC, viz. a value up to 90%, while it was even negative (-16%) in the anaerobic RBC. The aerobic biofilm has a superior ability to adsorb $COD_{colloidal\ matter}$. In the anaerobic biofilm apparently coarse suspended solids are converted (presumably by a hydrolysis process) to colloidal particles. Moreover, accordingly the aerobic RBC system also achieved a significantly higher removal efficiency of E.Coli.

The installation of an anaerobic RBC-stage in front of the aerobic RBC increased the overall removal efficiency of COD $_{total}$ only by 9.0 %, but it didn't improve the overall removal of E.Coli $_{total}$ and the nitrification rate as compared to single stage aerobic RBC system. Therefore from the results of this study we recommend to use an aerobic RBC as a post-treatment step for anaerobically pre-treated domestic sewage.

Chapter 4 describes the results of experiments dealing with the operation of the single and two stage RBC for post-treatment of a high quality UASB-effluent (UASB reactor at operational temperature of 30 °C). Both systems were operated at the same OLR of 14.5 $gCOD_{total}/m^2.d$ and at a HRT of 2.5 h but at different flow rates and different temperatures, viz. of 0.576 & 1.152 m^3/d and 24 & 17 °C in the single and two stage system respectively. In both systems the same residual effluent values were found for COD $_{total}$ (72 mg/l), for COD $_{suspended}$ (16 mg/l), for COD $_{colloidal}$ (5 mg/l) and for COD $_{soluble}$ (51 mg/l). Moreover also the removal efficiency of E.Coli was almost the same, viz. amounting to 94 %. However, the ammonia removal in the single stage RBC system amounted to 50 % of which 71% was nitrified compared to only 23 % in the two-stage RBC system. This better performance of the single stage system can be attributed to the higher operational temperature of the wastewater during the experiment in the single stage RBC system. In view of the results obtained, we recommend to use a single stage RBC system for COD removal and for a partial removal of ammonia and E.Coli for post-treatment of a high quality anaerobically pre-treated domestic sewage, viz. at imposed OLR's of approximately 14.5 g COD $_{total}$ $/m^2.d$ (10 g COD $_{biod.}$ $/m^2.d$) and HRT of approximately 2.5h. Such conditions prevail for well-designed UASB reactors operated at higher temperatures, as generally is the case in tropical countries.

We investigated the nitrogen removal from the nitrified effluent using a biofilm reactor, viz. an anoxic up-flow submerged bio-filter in a three stage process configuration, i.e. consisting of a segmental two stage aerobic RBC's and the biofilter. The nitrified effluent of the 2nd stage RBC was recycled to the anoxic reactor with the objective to get some denitrification. The results obtained reveal that the introduction of such an anoxic reactor as a 1st stage combined treating the nitrified effluent of the 2nd stage RBC is accompanied with a conversion of nitrate into ammonia, when the content of COD $_{biod.}$ in the UASB effluent is low. Latter is the case for high quality anaerobic effluents. This consequently would lead to the very undesirable situation that the ammonia needs to be nitrified twice, without achieving any substantial denitrification. Therefore, in such 'extreme' situations of a too high quality anaerobic effluent in terms of biodegradable COD content, the introduction of a separate anoxic reactor for denitrification, as final post-treatment step does not make any sense.

During the single stage RBC experiments presented in **Chapter 5** the UASB reactor was operated at two different operational temperatures viz. 30 and 11°C resulting in quite different COD $_{biod.}$ concentrations in the UASB effluent. The two (single stage) RBC's were operated at a constant HRT of 1.25 h but at different COD $_{biod.}$ loading rates, viz. of 17.7 and 36.8 g/m^2.d due to the highly different UASB effluent qualities. The results obtained clearly show that the residual values of COD $_{total}$, COD $_{suspended}$, COD $_{colloidal}$ and E.Coli are significantly lower at the lower imposed COD $_{biod.}$ loading rate of 17.7g COD $_{biod.}$ m^2.d^{-1}. Moreover, also the calculated sludge stability (VSS/MLSS) for the single stage RBC system operated at the low COD $_{biod.}$ loading rate was distinctly higher than found for the high COD $_{biod.}$ loading rate.

