Fixed-Biofilm Reactors applied to Waste Water Treatment and Aquacultural Water Recirculating Systems

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H. C. van der Plas,
in het openbaar te verdedigen
op woensdag 22 november 1989
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

STELLINGEN

1. Degelijk research werk verdient eerder toepassing in de praktijk dan herhaalde navolging door andere onderzoeksinstellingen.

2. Hoewel het ½-orde/0-orde kinetiek model goed werkbaar is gebleken voor biofilm reactoren, heeft Harremoës ten onrechte de resultaten van Pöpel gebruikt om de toepasbaarheid van het model aan te tonen bij de biologische zuivering van afvalwater dat een mengsel bevat van opgeloste en gesuspendeerde organische stof.

   Harremoës, P. (1978). Biofilm kinetics. In: Water Pollution Microbiology, R. Mitchell (Ed), Vol.2, Wiley, New York, 71-109.

3. Gezien de doorgaans stringente eisen die aquatische organismen stellen aan hun leefmilieu, gecombineerd met een directe uitscheiding van afvalstoffen in het omringende medium, verdient de productie van dierlijk eiwit in aquatische recirculatiesystemen, vanuit milieuhygienisch standpunt, verre de voorkeur boven de huidige productiesystemen voor landbouwhuisdieren.

4. Gelet op de huidige waterkwaliteit en het geringe uitzicht op een spoedige verbetering daarvan, kan de doelstelling "in 2000 weer zalm in de Rijn" het meest effectief bereikt worden door het frequent uitzetten van deze vissoort.

5. De door Rusten geponeerde hyperbolische functie ter beschrijving van CZV-eliminatie in een ondergedompeld filter dient te worden beschouwd als een toevallige "overall" beschrijving van gelijktijdig verlopende processen met 0-orde, ½-orde en 1-orde kinetiek.

   Rusten, B. (1984). Wastewater treatment with aerated submerged biological filters. J. Water Pollut. Control Fed., 56, 424-431.

6. Vanuit het standpunt dat over de problematiek van het vóórkomen van zware metalen in het milieu niet te licht gedacht, doch hieraan ook niet te zwaar getild dient te worden, biedt de huidige normering onvoldoende mogelijkheden voor een zinvolle sanering van verontreinigde locaties.

7. Gezien de huidige overbevissing van de Noordzee en de marktpositie van kweekvis, wordt de gevangen vis te duur en de gekweekte vis niet duur genoeg betaald.

8. In het vakgebied van de waterzuivering dient de nodige voorzichtigheid betracht te worden met het gebruik van oude zegswijzen. Zo schuurt zand vooral het riool en laten de meeste organische afvalstoffen zich zeer goed in een vat verzuren.

9. Het drastisch beperken van de mogelijkheden voor grensoverschrijdende afvaltransporten staat op gespannen voet met de Europese éénwording in 1992 en kan voor specifieke afvallen een doelmatige verwerking in de weg staan.

Wet chemische afvalstoffen, art. 16. Wetswijziging 7 juli 1988, Stb. 331, I.w.tr. 15 oktober 1988, Stb. 470.

Schot, J. van der (1989). De metamorfose van de oliegiganten. Nieuwe Beta, 8, 1-2.

10. Flexibiliteit is een rekbaar begrip.

Stellingen behorende bij het proefschrift "Fixed-biofilm reactors applied to waste water treatment and aquacultural water recirculating systems" van J. Bovendeur

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- Water treatment in aquacultural water recirculating systems,

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ABSTRACT

Bovendeur, J. (1989). Fixed-biofilm reactors applied to waste water treatment and aquacultural water recirculation systems. Thesis, Wageningen Agricultural University, Wageningen, The Netherlands, 168 p.

Fixed-biofilm waste water treatment may be regarded as one of the oldest engineered biological waste water treatment methods. With the recent introduction of modern packing materials, this type of reactor has received a renewed impuls for implementation in a wide field of water treatment.

In this thesis the possibilities are presented for fixed-film post-treatment of anaerobically digested domestic sewage and water reconditioning in aquacultural water recirculation systems. Emphasis is put on the nitrification process and removal processes for organic matter, using the ½-order/0-order kinetic model for fixed-biofilm processes as a starting point for the research.

With regard to waste water treatment biofilm COD removal kinetics for waste water, containing both suspended and dissolved organics, are discussed illustrating that the apparent kinetic order of the combined process and the reaction constant strongly depends on the ratio of suspended/dissolved COD. The overall conclusion was that a rotating biological contactor is especially suitable for this application, and that such reactor may be regarded as a reactor in which biochemical oxidation of dissolved organic substrate is combined with adsorption/flocculation processes of suspended organic matter.

The suitability of fixed-film reactors for application in aquacultural recirculation systems is demonstrated by the implementation of a nitrifying trickling filter in a pilot-scale recirculation system for the culture of the African catfish. The corresponding design philosophy behind this system is based on combining the waste production dynamics in the fish tank with the waste removal kinetics in the trickling filter. Attention is paid in this design philosophy to a) hydraulic loading of the primary clarifier, b) dimensions of the fixed-film reactor, c) water recirculation rate, and d) water exchange rate.

Refinements of the presented design philosophy can be found in anticipating the effects of simultaneous COD loading, dissolved oxygen levels, pH and salinity, which are discussed in separate chapters of this research.

It is concluded that the specific information generated in this thesis may be regarded as a valuable contribution to a more rational approach in design and operation of aerobic fixed-film reactors in the field of water treatment, as compared to the conventional, largely "folklore-based" approaches.

Key words _fixed-biofilm reactor, biological waste water treatment, nitrification, COD removal, removal mechanisms, reaction kinetics, design philosophy

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Backgrounds and applications

The term "fixed-biofilm reactor" may be regarded as a modern definition for one of the oldest engineered biological waste water treatment methods. The most widely used representative of this type of reactors is the trickling filter: low-rate trickling filters were first applied in the late 1800s in Europe and North America, using rock-packed filter media. During the last two decades light-weight plastic filter media combining a high specific surface area with a high porosity are succesfully introduced in trickling filtration as well as in rotating biological contacting. In the trickling filter the media is fixed, while the water flows over the attached biofilm simultaneously exposed to the ambient air, where in the rotating biological contactor the media rotates in the more or less stagnant water and air phase. The availability of those new biofilm attachment materials enabled a thorough modification of the conventional reactor construction and operation, overcoming many of the inherent disadvantages (e.g. low capacities and blocking of the filterbed) of the rock-packed treatment systems and thus raising again the popularity of the resulting high-rate fixed-biofilm waste water treatment reactors.

The most common application of fixed-film reactors is secondary treatment of industrial as well as domestic waste water, after a primary sedimentation step. Another application is upgrading aerobic secondary effluents, especially by rotating biological contactors (Marsh et al., 1981; Poon et al., 1981a,b). Since anaerobic treatment processes are succesfully applied as pretreatment, and may also possibly be applied to low strenght waste water (Lettinga et al., 1983), aerobic post-treatment of anaerobic effluents may become another important issue in waste water treatment, in order to meet the restrictions set for discharge of effluents. Waste water resulting from aquacultural activities are also succesfully treated by fixed-film systems

(Liao and Mayo, 1974), while even treatment of dissolved xenobiotica containing (industrial) waste water, leachates from sanitary landfills or groundwater, by fixed-film reactors is under investigation (Arvin and Harremoës, 1989). All these applications have in common that the pollutants are present in relatively low concentrations and have a low biomass yield. Therefore, in general a high cell residence time is required, which is one of the characteristic properties of fixed-film reactors.

Phenomena and modelling

However, for all possible applications there is a need for information on process mechanisms and kinetics, enabling a rational design and operation of the system. As a result of a design approach in the past, largely based on folklore, many emperical formules have been proposed, of which Roberts (1973) could list more than a dozen for trickling filtration for a 40 year period. "None of these comes even close to an adequate description of the phenomena involved, and they give very different results" (Harremoës, 1978). Therefore, more recently also conceptual biofilm models have been introduced, mathematically associating diffusional transport of dissolved substrates and reaction products with specific reaction kinetics in the active biofilm layer (Williamson and McCarty, 1976a,b; LaMotta, 1976a,b; Harremoës, 1978). The phenomena involved with fixed-biofilm performance are schematically illustrated in Fig. 1, on which Arvin and Harremoës (1989) commented: "The essential feature of this configuration is the need for the substrates and the reaction products as well to diffuse through the biofilm. This purely physical phenomenom has turned out to be crucial to the understanding of the performance of water purification in fixed-biofilm reactors". This statement has been elaborated thoroughly by the research group of Harremoës, combining the diffusion processes with reaction kinetics.

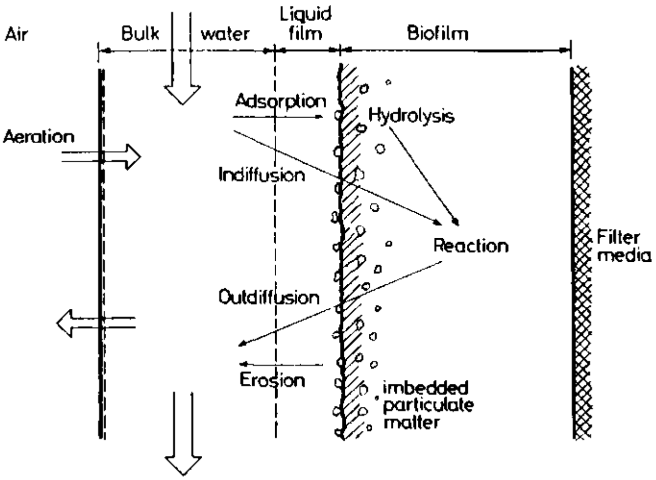

Fig. 1. Schematic illustration of the phenomena involved with fixed-biofilm performance (Harremoës and Gönenc, 1983)

After Arvin and Harremoës (1989): "In all modern descriptions of redox-processes performed by the active bacteria in the biofilm, the kinetics of substrate utilization is based on the gradual transition from first order to zero order kinetics as postulated by Monod and Michaelis-Menten. However, there is a marked difference in performance of the reactor depending on wether the substrate can penetrate the biofilm fully or partly (Harremoës, 1978). Starting from a constant diffusion coefficient and a constant volumetric reaction rate, the following remarks can be made on fixed-biofilm performance. There has to be a concentration gradient for the substrate enabling penetration of the biofilm by diffusion. If the removal rate per unit biofilm volume is high and the diffusion rate is low, the concentration of the substrate may reach zero and the substrate penetrates the film only partly: only a part of the biofilm is active and the rate of reaction per unit surface area of the biofilm is much lower (substrate diffusion limitation) than with a fully penetrated biofilm layer (metabolism or reaction limitation). It is the bulk concentration that determines the transition from fully to partly penetration. On the other hand, the diffusion resistance affects both the rate of removal and the order of the reaction, essentially as follows:

a first order reaction in the interior of the biofilm is converted into a first order bulk reaction at a reduced rate,

a zero order reaction in the interior of the biofilm remains a zero order bulk reaction if the biofilm is fully penetrated, but is converted into a half order bulk reaction if the biofilm is partly penetrated".