The efficiency of using a two-stage RBC for such highly different UASB-effluent qualities was compared at imposed overall COD $_{biod.}$ loading rate of 9 and 18 g m^{-2}.d^{-1} at the same HRT of 2.5 h. The results of these experiments reveal that the residual values for the various distinguished COD fractions in the final effluent were almost the same in both cases. However the residual value of E.Coli in the final effluent amounts to 3.4 x 10^5 at the higher COD $_{biod.}$ loading rate and to 7.6 x 10^4 /100ml for the lower one. The calculated nitrification rate in the 2nd stage of RBC system decreased from 1.56 to 1.1 gNO_3-N.m^{-2}.d^{-1} when imposing a COD $_{biod.}$ loading rate 11.3 in stead of 16 g m^{-2}.d^{-1}. The use of a higher quality UASB reactor effluent clearly improves the nitrification rate in the post-treatment system, as in fact could be expected. Consequently, depending on the practical need of nitrification, a two stage RBC can suffice for post-treatment of a poor quality UASB-effluent at imposed OLR as high as 18 g m^{-2}.d^{-1} and a short HRT of 2.5 h, because all the COD-fractions are removed quite well. This likely will still be the case at substantially higher OLR's.

Additionally, we used in the experiments described in Chapter 5 the 2nd stage of a two-stage system for a period of 9 days as a single stage RBC reactor by introducing the UASB effluent directly into this reactor. The idea of this experiment was to assess the effect on the nitrifying bacterial activity. During this 9.0 days period this 2nd stage reactor was fed at a high COD $_{biod.}$ loading rate of 13.2 g COD $_{biod.}$ m^{-2}.d^{-1}, and subsequently the system remained unfed for a period of 7.0 days, after which the RBC was again used as a two stage. The results of the experiments reveal an immediate deterioration of the nitrification process after exposing the reactor to a COD $_{biod.}$ loading rate of 13.2 g m^{-2}.d^{-1}, resulting in an increase of ammonia concentration in the effluent to 28 mg/l after 3h.

After returning to the original total COD $_{biod.}$ loading rate of 7 g COD $_{biod.}$ m^{-2}.d^{-1}, the nitrification process in the reactor (as a 2nd stage) recovered within 2-3 days. The ammonia concentration in the effluent dropped to 17 mg/l after 2 days and to 8 mg/l after

3 days. The calculated nitrification rate after 3 days feeding with low COD $_{biod.}$ loading rate amounted to 1.5 g NO_3-N/m^2.d.

The results of the experiment clearly show that after a feed – less period of 7 days and feeding with a high COD $_{biod.}$ loading rate for 9 days, the nitrification activity can be recovered after a total period of 16 days working under stress conditions.

In **Chapter 6** the research was focused on the treatment of effluent from UASB reactor operated at low temperatures of 11-14 °C, consequently a rather low effluent quality. First of all, we compared the performance of the single stage with the two-stage RBC for COD; E.Coli removal and nitrification at the same imposed OLR. The results obtained showed that the values for COD $_{total}$, COD $_{suspended}$ and COD $_{colloidal}$ in the final effluent of a two stage RBC were lower than those of the single stage. Moreover, the calculated nitrification rate in the single stage system was quite significantly lower compared to the two-stage system. The two-stage RBC system therefore provides substantially higher effluent concentrations of nitrate, viz. 26 and 7.0 mg l^{-1} respectively, as compared to single stage 5.3 and 1.7 mg l^{-1}. The heterotrophic bacteria dominate in the 1st stage and the autotrophic organisms in the 2nd stage. The results for E.Coli removal also show a superior performance of the two stages RBC over the single stage system. Based on these results, we concluded to recommend a two stage RBC system for post-treatment of the effluent of a conventional UASB reactor operating at a low temperature, especially for ammonia and E.Coli removal.

We operated the two stages RBC at different HRT's and OLR's. The removal efficiencies of COD $_{total}$, COD $_{suspended}$, COD $_{colloidal}$ and COD $_{soluble}$ improved slightly when imposing a higher HRT and a lower OLR, but the overall removal efficiency for ammonia and E.Coli were negatively affected by increasing the loading rates.

The major part of the E.Coli entrapped in coarse suspended matter (>4.4 µm) was removed by sedimentation or by entrapment sorption in the biofilm of the 1st stage of two stage RBC (99.66%). However, E.Coli present in the colloidal fraction (<4.4 - >0.45 µm) was predominantly eliminated in the 2nd stage of two stage RBC (99.78%).

Based on these results once again the two-stage RBC system looks the best solution for treating a poor UASB effluent quality. It comprises the most efficient system and for the combined COD removal and complete ammonia removal at low OLR of 6.5 gCOD/m^2.d and long HRT of 10 h.

The two stages RBC was examined for the effect of hydraulic shock loads on its performance in terms of COD removal, nitrification and E.Coli removal. The results obtained show that the decrease of HRT from 5.0 to 1.66h and an accompanying increase of the OLR from 13.7 to 52 gCOD/m^2.d didn't affect the COD removal efficiency. However the ammonia concentration in the effluent increased and consequently the nitrate concentration decreased. Moreover also the E.Coli removal efficiency declined.

After returning to the reference HRT of 5 h and OLR of 13.7 gCOD/m^2.d, the E.Coli and ammonia removal efficiency returned to their original values within 3.0h and 24.6 h. This leads to the conclusion that staging of the RBC makes the system quite resistant to shock loads.

The effluent of a 2nd stage RBC still cannot be used for unrestricted irrigation purposes, as the E.Coli concentration is still too high, at least relative to the very stringent (likely too stringent!) standards set by the WHO. In **Chapter 7** we therefore investigated the use of a three stage RBC for post-treatment for effluent from a rather poorly performing UASB reactor. For this purpose a three stage RBC system was first operated at a HRT of 3.0 h. Under these conditions the E.Coli count found in the final effluent that was still too high, but a HRT of 10h sufficed to get an E.Coli concentration almost complying with the WHO standards for unrestricted irrigation purposes. Regarding the considerable higher investments needed for that, and considering the likely far too stringent standards of WHO, it is highly questionable whether it should be attempted to comply with these standards in practice, particularly considering also the present situation of public health risks.

We attempted to assess the effect of effluent recirculation on the overall removal efficiency of E.Coli in a three stage RBC system. The results obtained reveal an improvement of the E.Coli reduction in the 1st stage of the RBC. It leads to a decrease of the average residual values in the final effluent from 0.2 x 10^4 to 9.8 x10^2/100ml. And so the E.Coli count in the final effluent with recirculation satisfy with WHO (1989) criteria for unrestricted irrigation. The results have shown that when a high removal is required for E.Coli, the best solution is to use three independent stages at HRT of 10 h.

CONCLUSIONS AND RECOMMENDATIONS

1. For tropical areas, with average sewage temperatures (22-30 °C), anaerobic pre-treatment proceeds efficiently, provided the system is well designed. The results of

this study obtained with high quality anaerobic effluents using RBC-systems revealed that:

- a single stage RBC system suffices for achieving a low COD $_{total}$ (70 mg/l) and for partial ammonia and partially E.Coli removal at imposed OLR of 14.5 $gCOD_{tot}/m^2.d$ and HRT of 2.5 h.
- a two-stage RBC system is needed for achieving a complete ammonia removal and a satisfactory removal of E.Coli. The 1st stage of the system can be applied at high OLR (e.g. values exceeding 51 g $COD/m^2.d$) for achieving COD removal, whereas the 2nd stage can be used at low OLR for nitrification.

2. In subtropical areas (average winter time wastewater temperature > 10 °C and summer time wastewater temperatures > 20 °C with an UASB effluent COD of 150-200 mg/l ,
- the required HRT for single stage RBC system should be >2.5 h and the organic loading rate should not exceed 20 g COD $_{total}$ $/m^2.d$ in order to achieve a residual COD value of 85 mg/l
- for complete ammonia and a better E.Coli removal a 2nd RBC stage is needed.