Harremoës (1978) showed that taking full account of the transition from first order to zero order kinetics as expressed by Monod and Michaelis-Menten, does not show significant differences for the practical range of applications. Therefore, under the reasonable simplification that for the common redox processes the intrinsic reaction is zero order, the combination of half order and zero order bulk reaction kinetics has been introduced as a useful approximation in describing fixed-biofilm mineralization of dissolved biodegradable organic matter, nitrification and denitrification (LaMotta 1976a,b; Harremoës, 1978, 1982; Riemer, 1978). The characteristic shape of a plot of substrate conversion rate per unit area versus substrate bulk concentration, according to half order/zero order kinetics, is shown in Fig. 2 for biofilm ammonia removal by nitrification.

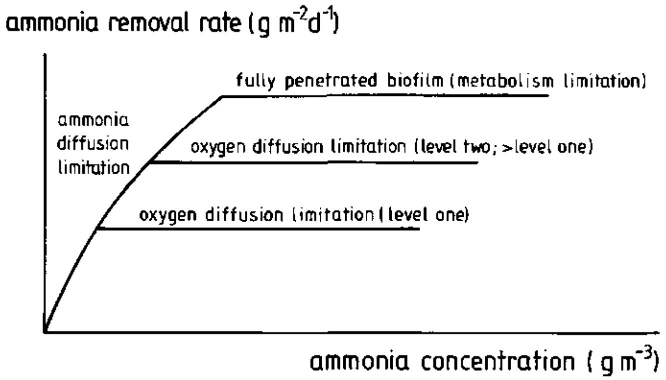

Fig. 2. Schematic illustration of the relation between the substrate removal rate per unit biofilm area and the concentration of substrates (both electron donor and acceptor), according to half order/zero order kinetics, applied to fixed-biofilm nitrification (after Jansen and Harremoës, 1984)

As a result of oxygen diffusion limitation at a relatively low level of dissolved oxygen (level one), zero order kinetics are met for ammonia concentrations exceeding a certain transition concentration. However, the ammonia removal rate per unit area can be increased, following the initial half order kinetics towards the ammonia concentration, by increasing the dissolved oxygen level in the bulk liquid (level two). In case both substrates are present in sufficiently high concentrations to ensure complete penetration of the biofilm, zero order kinetics are met as a consequence of metabolism limitation of the reaction.

This concept, further denoted as the ½-order/0-order kinetic model, plays a central role in this thesis. The model, however, is restricted to removal kinetics for truely dissolved substrates only, whereas most waste waters contain significant amounts of suspended substrate solids. Therefore, also attention is paid to the mechanisms of removal of particulate organic matter.

Aim

The aim of the present study is improvement and implementation of kinetic models in design and operation of fixed-biofilm reactors in the field of biological water treatment.

Scope and objectives

The scope of this thesis is focussed on the application of aerobic fixed-film reactors for removal and mineralization of organic matter and nitrification of ammonia nitrogen. Although the elimination of organic matter, both dissolved and suspended, plays an important role in waste water treatment, emphasis in the thesis is put on the nitrification kinetics of fixed-biofilm samples under various process conditions. It should be noted that the ½-order/0-order kinetic model already introduced, is adopted as a starting point in this research on biofilm performance.

The scope of this thesis is realized by research on:
-domestic waste water treatment, especially post-treatment of anaerobically digested domestic sewage,
-proces water treatment in aquacultural water recirculating systems.

The information generated for the treatment of these selected waste waters is judged to be applicable to the treatment of other relevant types of waste water.

The increasing importance of anaerobic (pre)treatment of waste water is the result of the specific benefits of the process, such as low requirements of energy and land area, and simple reactor construction. However, post-treatment of the anaerobic effluent will be required in many cases in order to meet the restrictions set for effluent discharge. The original benefits of the pre-treatment should be supported by the successive post-treatment; fixed-biofilm reactors are believed to meet this criterion. From a research point of view, studying fixed-biofilm post-treatment of anaerobic effluent provided a good possibility to focus on the mechanisms and kinetics of the removal of dissolved and particulate organic matter.

Since fisheries resources are world-wide reaching maximum levels of sustainable catches, while the demand of fish products still increases, aquaculture is becoming increasingly important as a means of meeting the expected future shortfall in supply of fisheries products. It is expected that the world aquaculture production will rise to 26 million tons by the end of the century, versus a present aquacultural production of about 10 million tons (Jones, 1986). Although about 75% of the worlds finfish-production is realized in fresh water, marine fish farming is growing rapidly in Japan and Europe and is expected to increase in importance. The issue of water treatment in aquaculture for this study was introduced by the Department of Fish Culture and Fisheries of the Wageningen Agricultural University. The required water reconditioning is a consequence of the ambition to realize a high degree of water and energy conservation by recirculation of the heated water in the culture of warm water fish species. Since the removal of ammonia was selected as the key to this problem, research on fixed-film nitrification is concentrated in this application. In combination with the introduction of new fish species suitable for aquaculture, such as the African catfish introduced by the Department of Fish Culture and Fisheries (Hogendoorn, 1983), and some already existing culture of the European eel and the rainbow trout, the introduction of a reliable water recirculating system is a further stimulation of the development of fish culture in the Netherlands, especially the culture of warm water species.

Notes on thesis structure

The chapters in this thesis are all presented as independent contributions, each of which forms a part that can be read apart from the others. The presented chapters may be centred around the two applications: domestic waste water treatment (Chapter 2 and 3), and aquacultural process water treatment (Chapter 4,5,6 and 7). The coherency of the various chapters is indicated in the following review: In Chapter 2 introductory experiments on COD removal and nitrification are presented for post-treatment of anaerobic effluent using a trickling filter and rotating biological contactor on pilot-scale. Chapter 3 essentially concerns the mechanisms and kinetics of COD removal and mineralization, discussed for a rotating biological contactor biofilm sample loaded with domestic sewage and anaerobic effluent. The results are extrapolated for fixed-biofilm performance in general, and related to the ½-order/0-order kinetic model in combination with adsorption of particulate organic matter. In Chapter 4 a design philosophy is presented for aquacultural water recirculating systems, based again on the ½-order/0-order kinetic model for fixed-film nitrification; the feature of this chapter is the link between waste production dynamics and waste removal kinetics in relation to certain water quality requirements; emphasis is put on nitrification kinetics in trickling filters. The nitrification kinetics are further elaborated in Chapter 5,6 and 7, dealing with the effects of factors inherent to the proposed system configuration of Chapter 4: simultaneous organic matter elimination by the fixed-biofilm (Chapter 5 and 6, linked to Chapter 2), pH and dissolved oxygen levels (Chapter 6), and effect of salinity (Chapter 7), with respect to marine aquaculture systems. Finally, in Chapter 8 some of the observed phenomena are considered in a wider context, linking the experimental results of the various research parts and the applications concerned.

References

Arvin, E. and Harremoës, P., 1989. Concepts and models for biofilm reactor performance. In: Technical advances in biofilm reactors, International EWPCA-IAWPRC Conference, Nice, France, 4-6 April 1989, 191-212.

Harremoës, P., 1978. Biofilm kinetics. In: R. Mitchell (Editor), Water Pollution Microbiology, Vol. 2. Wiley, New York, NY, 71-109.

Harremoës, P., 1982. Criteria for nitrification in fixed film reactors. Water Sci. Technol., 14, 167-187.

Harremoës, P. and Gönenc, E.I., 1983. The applicability of biofilm kinetics to rotating biological contactors. In: Rotating biological discs, International EWPCA-IAWPRC Seminar, Feldbach, BRD, 1983, 19-39. Gesellschaft zur Förderung der Abwassertechnik, e.V., St. Augustin, BRD.

Jansen, I. la Cour and Harremoës, P., 1984. Removal of soluble substrates in fixed biofilms. Water Sci. Techn., 17, 1-41.

LaMotta, E.J., 1976a. Kinetics of growth and substrate uptake in a biological film system. Appl. envir. Microbiol., 31, 286-293.

LaMotta, E.J., 1976b. Internal diffusion and reaction in biological films. Envir. Sci. Technol., 10, 765-769.

Lettinga, G., Roersma, R. and Grin, P., 1983. Anaerobic Treatment of Raw Domestic Sewage at Ambient Temperatures Using a Granular Bed UASB Reactor. Biotech. & Bioeng., XXV, 1701-1723.

Liao, P.B. and Mayo, R.D., 1974. Intensified fish culture combining water reconditioning with pollution abatement. Aquaculture, 1, 317-335.

Marsh, D., Benefield, L., Bennet, E., Linstedt, D. and Hartman, R., 1981. Coupled trickling filter-rotating biological contactor nitrification process. J. Water Pollut. Control Fed., 1469-1480.

Poon, C.P.C., Chin, H.K., Smith, E.D. and Mikuchi, W.J., 1981a. Upgrading with rotating biological contactors for BOD removal. J. Water Pollut. Control Fed., 53, 474-481.

Poon, C.P.C., Chin, H.K., Smith, E.D. and Mikuchi, W.J., 1981b. Upgrading with rotating biological contactors for ammonia nitrogen removal. J. Water Pollut. Control Fed., 53, 1158-1165.

Riemer, M., 1977. Kinetics of denitrification in submerged filters. Thesis, Technical University of Denmark, Lyngby, Denmark, 213 pp.