3. In moderate climate areas (average sewage temperature can occasionally (a few days) drop down to values as low as 4 °C in winter while they can raise to 20 °C in the summer, The performance of conventional UASB reactors will be rather poor during wintertime. The results of our investigations with a poor quality anaerobic effluent revealed that,
- the required HRT for a single stage RBC system should be prolonged to 5 h and the imposed OLR reduced to values below 13 $gCOD/m^2.d$ in order to achieve a residual effluent COD value of 76 mg/l.
- the single stage RBC system can be operated in different ways, i.e. during summer time with temperatures around 20 °C and better UASB effluent quality, the single stage system can be operated at higher OLR and lower HRT.
- A two stages RBC can be operated in several ways: 1) at HRT of 2.5 h and OLR of 24 g $COD/m^2.d$ to achieve a highly efficient removal of COD. 2) at HRT > 5 h and OLR < 13 $gCOD/m^2.d$.for achieving up to 67% ammonia removal and up to 99% removal of E.Coli, 3) at HRT > 10 h and OLR < 6.5 $gCOD/m^2.d$ for a complete ammonia removal and up to 99.5 % E.Coli removal

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SAMENVATTING, SLOTDISCUSSIE, CONCLUSIES EN AANBEVELINGEN

Steeds vaker wordt onderkend dat voor de bescherming van het milieu en de terugwinning van grondstoffen anaërobe zuiveringstechnologie zou moeten worden toegepast (Lettinga, 1997). Anaërobe zuivering, eventueel in combinatie met andere methoden, is een duurzaam proces dat niet alleen toepasbaar is in ontwikkelingslanden maar ook in geindustrialiseerde landen. Het proces wordt in trposche landen met succes voor de behandeling van huishoudelijk afvalwater toegepast en ook in subtropische en gematigde regio's zijn bemoedigende resultaten geboekt.

Anaërobe zuivering is vooral geschikt voor de verwijdering van organische stof (uitgedrukt als chemische zuurstofverbruik oftewel CZV). Indien eventueel resterende CZV, ammonium-stikstof, S^{2-} en pathogenen moeten worden verwijderd is daar een geschikte nazuivering voor vereist. Door verschillende onderzoekers (van der Steen *et al.*, 1998 en 1999, Augusto *et al.*, 2000, Frassinetti *et al.*, 2000, El-Gohary, 1998) is onderzocht of hier algenvijvers voor kunnen worden gebruikt. Echter, deze vijvers hebben als belangrijke nadelen dat zij veel oppervlak in beslag nemen, er veel overbodig slib wordt geproduceerd, door verdamping veel water verloren gaat, lange verblijftijden nodig zijn en gedurende de winter de verwijderingspercentages laag zijn.

Er bestaat een toenemende belangstelling om biofilmsystemen te gebruiken voor de nabehandeling van anaëroob effluent, zoals oxidatiebedden (Bovendeur *et al.*, 1990), ondergedompelde biofilters (Goncalves *et al.*, 1998 en 1999) granulaire biofilters, gefluïdiseerde systemen (Collivignarelli, 1990), enzovoort. Elk van deze systemen heeft zijn specifieke voor- en nadelen. Zo moeten oxidatiebedden groot worden uitgevoerd omdat het te behandelen water anders niet goed over het gehele oppervlak verdeeld kan worden. Statische granulaire biofilters worden discontinue bedreven omdat ze regelmatig moeten worden teruggespoeld. Gefluïdiseerde reactoren zijn hydraulisch nogal instabiel. Volgens Bovendeur *et al.* (1990) is een biorotor daarentegen een uitstekend systeem voor de nabehandeling van effluent van UASB reactoren. Biorotoren zijn compact, staan lange slibleeftijden en hoge volumetrische belastingen toe en het energieverbruik is laag. Daarnaast zijn biorotoren eenvoudig te bedienen en gedraagt het zuiveringsproces zich erg stabiel.

Gezien deze voordelen werd in het kader van de studie die in dit proefschrift wordt beschreven de toepassing van biorotoren voor de nazuivering van verschillende

kwaliteiten UASB effluent onderzocht. De de nadruk lag hierbij vooral op de verwijdering van CZV, ammonium-stikstof en E.Coli.