Roberts, J., 1973. Towards a better understanding of high rate biological film flow reactor theory. Wat. Res., 7, 1561-1588.

Williamson, K. and McCarty, P.L., 1976a. A model of substrate utilization by bacterial films. J. Water Pollut. Control Fed., 48, 9-24.

Williamson, K. and McCarty, P.L., 1976b. Verification studies on the biofilm model for bacterial substrate utilization. J. Water Pollut. Control Fed., 48, 281-296.

CHAPTER 2

AEROBIC POST-TREATMENT OF ANAEROBICALLY DIGESTED MUNICIPAL WASTE WATER BY A TRICKLING FILTER AND A ROTATING BIOLOGICAL CONTACTOR

In: Anaerobic Treatment a Grown-Up Technology, Proc. Int. EWPCA-NVA Conference, 15-19 September 1986, Amsterdam, The Netherlands, p. 385-397. Industrial Presentations (Europe) B.V., Schiedam, The Netherlands

AEROBIC POST-TREATMENT OF ANAEROBICALLY DIGESTED MUNICIPAL WASTE WATER BY A TRICKLING FILTER AND A ROTATING BIOLOGICAL CONTACTOR

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ABSTRACT

In an introductory study two types of aerobic fixed-biofilm reactors have been used on pilot-scale for post-treatment of anaerobically digested domestic waste water: a trickling filter and a rotating biological contactor, both in two-stage systems. Emphasis in the post-treatment is put on additional removal of organic matter from the anaerobic effluent and ammonium removal by nitrification. Both types of reactor showed reasonable performance for both processes, where the rotating biological contactor showed higher average removal rates per unit area than the trickling filter: circa 35 g $m^{-2}d^{-1}$ COD and 5 g $m^{-2}d^{-1}$ respectively for the removal of COD, and 2.4 g $m^{-2}d^{-1}$ NH_4-N and 0.3 g $m^{-2}d^{-1}$ respectively for the nitrification. Concerning the removal of organic matter, distinction is made between dissolved COD and particulate COD, indicating that different mechanisms are responsable for removal from the water phase. As a result it has to be concluded that significant amounts of non-biodegradable particulate organic matter may be removed, especially by the rotating biological contactor. Since significant nitrification is observed in the last stages or compartments of the systems, the interfering role of COD removal in the nitrification process is illustrated once more.

Key words _biological waste water treatment, COD removal,
nitrification, fixed-film reactor, post-treatment of
anaerobic effluents

INTRODUCTION

Anaerobic digestion has proven to be a feasible treatment of medium and high strength agro-industrial waste water (Lettinga, 1978). Recent research work, however, demonstrates that anaerobic treatment processes are also successfully applicable at ambient temperatures to low strength waste water, e.g. domestic sewage, in tropical areas (Louwe Kooymans et al., 1985; Schellinkhout et al., 1985) as well as under moderate climatological conditions (Grin et al., 1983; Lettinga et al., 1983; Genung et al., 1985). On the other hand, anaerobic waste water treatment generally should be considered as a pretreatment, i.e. a supplementary (aerobic) treatment is required to meet the restrictions (in terms of oxygen demands and ammonium levels) set for discharge of effluents into receiving waters (Lettinga et al., 1981; Souza et al., 1984; Louwe Kooymans et al., 1985; Yoda et al., 1985). Therefore, the benefits of anaerobic waste water treatment can only fully be realized if its post-treatment is also reliable, simple to construct and does not involve high energy costs. When the desired post-treatment must be constructed in a limited land area, a high hydraulic loading rate is required. In such cases fixed-film processes are especially suitable for post-treatment of low strength anaerobic effluents, because high cell residence times are easily maintained. For these reasons a trickling filter and a rotating biological contactor have been selected for research on post-treatment of anaerobically digested municipal waste water, with emphasis on the supplementary removal of COD and Kjeldahl-N, especially the conversion of ammonium nitrogen by nitrification.

EXPERIMENTAL

Apparatus and operation

Trickling filter

The main structure of the trickling filter installation included two plexiglass columns and a final settling tank. The two columns had a square cross-sectional area of 0.16 m^2 and heights of 4.3 m and 2.3 m respectively, providing a total effective filter volume of 0.86 m^3. A lightweight plastic filter medium has been employed (Filterpack CR50, Mass Transfer International, Heversham, Cumbria, U.K.), having a specific surface area of about 200 m^2m^{-3}, when packed in the described columns. Both columns were equipped with a liquid distributor device at the top and sampling ports at intervals of 1 m. The columns were connected in series and could be seperated by an intermediate settling tank. The attached biomass was provided with air-oxygen by forced downflow aeration at a constant flow rate of 4.7 m^3h^{-1}. The waste water flow rate was kept at a constant value of 0.225 m^3h^{-1}, while the effluent recirculation flow rate has been changed once during the experimental period (7 months), to obtain sufficiently high surface loading rates (superficial liquid velocity) of the trickling filters, viz. 2.9 and 5.6 $m^3m^{-2}h^{-1}$. The surface loading rate of the final settling tank was 0.3 $m^3m^{-2}h^{-1}$ and 1 $m^3m^{-2}h^{-1}$ for the settling tank in between the trickling filter columns, when in operation. A schematic diagram of the main items of the trickling filter installation is given in Fig. 1a.

Rotating biological contactor (RBC)

The main arrangements of the rotating biological contactor installation were two identical units connected in series, with settling tanks following each unit (Fig. 1b). Both rotating biological contactor units contained 10 polystyrene-foam disks providing a total effective surface of 5.2 m^2 of which 35% was constantly submerged. The disks were mounted on a steel shaft, rotating at 12-18 rpm in a tank containing 0.036 m^3 waste water. The first unit was loaded during the experimental period (8 months) at waste water flow rates up to 0.15 m^3h^{-1}. The performance of the second unit has been examined during 2.5 month, receiving up to 0.013 m^3h^{-1} of the settled first stage effluent, resulting in both low COD and hydraulic loading rates. The maximum surface loading rates of the first and second stage settling tanks were 3 and 0.4 $m^3m^{-2}h^{-1}$ respectively.

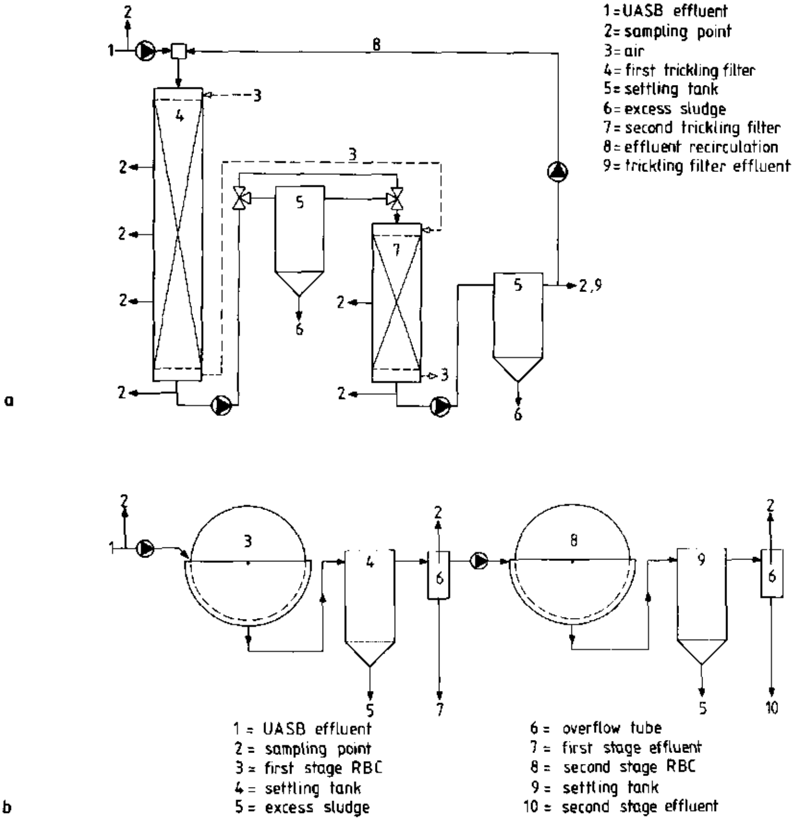

Fig. 1. Schematic diagrams of the post-treatment installations, a. trickling filter, b. rotating biological contactor

Waste water characteristics

All experiments were carried out with anaerobically pretreated raw domestic sewage of the village Bennekom, which has a combined sewer system. The anaerobic pretreatment was performed by a 6 m^3 upflow anaerobic sludge blanket (UASB) reactor, which has been described in detail by Grin et al. (1983, 1985). The UASB effluent can be characterized by concentrations of about 300 g m^{-3} total COD (range 100-500 g m^{-3}), 200 (60-300) g m^{-3}

dissolved COD, 60 (15-90) g m^{-3} Kjeldahl-N, 55 (12-80) g m^{-3} NH_4-N and a COD/BOD_5 ratio of 2.4-2.9. The complete wastewater treatment was conducted in an experimental hall at the ambient temperatures of the sewage resulting in wastewater temperatures during the post-treatment experiments varying from 12-20°C.

Sampling and analyses

The sampling points are indicated in Fig. 1. After a start-up period of 14 days the performance of the trickling filter installation was examined by daily grab samples of the UASB effluent, trickling filter effluent and 21 profile measurements. During the main experimental period, the rotating biological contactor plant performance was followed by analyses on 24 h composite samples stored at 4°C. This period was followed by a period during which daily analyses were made on grab samples.

The analyses of COD, Kjeldahl-N, ammonium, nitrite, nitrate, sulphide and sulphate were carried out according to Standard Methods. Dissolved COD was determined after filtration (Schleiden & Schuell paper filters 7.4 μm) and dissolved oxygen was measured using an electrode.

RESULTS AND DISCUSSION

COD removal rates

The COD loading rate of the complete trickling filter (without an intermediate settling tank) showed values of 1-4 kg $m^{-3}d^{-1}$ (5-20 g $m^{-2}d^{-1}$), due to fluctuating COD concentrations of the UASB effluent, whereas the first stage rotating biological contactor was loaded at rates ranging from 30-400 g $m^{-2}d^{-1}$ as a result of both fluctuating UASB effluent concentrations and varying UASB effluent flow rates. Note that the figures for the trickling filter rates in g $m^{-2}d^{-1}$ are accounted for the total provided medium surface area. The COD removal rates in relation to these loading rates are given in Fig. 2. Generally, the trickling filter removed about 50-60% of the COD-load (Fig. 2a), and occasionally showed very poor COD removal efficiences. No distinction was noticed between the employed hydraulic surface loading rates of the trickling filter. For COD loading rates ranging from 30-80 g $m^{-2}d^{-1}$ the first stage rotating biological contactor showed COD removal efficiencies almost similar to those of the trickling filter (Fig. 2b).

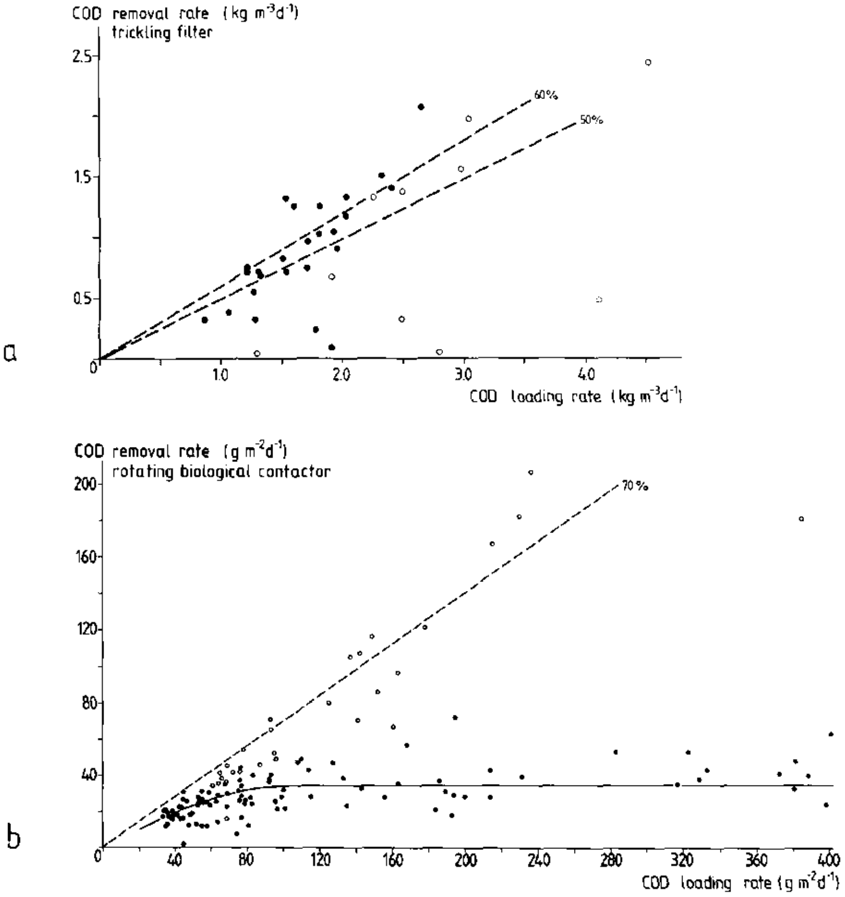

Fig. 2. Total-COD removal rate versus total-COD loading rate, a. trickling filter, b. first stage rotating biological contactor. UASB effluent total-COD/dissolved-COD: ○ ratio ≥ 2, ● ratio < 2; dotted lines indicate some hypothetical removal efficiencies

However, higher loading rates, up to 400 g $m^{-2}d^{-1}$, resulted in either removal rates directly proportional to the loading rate or a constant removal rate of approximately 40 g $m^{-2}d^{-1}$. This phenomenon can be explained by taking into account the suspended solids content of the UASB effluent. Introducing a total-COD/ dissolved-COD ratio ≥ 2 as an arbitrary criterion for high contents of suspended solids (SS), Fig. 2b clearly shows the correspondence of such high SS contents and high loading rates with COD removal rates directly proportional to the loading rate, resulting in a casual COD removal efficiency of about 70%. It is also shown that a constant removal rate of circa 40 g $m^{-2}d^{-1}$, independent on the loading rate, is only found when the UASB effluent contains relatively small amounts of suspended solids (ratio < 2).

On the other hand, the introduction of the total-COD/dissolved-COD ratio to the results of the trickling filter did not consequently show high COD removal efficiencies for high contents of suspended solids (Fig. 2a). On the contrary, some very poor removal efficiencies corresponded with relatively high SS contents of the UASB effluent. Therefore, it may be concluded that the attached biomass in the rotating biological contactor is able to resist and able to remove particulate organics more effectively than the attached biomass in the trickling filter.

A further comparison of the trickling filter and rotating biological contactor performance is made, leaving the SS-COD out of consideration. Since a trickling filter is a plug-flow reactor the overall removal rate can be differentiated into removal rates per column segment. The mean dissolved-COD removal rates per unit area in 1 m-segments of the trickling filter columns showed a dramatic decrease from 6.0 to 1.1 g $m^{-2}d^{-1}$ in the top and bottom 1 m-segments of the column, respectively (Fig. 3a). The observed dissolved-COD removal rates per segment are plotted versus the dissolved-COD loading rates per segment in Fig. 3b. The general structure of the Fig. indicates removal rates directly proportional to the loading rate of the trickling filter (dotted line, $r^2 = 0.81$), indicating an organic substrate limited biofilm in all column segments: removal of organic substrate in an upper segment results in a lower loading rate of the lower segment, and by that, also in a lower removal rate as is shown in Fig. 3a.

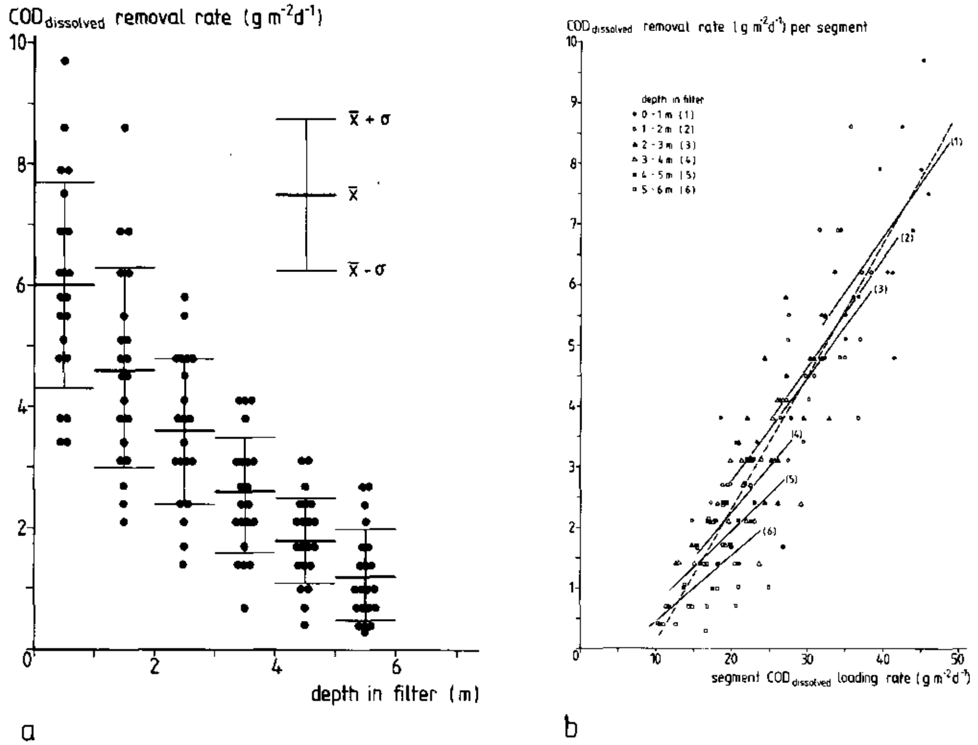

Fig. 3. Trickling filter: dissolved-COD removal rate per segment versus a. depth in combined filter, b. dissolved-COD loading rate per segment.

Furthermore, the segment removal and loading rates are linearly correlated to each other in Fig. 3b. According to the decreasing slopes, it is also shown that the attached biofilm removed a continually decreasing fraction of the loaded COD with increasing depth in the trickling filter, indicating that the organic substrate limited biofilm kinetics are also the result of a continually diminishing fraction of (easily) biodegradable organics remaining in the effluent during its filter passage.

Note that the trickling filter is arbitrarily divided into 1 m segments, resulting in loading rates having no absolute quantitative meaning and therefore the slopes of the straight lines in Fig. 3b only indicate relative differences.

As was to be expected, the dissolved-COD removal rates of the rotating biological contactor in relation to the dissolved-COD loading rate (Fig. 4) resembled very well the total-COD removal rates observed for COD loads having low SS contents (Fig. 2b).

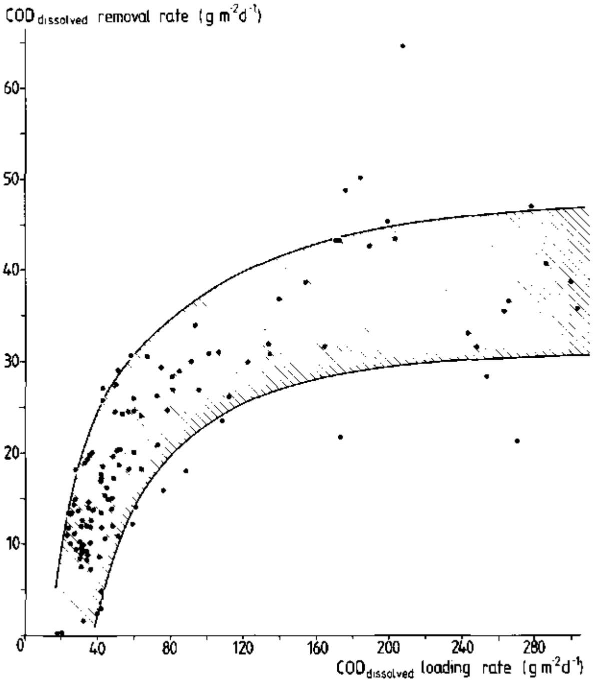

Fig. 4. First stage rotating biological contactor: dissolved-COD removal rate versus dissolved-COD loading rate.