Hoofdstuk 2 beschrijft onderzoek naar de wijze waarop in een biorotor E. Coli uit UASB effluent wordt verwijderd. De resultaten van een aantal verkennende batchexperimenten gaven aan dat deze verwijdering volgens een eerste-orde kinetiek verloopt. Daarnaast werd het effect van factoren zoals roersnelheid, het optreden van sedimentatie, de zuurstofconcentratie, de pH, het type dragermateriaal en de toediening van een kationisch polymeer onderzocht. E. Coli wordt waarschijnlijk verwijderd door achtereenvolgende adsorptie aan de biofilm en sedimentatie. Volgens Omura *et al.* (1989) heeft bacteriële afsterving slechts een geringe invloed op de verwijdering. Tevens werd geconstateerd dat in een aërobe biorotor (zuurstofconcentraties variërend tussen 3.3 en 8.7 $mg \cdot l^{-1}$) veel hogere verwijderingsrendementen voor E. Coli mogelijk zijn dan in een anaërobe biorotor. Binnen een pH-bereik van 6.5 tot 9.3 werd geen significante invloed van de pH op de verwijdering van E. Coli geconstateerd. Het effect van het type dragermateriaal op E. Coli verwijdering manifesteert zich waarschijnlijk pas zodra er een biofilm is ontwikkeld, hetgeen overeenkomt met resultaten van Ueda en Horan (2000). De toediening van een kationisch polymeer resulteerde in een snellere adsorptie en daarmee in een verbeterde E. Coli verwijdering.

In **hoofdstuk 3** worden de prestaties van een anaërobe en aërobe biorotor met elkaar vergeleken voor de behandeling van UASB effluent. Beide biorotoren werden met een hydraulische verblijftijd (HVT) van 2.5 h en een organische belasting (OB) van 14.5 g $CZV \cdot m^{-2} \cdot d^{-1}$ bedreven. Uit de resultaten bleek dat de aërobe biorotor veel beter presteerde (respectievelijk 56 en 28% verwijdering van totaal en opgelost CVZ) dan de anaërobe biorotor (respectievelijk 23 en 14% verwijdering van totaal en opgelost CZV). Het meest opvallende was het hoge rendement van de aërobe biorotor voor colloïdaal CZV (90%). In de anaërobe biorotor daarentegen trad geen verwijdering van colloïdaal CZV op maar werd zelfs wat colloïdaal materiaal geproduceerd (16%). Blijkbaar kan een aërobe biorotor colloïdaal materiaal invangen maar wordt in een anaërobe biorotor een deel van het gesuspendeerd materiaal uit het afvalwater (door hydrolyse) in colloïdaal materiaal omgezet. Niet alleen CZV maar ook E. Coli werd in de aërobe biorotor veel beter verwijderd dan in de anaërobe biorotor.

De installatie van een anaërobe biorotor vóór de aërobe biorotor verhoogde de verwijdering van totaal CZV slechts met 9% en gaf geen significante verbetering van de verwijdering van E. Coli en ammonium.

Hoofdstuk 4 beschrijft de resultaten van experimenten waarin de prestaties van één enkele biorotor worden vergeleken met de prestaties van twee biorotoren die in serie zijn geschakeld. In beide gevallen werd effluent van een UASB reactor behandeld.

Omdat deze UASB bij 30 °C werd bedreven was dit effluent van redelijk goede kwaliteit. Beide systemen werden belast met 14.5 g CZV·m^{-2}·d^{-1} en een HVT van 2.5 uur. Om dit te bewerkstelligen waren de debieten verschillend, respectievelijk 0.587 en 1.152 m^3·d^{-1} voor de eentraps en tweetraps biorotor. De temperatuur in het eentrapssysteem was wat hoger (24 °C) dan in het tweetrapssysteem (17 °C). De systemen produceerden dezelfde effluentkwaliteit met betrekking tot totaal CZV (72 mg·l^{-1}), gesuspendeerd CZV (16 mg·l^{-1}), colloïdaal CZV (5 mg·l^{-1}) en opgelost CZV (51 mg·l^{-1}). Ook het verwijderingspercentage van E. Coli was in beide gevallen gelijk (94%). Echter, de verwijdering van ammonium in het eentrapssysteem (50% waarvan 71% door nitrificatie), was aanzienlijk beter dan in het tweetrapssysteem (23%). Waarschijnlijk kan het verschil worden verklaard door de hogere temperatuur in het eentrapssysteem. Indien onder tropische omstandigheden het effluent van een UASB reactor moet worden ontdaan van CZV en E. Coli en een gedeeltelijke verwijdering van ammonium wordt nagestreefd, bevelen we op basis van de resultaten aan om daar een eentraps biorotorsysteem voor te gebruiken.