Strongly increasing removal rates were observed for increasing loading rates up to about 80 g $m^{-2}d^{-1}$, indicating that also the rotating biological contactor biofilm has been operated under organic substrate limiting conditions. This limitation seemed to be abolished when the dissolved-COD loading rate exceeded about 100 g $m^{-2}d^{-1}$, resulting in removal rates fluctuating from 30-45 g $m^{-2}d^{-1}$. Primarily as a result of higher dissolved-COD loading rates per unit area, it appears from Fig. 3 and 4 that the first stage rotating biological contactor showed higher dissolved-COD removal rates per unit area than the trickling filter.

The excess sludge production was strongly affected by the COD loading rate: a mean value of the net sludge yield was found to be 0.57 g sludge-COD per g COD removed, for the first stage rotating biological contactor, when loaded at rates of 40-80 g $m^{-2}d^{-1}$ with low SS-COD containing UASB effluent. Few observations for the trickling filter biomass, however, suggested a net sludge yield of about 0.06 g g^{-1} for COD loading rates ranging from 1-2 kg $m^{-3}d^{-1}$ (5-10 g $m^{-2}d^{-1}$).

Nitrogen conversion rates

General aerobic microbiological conversion processes of waste water Kjeldahl-N are immobilization in bacterial tissues and biological oxidation to nitrite and nitrate. In this article Kjeldahl-N removal is defined as the result of these two processes together, and Kjeldahl-N oxidation is defined as the result of the nitrification processes. It should be noted hereby that in case of anaerobic effluents, Kjeldahl-N is almost equivalent to ammonium-N.

The Kjeldahl-N removal rates of the examined fixed-film reactors are plotted versus the Kjeldahl-N loading rate in Fig. 5. The overall removal rates of the trickling filter (Fig. 5a) varied strongly (20-200 g $m^{-2}d^{-1} \cong$ 0.1-1.0 g $m^{-2}d^{-1}$), and showed little, if any, correspondence with Kjeldahl-N loading rates up to 600 g $m^{-3}d^{-1}$ (3 g $m^{-2}d^{-1}$). As described before, generally the total-COD loading rates ranged from 1-4 kg $m^{-3}d^{-1}$ (5-20 g $m^{-2}d^{-1}$).

The Kjeldahl-N removal rate of the second trickling filter column was not affected by putting an intermediate settling tank into operation and the Kjeldahl-N removal efficiencies seldom exceeded about 50%.

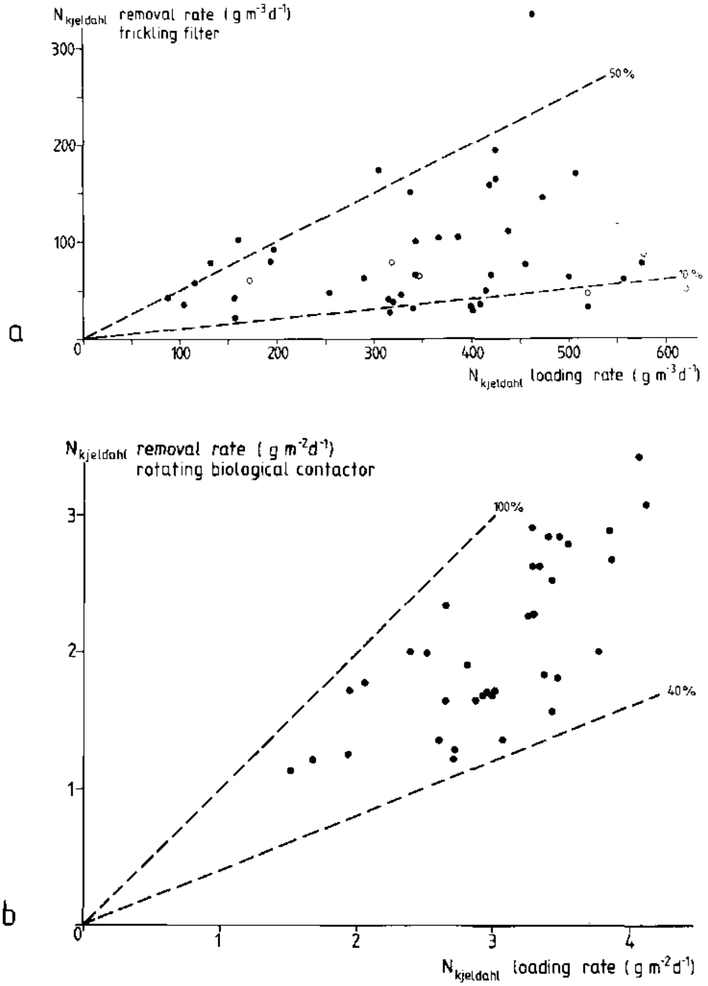

Fig. 5. Kjeldahl-N removal rate versus Kjeldahl-N loading rate, a. trickling filter (● no intermediate settling tank, O intermediate settling tank), b. second stage rotating biological contactor; dotted lines indicate some hypothetical removal efficiencies

The first stage rotating biological contactor operated with extremely low Kjeldahl-N removal efficiences, therefore only the removal rates observed for the second stage rotating biological contactor are given in Fig. 5b. The lower removal rates shown in Fig. 5b are the result of measurements during the start-up period of the reactor. Therefore, an increasing removal rate with increasing loading rates due to higher ammmonium concentrations, may only be expected for the measurements carried out after the maximum capacity was reached. The maximum removal rate showed a value of circa 3 g $m^{-2}d^{-1}$, involving 70-90% removal efficiencies. The total-COD loading rate fluctuated from about 4 to 14 g $m^{-2}d^{-1}$, due to fluctuating COD concentrations of the UASB effluent, varying effluent flow rates and varying COD removal efficiencies of the first stage rotating biological contactor.

The low COD loading rates of both trickling filter and rotating biological contactor resulted in little Kjeldahl-N immobilization in excess sludge. After correction of the Kjeldahl-N removal rates for the immobilization in excess sludge, average Kjeldahl-N oxidation rates were calculated, viz. 0.32 g $m^{-2}d^{-1}$ for the trickling filter and 2.4 g $m^{-2}d^{-1}$ for the second stage rotating biological contactor (at the end of the experimental period). The high oxidation rate of the rotating biological contactor in comparison with the trickling filter may be surprising, since the COD and Kjeldahl-N loading rates per unit surface area did not differ that much for the two experiments. However, as a result of the first stage COD removal efficiency the second stage rotating biological contactor received effluent with a mean dissolved-COD/ Kjeldahl-N ratio of 1.8, whereas the trickling filter was loaded with UASB effluent having a mean dissolved-COD/Kjeldahl-N ratio of 3.3. As a result of the COD removal in the upper parts of the trickling filter column (Fig. 3), this ratio will rapidly decline during the filter passage, so only the lower parts of the column are loaded comparable to the second stage rotating biological contactor.

The calculated average overall Kjeldahl-N oxidation rate of the trickling filter does not agree with the observed oxidation rate based on the actual concentrations of nitrite and nitrate in the final effluent. This observed net Kjeldahl-N oxidation rate is given in Fig. 6 for the 1 m-segments of the trickling filter, showing a negative net oxidation rate for the top 1 m-segment and increasing positive values with increasing depth in the filter bed (Fig. 6a).

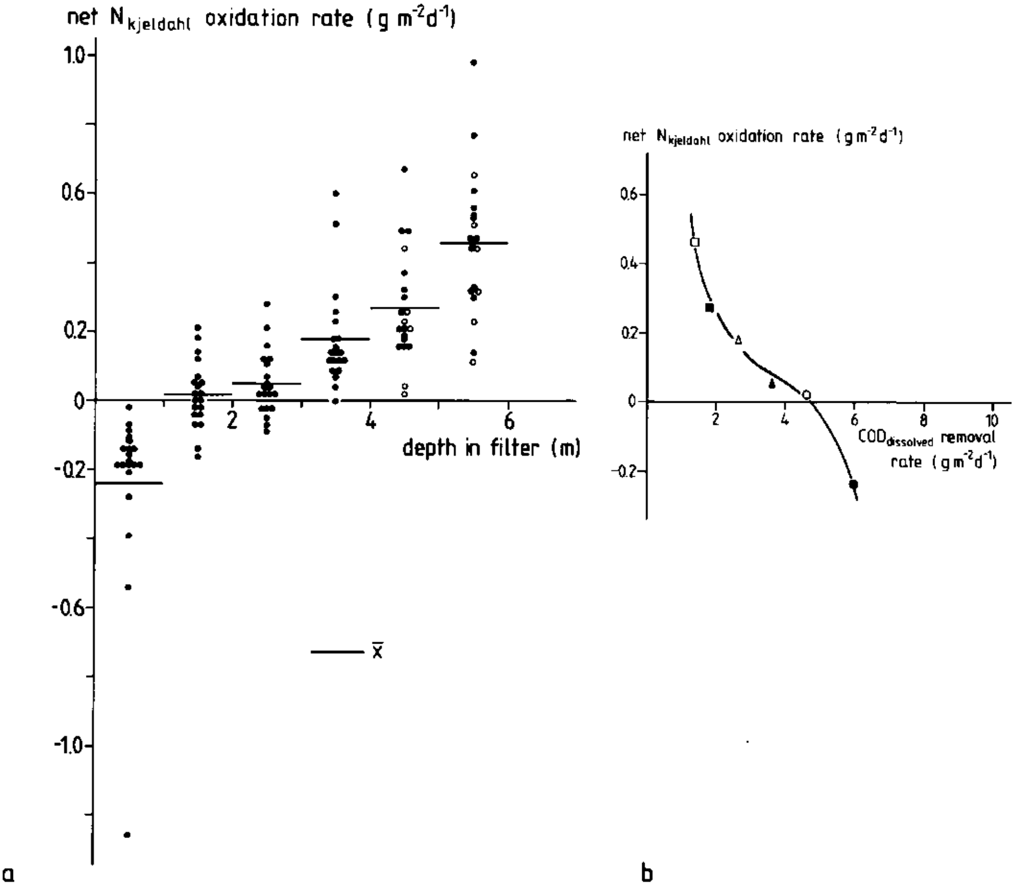

Fig. 6. Trickling filter: net Kjeldahl-N oxidation rate per segment versus a. depth in filter (● no intermediate settling tank, ○ intermediate settling tank), b. dissolved-COD removal rate per segment —mean values (depth in filter: ● 0-1 m, ○ 1-2 m, ▲ 2-3 m, Δ 3-4 m, ■ 4-5 m, □ 5-6 m).

The negative net Kjeldahl-N oxidation rate in the upper 1 m segment indicates the appearance of denitrification in the trickling filter, due to the recirculation of the final effluent. From the net Kjeldahl-N oxidation rates per 1 m-segment an overall net oxidation rate of 0.13 g $m^{-2}d^{-1}$ is calculated, showing that 40% of the oxidized Kjeldahl-N is denitrified in the trickling filter columns. In connection with the organic substrate limited COD removal rates (Fig. 3), it is most likely that denitrification is restricted to the upper segments, and that the average net Kjeldahl-N oxidation rate found for the lowest 1 m-segment is close to the actual gross Kjeldahl-N oxidation rate per unit area in that particular part of the trickling filter bed. Notwithstanding a loading rate and a dissolved-COD/ Kjeldahl-N ratio almost similar to those of the second stage rotating biological contactor, the observed Kjeldahl-N oxidation rate in the lower regions of the trickling filter is significant lower, problably due to the different hydraulic characteristics (Harremoës, 1978). In Fig. 6b the net Kjeldahl-N oxidation rate is plotted versus the dissolved-COD removal rate for the various 1 m-segments. However, it should be emphasized that the curve shown is the combined result of the effect of COD removal on the nitrification process, and of the observed appearance of denitrification, especially in the upper segments. Therefore, Fig 6b suggests a too progressive relationship between the net nitrification and the simultaneous dissolved COD-removal.

Nitrogen balances of the rotating biological contactor did not indicate the occurrence of denitrification, at least not at a significant scale. Based on the trickling filter performance it is obvious that denitrification, if desired, is easily introduced to the rotating biological contactor performance by recirculation of the second stage effluent to the first stage rotating biological contactor.

Supplementary observations

Dissolved oxygen is measured because of its role in fixed-film oxidation kinetics (Harremoës, 1978,1982). The reported COD and

Kjeldahl-N removal rates of the trickling filter were observed in combination with dissolved oxygen concentrations of 7-9 g m^{-3} for all 1 m-segments of the columns. Dissolved oxygen concentrations in the rotating biological contactor tanks generally showed values of 3-4 g m^{-3} with occasional maximum values of about 6 g m^{-3}. The fluctuations of the dissolved-COD removal rate for high dissolved COD loading rates (Fig. 4) are partly the result of fluctuations of the dissolved oxygen concentration.

The UASB effluent contained about 5-25 g m^{-3} sulphide, which was effectively oxidized into sulphate in both fixed-film reactors. As a result the UASB effluent had lost its bad smell after the post-treatment.

Few observations of the dry matter content of the biofilm resulted in round figures, viz. 10-20 g m^{-2} and 20-35 g m^{-2} for the trickling filter and the rotating biological contactor respectively. The difference may be explained by different loading rates as well as different hydraulic characteristics of the installations. Excess sludge produced by the attached biomass was obtained from the settling tanks for the determination of the sludge volume index (SVI), resulting in 60 ml g^{-1} and < 50 ml g^{-1}, again respectively for the trickling filter and the rotating biological contactor.

SUMMARY AND CONCLUSIONS

Based on the reported results it can be concluded that aerobic fixed-film processes are suitable for high-rate post-treatment of anaerobically digested municipal waste water at ambient temperatures of 12-20°C:

About 60% COD removal and 25% Kjeldahl-N removal can be achieved by trickling filtration, applying a high hydraulic loading rate ($6\ m^3m^{-3}d^{-1} \cong 0.03\ m^3m^{-2}d^{-1}$, flow rate/internal surface area), high hydraulic superficial loading rates ($2\text{-}6\ m^3m^{-2}h^{-1}$), depth of the filter bed 6 m and COD loading rates from 1.5 - 2.0 kg $m^{-3}d^{-1}$

(7.5 - 10.0 g $m^{-2}d^{-1}$). Such COD loading rates implicate organic substrate limited dissolved-COD removal rates and low Kjeldahl-N removal efficiences. The trickling filter was not able to handle succesfully peak-loads of SS-COD. Excess sludge production is low and the sludge settling proporties are good (SVI 60 ml g^{-1}).

Post-treatment by a two stage rotating biological contactor installation with respective COD loading rates of 80 and 10 g $m^{-2}d^{-1}$, results in about 70% COD removal and 60-80% Kjeldahl-N removal efficiencies. The hydraulic loading rate of such an installation is 0.04 $m^3m^{-2}d^{-1}$ (flow rate/surface area). As a result of the high first stage COD loading rate the excess sludge production is high (0.57 g SS-COD g^{-1} COD removed), the sludge is highly septic and its settling properties are extremely good (SVI < 50 ml g^{-1}). The rotating biological contactor is able to handle very well any peak SS-COD load, due to occasional sludge wash-out of the UASB reactor.

Denitrification is easily induced in aerobic fixed-film reactors for post-treatment of anaerobic effluents by effluent recirculation, in spite of high dissolved oxygen concentrations in the bulk liquid. Trickling filter effluent recirculation resulted in about 40% removal of the oxidized Kjeldahl-N by denitrification.

Since the COD/BOD ratio of the UASB effluents amounts about 2.7, the observed post-treatment COD removal efficiencies (60-70%) indicate that the removal of non-biodegradable organic matter is significant, viz. 20-30% of the removed COD.

Based on the observed post-treatment removal efficiencies and an assumed COD removal efficiency of the anaerobic pretreatment of about 70%, an overall COD removal efficiency of about 90% can be achieved for the complete waste water treatment. The overall Kjeldahl-N removal efficiency fully depends on the choise of the post-treatment method and its (COD) loading rate.

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REFERENCES

Genung, R.K., Donaldson, T.L. and Reed, G.D., 1985. Pilot-Scale Development of Anaerobic Filter Technology for Municipal Wastewater Treatment. In: M.S. Switzenbaum (Ed), Proceedings of the Seminar/Workshop Anaerobic Treatment of Sewage, Amherst, 127-159.

Grin, P.C., Roersma, R.E. and Lettinga, G., 1983. Anaerobic Treatment of Raw Sewage at Lower Temperatures. Proceedings of the European Symposium on Anaerobic Waste Water Treatment, Noordwijkerhout, 335-347.

Grin, P.C., Roersma, R.E. and Lettinga, G., 1985. Anaerobic Treatment of Raw Domestic Sewage in UASB-Reactors at Temperatures from 9-20°C. In: Proceedings of the Seminar/Workshop Anaerobic Treatment of Sewage, Amherst, 109-124.

Harremoës, P., 1978. Biofilm Kinetics. In: R. Mitchell, Editor, Water Pollution Microbiology, Vol. 2, Wiley and Sons, New York, NY, 71-109.

Harremoës, P., 1982. Criteria for Nitrification in Fixed Film Reactors. Water Science and Technology, 14, 167-187.

Lettinga, G., 1978. Feasibility of anaerobic digestion for the purification of industrial waste waters. In: Proceedings of the 4th European Sewage and Refuse Symposium EAS, Munich.

Lettinga, G., Roersma, R., Grin, P., De Zeeuw, W., Hulshof Pol, L., Van Velzen, L., Hobma, S. and Zeeman, G., 1982. In: Hughes et al. (Eds), Proceedings of the Anaerobic Treatment of Sewage and Low Strength Waste Waters. 2nd Symposium on Anaerobic Digestion, Travemünde, 1981, 271-291. Elsevier Biomedical Press B.V., Amsterdam, The Netherlands.

Lettinga, G., Roersma, R. and Grin, P., 1983. Anaerobic Treatment of Raw Domestic Sewage at Ambient Temperatures Using a Granular Bed UASB Reactor. Biotech. & Bioeng., XXV, 1701-1723.

Louwe Kooymans, J., Lettinga, G. and Rodriquez, G., 1985. The UASB process for domestic wastewater treatment in developing countries. Water Sci., 39, 437-451.

Schellinkhout, A., Lettinga, G., Van Velsen, L., Louwe Kooymans, I. and Rodriguez, G., 1985. The Application of the UASB-Reactor for the Direct Treatment of Domestic Wastewater under Tropical Conditions. Proceedings of Seminar/Workshop Anaerobic Treatment of Sewage, Amherst, 259-276.

Souza, M. de, Toledo, A., Rocha, B. and Vianna, G., 1984. Tratamento Final da Vinhaca Biodigerida, para Reaproveitamento ou Lancamento em Rios. Proceedings of the 3° Congresso Nacional da STAB V Convencao da ACTALAC, Sao Paoulo, 465-473.

Yoda, M., Hattori, M. and Miyaji, Y., 1985. Treatment of Municipal Wastewater by Anaerobic Fluidized Bed: Behaviour of Organic Suspended Solids in Anaerobic Reactor. Proceedings of the Seminar/Workshop Anaerobic Treatment of Sewage, Amherst, 161-196.