De mogelijkheid om stikstof te verwijderen werd onderzocht door het effluent van een tweetraps biorotor over een opwaarts doorstroomde denitrificatiereactor te recirculeren. Indien het effluent van de UASB reactor weinig biodegradeerbare CZV bevatte bleek dat een deel van het gerecirculeerde nitraat werd omgezet in ammonium. Omdat dit betekent dat ammonium tweemaal moet worden genitrificeerd zonder dat denitrificatie optreedt lijkt onder tropische omstandigheden de implementatie van een denitrificatiereactor niet erg zinvol te zijn.

In **hoofdstuk 5** werd de UASB reactor bij twee verschillende temperaturen bedreven, 30 en 11 °C, hetgeen resulteerde in zeer verschillende effluentconcentraties biodegradeerbare CZV. Het anaërobe effluent werd behandeld in twee verschillende biorotoren met dezelfde HVT (2.5 h). Door de verschillen in de kwaliteit van het anaërobe effluent behandeleden ze een verschillende OB van 17.7 g CZV·m^{-2}·d^{-1} bij 30 °C en 36.8 g CZV·m^{-2}·d^{-1} bij 11 °C. Voor alle belangrijke parameters (totaal CZV, gesuspendeerde CZV, colloïdale CZV en E. Coli) leverde het laagbelaste systeem een aanzienlijk betere prestatie dan het hoogbelaste systeem. Daarnaast bleek het slib van het laagbelaste systeem beter gestabiliseerd te zijn (VSS/SS=0.63) dan het slib van het hoogbelaste systeem (VSS/SS=0.74).

Daarnaast werd de prestatie van een tweetraps biorotor voor de behandeling van het hierboven genoemde anaërobe effluent onderzocht. De HVT was constant 2.5 h maar er werd een OB tussen 9 en 18 g CZV·m^{-2}·d^{-1} toegepast. Deze verschillen in belasting gaven geen verschillen in de effluentconcentratie CZV. Echter, bij hoge belasting was de concentratie E. Coli in het effluent (3.4×10^5 per 100 ml) hoger dan bij lage belasting (7.6×10^4 per 100 ml). Bovendien bleek de nitrificatiesnelheid in de tweede trap te dalen van 1.6 naar 1.1 g NO_3-N·m^{-2}·d^{-1} zodra de belasting met biodegradeerbare CZV werd

verhoogd van 11.3 naar 16 $g \cdot m^{-2} \cdot d^{-1}$. Dit geeft aan dat, zoals verwacht, bij een betere werking van de UASB en daarmee een lagere concentratie aan biodegradeerbare CZV in het anaërobe effluent, de nitrificatie veel efficiënter verloopt. Dus kan, afhankelijk van de behoefte aan nitrificatie, een tweetraps biorotor voldoen voor de nabehandeling van UASB effluent van slechte kwaliteit. Bij een OB van 18 $g \cdot m^{-2} \cdot d^{-1}$ en een HVT 2.5 h worden alle CZV fracties redelijk goed verwijderd. Overigens is het de verwachting dat dit ook bij aanzienlijk hogere OBen nog het geval is.

Tevens werd in hoofdstuk 5 de tweede trap van een tweetraps biorotor gedurende 9 dagen als een eentraps systeem bedreven door deze direct met UASB effluent te voeden. Het achterliggende idee was om het effect van een plotselinge verhoging van de OB op de nitrificatie te onderzoeken. Nadat gedurende 9 dagen de reactor op deze manier bij een OB van 13.2 g biodegradeerbare $CZV \cdot m^{-2} \cdot d^{-1}$ was bedreven, werd de aanvoer naar de reactor gedurende 7 dagen onderbroken. Hierna werd de reactor weer gewoon als tweede trap van een tweetraps biorotor systeem in gebruik genomen. De resultaten lieten zien dat direct na de verhoging van de OB de ammoniumconcentratie binnen 3 uur in het effluent toenam tot 28 $mg \cdot l^{-1}$. Nadat de oorspronkelijke belasting van 7 g biodegradeerbare $CZV \cdot m^{-2} \cdot d^{-1}$ was hersteld, herstelde de nitrificatie zich binnen 2 tot 3 dagen en daalde de ammoniumconcentratie in het effluent naar 8 $mg \cdot l^{-1}$. De bijbehorende nitrificatiesnelheid was 1.5 g $NO_3\text{-}N \cdot m^{-2} \cdot d^{-1}$. De resultaten laten duidelijk zien dat de nitrificatie snel herstelt na een stressperiode van achtereenvolgens een verhoogde OB en een periode zonder voeding.