CHAPTER 3

MECHANISMS AND KINETICS OF COD REMOVAL AND SIMULTANEOUS OXYGEN CONSUMPTION IN A ROTATING BIOLOGICAL CONTACTOR BIOFILM

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MECHANISMS AND KINETICS OF COD REMOVAL AND SIMULTANEOUS OXYGEN CONSUMPTION IN A ROTATING BIOLOGICAL CONTACTOR BIOFILM

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ABSTRACT

A study on aerobic biofilm COD removal and simultaneous respiration is made using a Wazu respirationmeter unit. In a closed recirculating system completely submerged RBC biofilm samples have been examined in short-term batch experiments, using the corresponding waste water or dissolved organic substrate (acetate). The removal of dissolved organic substrate and the respiration were found to answer the ½-order/0-order kinetic model, whereas the removal of suspended solids COD (COD_s) showed 1-order kinetics. Under conditions usually met in practice the 0-order rates for dissolved COD (COD_d) removal and respiration were 30-40 g $m^{-2}d^{-1}$ and 10-16 g $m^{-2}d^{-1}$, respectively; in absence of organic substrate a certain background respiration was observed (3.5-9.0 g $m^{-2}d^{-1}$). As a result of the 1-order kinetics over a wide concentration range, extremely high COD_s removal rates were observed even exceeding 300 g $m^{-2}d^{-1}$. The difference between the maximum respiration and the COD_s removal rates is attributed to physical biofilm adsorption processes. Biofilm COD removal kinetics for waste water containing both COD_s and COd_d are discussed, illustrating that the kinetic order of the combined process and the reaction constant strongly depends on the COD_s/COD_d ratio. Conclusion is that a RBC biofilm loaded with a COD_s/COD_d mixture oxidizes some dissolved organic matter and acts as a catalyst in the coagulation and flocculation of fine suspended solids.

Key words –COD removal, respiration, biofilm reaction kinetics, biological waste water treatment, fixed-biofilm reactor

INTRODUCTION

Fixed-biofilm reactors are widely used as treatment systems for liquid organic wastes originating from industrial as well as domestic sources. The most common application is secondary treatment after sedimentation. A relatively new application is upgrading aerobic secondary effluents by means of rotating biological contactors (RBC) (Marsh et al., 1981; Poon et al., 1981a,b). Since anaerobic treatment processes are also succesfully applied as pretreatment, even to low strength waste water (Lettinga et al., 1983), post-treatment of anaerobic effluents may become another important issue in waste water treatment in order to meet the restrictions set for discharge of effluents. Bovendeur and Klapwijk (1986) showed that this goal too could be achieved using the aerobic fixed-biofilm process, supporting the benefits of the anaerobic pretreatment, viz. low energy requirement.

However, for all possible applications there is a need for information on process mechanisms and kinetics enabling a rational design and operation. As a result many emperical formulas have been proposed, of which Roberts (1973) could list more than a dozen in the field of trickling filtration for a 40 year period. On the other hand, more recently also conceptual biofilm models have been introduced, mathematically associating diffusional transport of dissolved substrates, and the active biofilm thickness with specific reaction kinetics (Williamson and McCarty, 1976a,b; LaMotta, 1976a,b; Famularo et al., 1978; Harremoës, 1978). It was shown that the biofilm reaction rate per unit area could be described by first-order, half-order, and zero-order kinetics in relation to the bulk substrate concentration.

These models, however, deal with the removal kinetics for soluble substrates only, whereas most waste waters in practice contain significant amounts of suspended substrate solids. Previous research on RBC performance treating anaerobically digested sewage (Bovendeur and Klapwijk, 1986) showed either total organic removal rates directly proportional to the loading rate, even at extremely high loadings, or a constant removal rate was found independent of the loading rate. This phenomenon could be explained by taking into account the suspended solids content of the feed: high suspended solids contents corresponded with the proportional removal rates and loading independent removal rates were found for low contents of suspended solids. This was confirmed by examining the relationship between removal and loading rates based on filtrated water samples. Similar results were reported by Rusten (1984) for aerated submerged filters loaded with presettled waste water, where the relation between total organic removal rate and organic load was modelled with a hyperbolic function having a very slight curve. Moreover, there are strong indications by the work of Särner (1980, 1986) that the removal of particulate and dissolved organics interact in the sense that particle adsorption on biofilm surfaces decreases the removal rate of dissolved organics.

The present study is a contribution to the discussion on the mechanisms and kinetics of organic matter removal in fixed-biofilm reactors by combining RBC kinetics for organic matter elimination processes with the simultaneous respiration, and substrate concentrations in the bulk liquid. Emphasis is put on the application of RBC for post-treatment of anaerobic effluents containing both dissolved and suspended COD.

MATERIALS AND METHODS

Rotating biological contactor units

Two identical laboratory-scale RBC units were used. Each unit consisted of 10 disks of expanded polystyrene (disk diameter 0.20 m; thickness 0.01 m; disk spacing 0.02 m) providing a total effective biofilm surface of 0.69 m^2, of which 50% was continuously submerged. The disks were mounted on a horizontal steel shaft, rotating in a triangular trough (total effective liquid volume 0.066 m^3). The rotational speed of the disks was 25 rpm, resulting in a peripheral velocity of 0.26 m s^{-1}.

Biofilm growing conditions

Non-nitrifying RBC biofilm material was obtained by continuous loading the two RBC units with two types of waste water (hydraulic loading rate 0.21 $m^3m^{-2}d^{-1}$, hydraulic residence time 1 h). One RBC unit was loaded with raw domestic sewage, anaerobically digested in a 6 m^3 UASB reactor (Upflow Anaerobic Sludge Blanket) described in detail by Grin et al. (1983) and De Man et al. (1986). The resulting biofilm sample is denoted as "UASB effluent biofilm". The other RBC unit was loaded directly with the presettled domestic sewage, thus producing a "domestic sewage biofilm" sample. Basic loading conditions varied between 60 and 90 g $m^{-2}d^{-1}$ total COD (Chemical Oxygen Demand), depending on the strength of the incoming sewage (combined sewer system). The ambient temperature of the sewage and the UASB effluent as well varied between 12 and 20°C. After reaching a relatively constant COD removal performance, the biofilm samples were alternately used for experiments in the biofilm monitoring system.

Biofilm monitoring system

The biofilm monitoring system is composed of a biofilm reactor unit (total effective liquid volume 0.025 m^3) connected to a respirationmeter unit (Fig. 1). Dissolved oxygen levels in the reactor unit were controlled by supply of air in the aerator. The water recycle flow between reactor vessel and aerator was set at 0.25 m^3h^{-1} and checked daily. A complete set of disks including the shaft was taken out of the trough of the RBC units and placed vertically in the reactor vessel. At the start of an experiment the reactor vessel is closed without enclosure of air. The attached RBC biofilm sample is examined batch-wise in the system, the disks being completely submerged and rotating at 25 rpm, during several hours at a constant temperature, set by the ambient temperature of the waste water. The RBC biofilm COD removal rate is determined from watersamples taken at certain time intervals (0.25, 0.50, or 1.0 h), and analysed for COD.

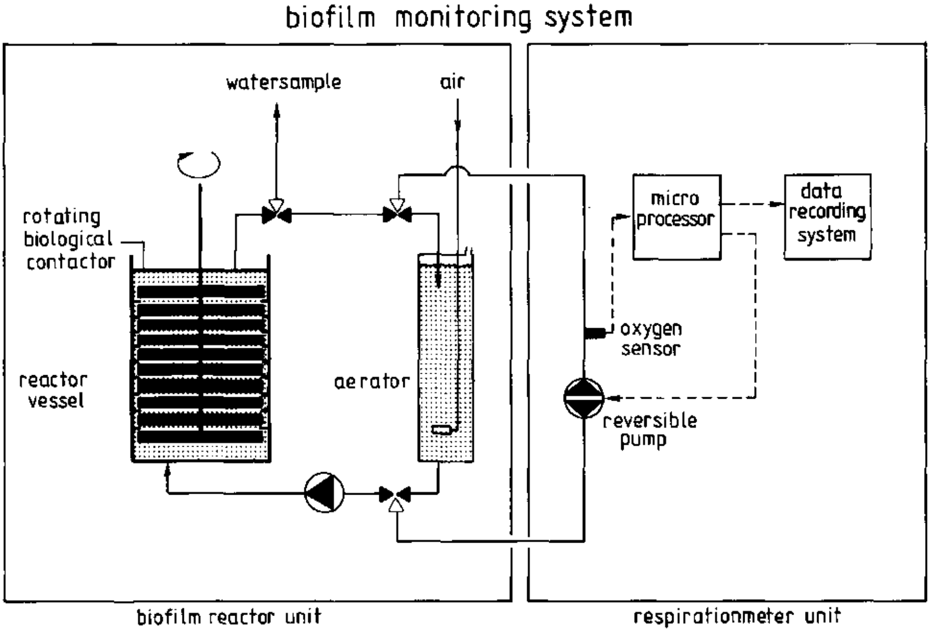

Fig. 1. Schematic presentation of the biofilm monitoring system

The overall oxygen consumption by the biofilm sample, denoted as respiration, is monitored by the respirationmeter unit (Fig. 1). Dissolved oxygen is measured periodically in the inflow and outflow of the reactor vessel using only one oxygen sensor. This procedure is realised by alternately changing the flow direction in the connecting bypass using a reversible pump. The oxygen sensor is connected to a modular microprocessor system, calculating the respiration rate and controlling the reversible pump. Complete respiration measurements are executed every 4 minutes. The respirationmeter unit used (Wazu respirationmeter, patent pended), and its operation and possibilities are described in more detail by Klapwijk et al. (1987), and Spanjers and Klapwijk (1987). The biofilm respiration is obtained by correcting the measured respiration for the oxygen consumption by the waste water itself (determined in the biofilm monitoring system in absence of a biofilm sample), which is relatively small. The disks including the attached biofilm sample are replaced in the original RBC unit immediately after termination of the experiment, and left there for adaptation during several days, before a new test is performed. Before each test the walls of the complete monitoring system are cleaned, disinfected, and rinsed with tap water. The amount of oxygen involved with COD removal, i.e. COD oxidation, is determined by combining the respiration data and the COD removal data. The biofilm rates for COD removal and respiration are expressed as g $m^{-2}d^{-1}$.

Experimental set-up

The general COD removal performance of the biofilm has been examined in the RBC units under biofilm growing conditions by grab samples of the RBC feed and RBC effluent over a 1h period. Detailed batch-wise biofilm monitoring experiments were performed loading the RBC biofilm sample with the corresponding type of waste water. The UASB effluent biofilm sample has also been loaded with dissolved sodium acetate as a model substrate for verification purposes of both the biofilm monitoring system and dissolved organic substrate removal performance. In some experiments extremely high suspended solids contents of the UASB effluent were applied, achieved by shaking the pipes connecting the UASB reactor and the RBC units. The absence of nitrifying micro-organisms in the biofilm samples has been checked regularly.