Het onderzoek in **hoofdstuk 6** was gericht op de nabehandeling van het effluent van een UASB reactor die werd bedreven bij lage temperaturen (11-14 °C). Als gevolg van de lage temperatuur was de kwaliteit van dit effluent relatief slecht. De nazuivering van het UASB effluent met een eentraps biorotor werd vergeleken met de behandeling door een tweetraps biorotor. Hierbij stonden CZV en E. Coli verwijdering en nitrificatie centraal. Beide systemen werden bij een hoge en een lage OB bedreven. De effluentconcentraties totaal CZV, gesuspendeerd CZV en colloïdaal CZV waren lager voor het tweetrapssysteem dan voor het eentrapssysteem. Ook de nitrificatie in het tweetrapssysteem verliep aanzienlijk beter. Effluentconcentraties nitraat in het tweetrapssysteem waren 26 $mg \cdot l^{-1}$ bij lage OB en 7 $mg \cdot l^{-1}$ bij hoge OB. In het eentrapssysteem waren deze 5.3 $mg \cdot l^{-1}$ bij lage OB en 1.7 $mg \cdot l^{-1}$ bij hoge OB. In de eerste trap van het tweetrapssysteem domineren heterotrofe bacteriën en in de tweede trap autotrofe micro-organismen. Ook met betrekking tot de verwijdering van E. Coli gaf het tweetrapssysteem veel betere resultaten dan het eentrapssysteem. Gebaseerd op deze resultaten wordt aanbevolen om een tweetraps biorotor te gebruiken voor de nabehandeling van UASB reactoren die bij lagere temperaturen worden bedreven, vooral indien niet allen CVZ-verwijdering maar ook nitrificatie en E. Coli verwijdering belangrijk zijn.

De tweetraps biorotor werd bij een aantal verschillende OBen en HVTen bedreven. De verwijderingspercentages voor totaal CZV, gesuspendeerd CZV en colloïdaal CZV verbeterden licht bij een langere verblijftijd en lagere belasting. De nitrificatie en E. Coli verwijdering werden negatief beïnvloed door hogere belastingen.

Het grootste deel van de E. Coli die in de gesuspendeerde stof (> 4.4 μm) was opgenomen werd door bezinking verwijderd of door adsorptie vastgelegd in de biofilm van de eerste trap van een tweetraps biorotor (99.7%). Echter, E. Coli die zich in de colloïdale fractie (<4.4 - > 0.45 μm) bevindt werd voornamelijk in de tweede trap verwijderd (99.8%).

Wederom laten de resultaten zien dat het tweetrapssysteem de beste oplossing lijkt voor de nabehandeling van UASB effluent, ook als dat van slechte kwaliteit is. Voor CZV verwijdering wordt een OB van 24 g $CZV \cdot m^{-2} \cdot d^{-1}$ en een HVT van 2.5 uur aanbevolen. Voor gecombineerde CVZ verwijdering en nitrificatie wordt een OB van 6.5 g $CZV \cdot m^{-2} \cdot d^{-1}$ en een HVT van 10 uur aanbevolen.

In de tweetraps biorotor werd ook onderzoek gedaan naar het effect van een hydraulische schokbelasting op de CZV verwijdering, nitrificatie een E. Coli verwijdering. Een verlaging van de HVT van 5.0 naar 1.6 h, oftewel een verhoging van de OB van 13.7 naar 52 g $CZV \cdot m^{-2} \cdot d^{-1}$, had geen zichtbaar effect op de CZV verwijdering. Echter, de concentratie ammonium in het effluent nam toe en ook de verwijdering van E. Coli verslechterde. Nadat de oorspronkelijke HVT en OB waren hersteld, keerden zowel de nitrificatie als de E. Coli verwijdering respectievelijk binnen 3 en 24 h op hun oorspronkelijke niveau terug. Dit geeft aan dat het tweetrapssysteem relatief ongevoelig is voor schokbelastingen.

Ondanks de goede resultaten is het effluent van de tweetraps biorotor nog niet geschikt voor irrigatiedoeleinden omdat de concentratie E. Coli nog steeds hoger is dan de zeer strenge eis die door de WHO wordt voorgeschreven. In **hoofdstuk 7** werd daarom een drietraps biorotor onderzocht voor de behandeling van UASB effluent van matige kwaliteit. Bij een HVT van 3 h werd nog steeds niet aan de E. Coli eis voldaan, maar bij een HVT van 10 h was dit bijna het geval. Gezien de grote investeringen die moeten worden gedaan om aan de WHO eisen te voldoen en de grote waterschaarste die momenteel heerst, moet serieus worden afgevraagd of in de praktijk wel geprobeerd moet worden om aan deze eisen te voldoen.
Tevens is in hoofdstuk 7 het effect van recirculatie op de verwijdering van E. Coli in het drietrapssysteem onderzocht. De recirculatie geeft een duidelijke verbetering van de E. Coli verwijdering in de eerste trap. Ook het effluent van het hele systeem vertoont een daling zien van de concentratie E. Coli van 0.2×10^4 naar 9.8×10^{-2} per 100 ml effluent. Dit laatste getal voldoet aan de WHO eis (1989) voor onbeperkte irrigatie.

CONCLUSIES EN AANBEVELINGEN

1. Voor tropische regio's met gemiddelde afvalwatertemperaturen (22-30 °C) voldoet anaërobe zuivering uitstekend, aangenomen dat het systeem goed is ontworpen. De resultaten van deze studie hebben laten zien dat onder die omstandigheden:

- met een eentraps biorotor lage effluent CZV concentraties bereikt kunnen worden (< 70 $mg \cdot l^{-1}$) en bij een OB van 14.5 g $CZV \cdot m^{-2} \cdot d^{-1}$ en een HVT van 2.5 h gedeeltelijke ammonium- en E. Coli verwijdering mogelijk zijn.
- een tweetraps biorotor nodig is om volledige nitrificatie en goede E. Coli verwijdering te bewerkstelligen. De eerste trap kan bij hoge OB (> 51 g $CZV \cdot m^{-2} \cdot d^{-1}$) bedreven worden ten behoeve van CZV verwijdering en de tweede trap bij een lagere OB zodat nitrificatie optreedt.

2. In subtropische regio's, met een gemiddelde afvalwatertemperatuur in de winter hoger dan 10 °C en in de zomer hoger dan 20 °C, zal het UASB effluent een CZV concentratie hebben van 150-200 $mg \cdot l^{-1}$. Onder die omstandigheden:

- moet de HVT voor een eentraps biorotor groter zijn dan 2.5 h en mag de OB niet hoger zijn dan 20 g $CZV \cdot m^{-2} \cdot d^{-1}$ om een effluent CZV concentratie van 85 $mg \cdot l^{-1}$ te kunnen bewerkstelligen.
- is voor volledige nitrificatie en een goede E. Coli verwijdering een tweetraps biorotor vereist.

3. Onder gematigd klimatologische omstandigheden, waarbij de afvalwatertemperatuur in de zomer hoger kan zijn dan 20 °C maar de UASB reactor soms slecht zal presteren omdat in de winter de temperatuur een aantal dagen achter elkaar lager is dan 4 °C, geld dat:

- de HVT van een eentraps biorotor verlengd moet worden tot 5 h en de OB moet worden verlaagd naar 13 g $CZV \cdot m^{-2} \cdot d^{-1}$, zodat een effluent CZV concentratie van 76 $mg \cdot l^{-1}$ wordt verkregen.
- in de zomer bij temperaturen rond 20 °C een eentraps biorotor systeem bij een kortere HVT en een hogere OB kan worden bedreven dan in de winter.
- een tweetraps biorotor op verschillende manieren worden bedreven : 1) bij een HVT van 2.5 h en een OB van 24 g $CZV \cdot m^{-2} \cdot d^{-1}$ zodat een goede verwijdering van CZV wordt verkregen en 2) bij een HVT van meer dan 5 h en een OB lager dan 13 g $CZV \cdot m^{-2} \cdot d^{-1}$ zodat 67% ammonium wordt verwijderd en 99% E. Coli of 3) bij een HVT die groter is dan 10 h en een OB die lager is dan 6.5 g $CZV \cdot m^{-2} \cdot d^{-1}$ ammonium volledig wordt verwijderd en meer dan 99.5% E. Coli verwijdering mogelijk is.

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