Analyses

COD was analysed according to Standard Methods (American Public Health Association, 1980). Distinction was made between total COD (COD_t), COD after filtration (COD_f) using Schleiden & Schuell paper filters (fraction < 7.4 µm), and dissolved COD (COD_d) after filtration using Schleiden & Schuell membrane filters (fraction

< 0.45 µm). Suspended solids COD (COD_s) was calculated as COD_t minus COD_f, and colloidal COD (COD_c) as COD_f-COD_d. Acetate was determined by gas chromatography using a Packard Becker Model 427 equipped with a 2m column and flame ionisation detector. The column was packed with Super Copolt Fluorad (100-200 mesh) and the carrier gas used was nitrogen gas.

RESULTS

COD removal performance in batch experiments

The removal rates per unit biofilm area for COD_s, COD_f, and COD_d in UASB effluent are plotted versus the concentration of the corresponding COD fraction in Fig. 2. Distinction is made between measurements in the RBC unit (50% submergence) and measurements in the biofilm monitoring system (100% submergence),

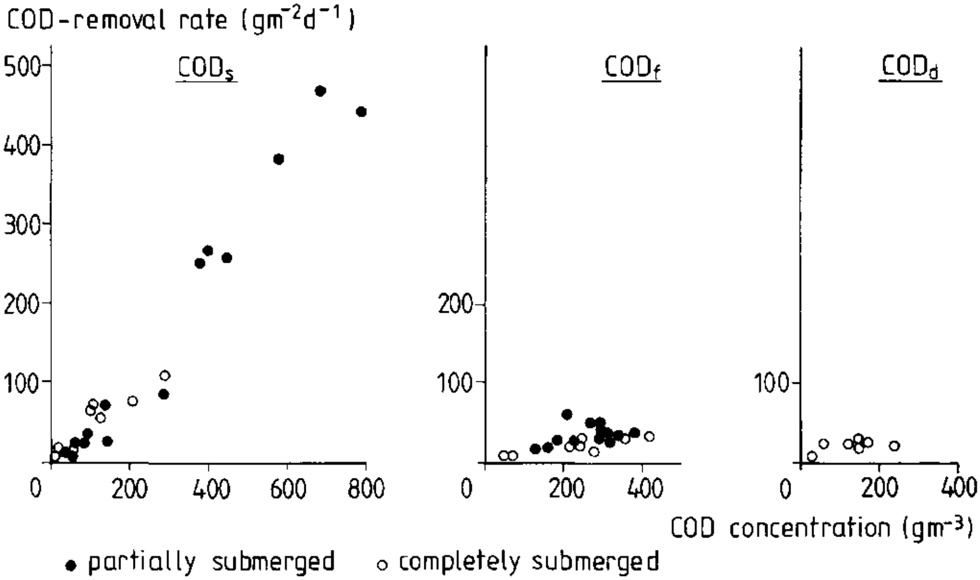

Fig. 2. Removal rates (UASB effluent biofilm) for COD_s, COD_f, COD_d related to the concentration of the corresponding COD fraction in UASB effluent

showing little, if any, difference. Characteristic distinction was found for COD_s removal performance in comparison to COD_f or COD_d removal performance.

Simultaneous COD removal and respiration performance

Two examples of the output of the biofilm monitoring experiments are given in Fig. 3 and 4. In Fig. 3 the cumulative removal of acetate and the respiration, as well as the respiration rate plotted versus time for the UASB effluent biofilm sample fed with acetate. The total respiration followed the course of the acetate removal, indicating that acetate removal is mainly the result of a biochemical process, especially acetate oxidation.

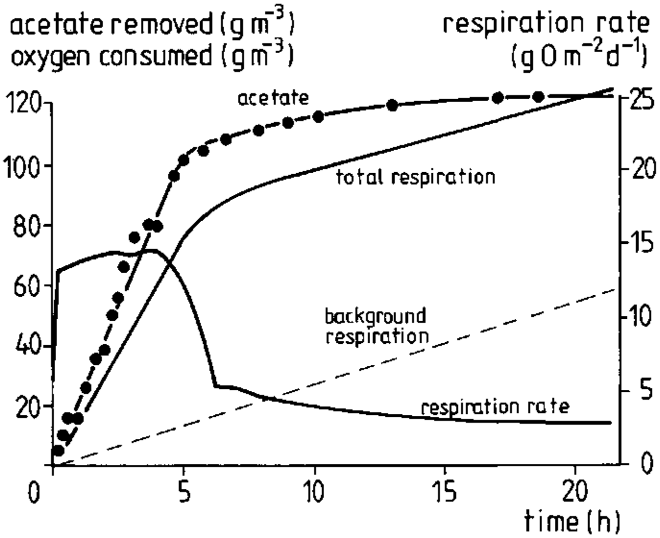

Fig. 3. Acetate removal performance and simultaneous respiration of the UASB effluent biofilm sample

The respiration rate reached a more or less constant high level during about 4 hours, starting almost immediately after initiating the experiment. After this period the respiration rate decreased strongly untill a certain background respiration rate was reached. Fig. 4 shows the same relations for the UASB

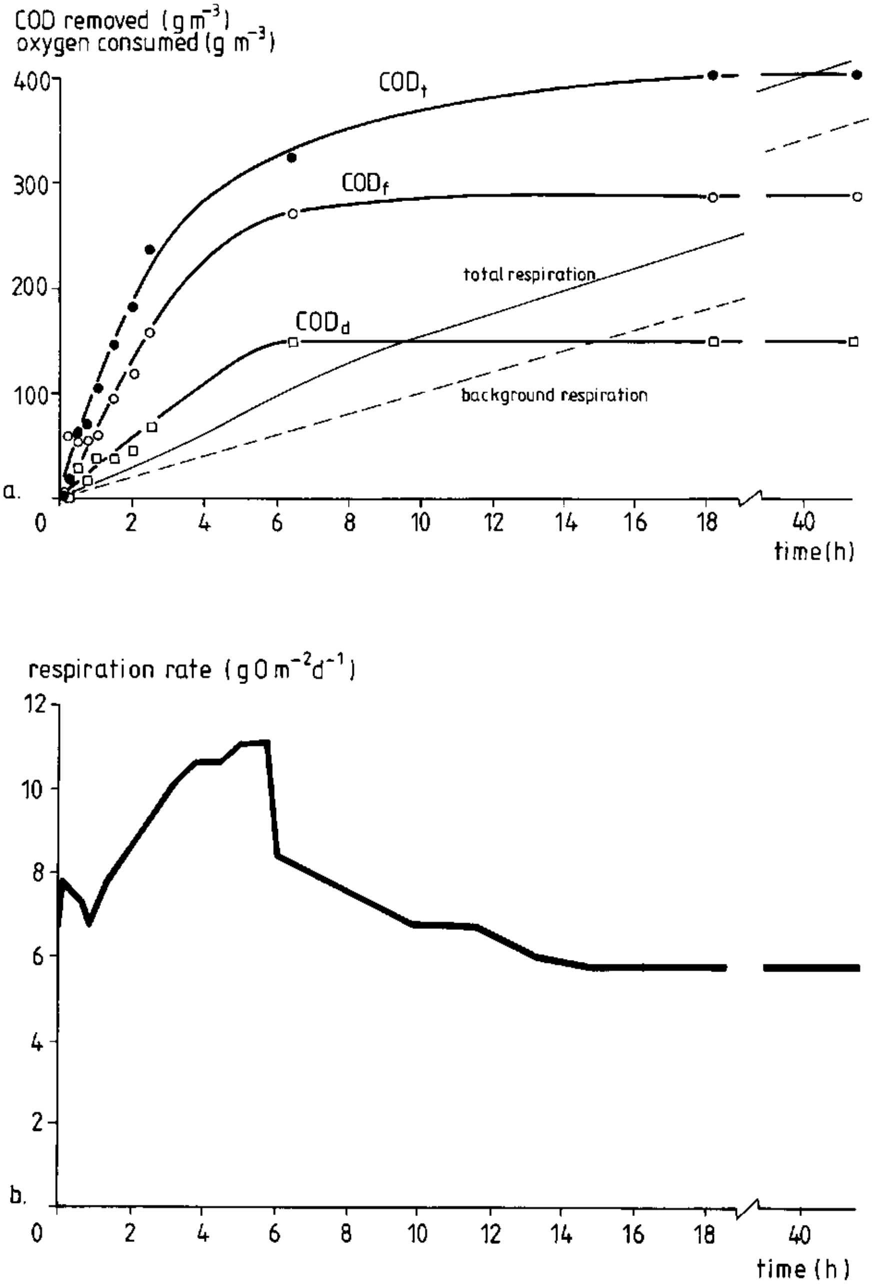

Fig. 4. COD removal performance, oxygen consumption (Fig. 4a) and respiration rate (Fig. 4b) of the UASB effluent biofilm sample submerged in the corresponding effluent

effluent biofilm sample fed with the corresponding effluent. In contrast to the acetate experiments, a minor effect was observed for the initial increase of the respiration rate on total respiration (Fig. 4a), due to a less rapid increase of the respiration rate after initiating the experiments (Fig. 4b). The background respiration rate was found to be constant over an extended period. The background and maximum levels for the respiration rates observed for the different combinations of biofilm samples and effluents are presented in Table 1.

Table 1. Background and maximum levels of the respiration rate (µ ± SD (n)), measured in two series (a, b) of experiments

Biofilm sample		UASB effluent biofilm		domestic sewage biofilm
Organic substrate		acetate	UASB effluent	raw domestic sewage
Background respiration rate ($gm^{-2}d^{-1}$)	a	3.5 ± 1.23 (14)		9.0 ± 2.97 (8)
	b	5.2 ± 0.50 (5)		
Maximum respiration rate ($gm^{-2}d^{-1}$)	a	13.4 ± 1.21 (2)	10.1 ± 3.30 (12)	16.2 ± 4.53 (8)
	b	12.6 ± 1.27 (2)	11.3 ± 0.86 (5)	

Effect of substrate concentration

The relation between the dissolved organic substrate removal rate and the respiration rate on one hand, and the bulk concentration of both dissolved organic substrate and dissolved oxygen on the other hand is shown in Fig. 5 for all experiments in which the UASB effluent biofilm is fed with acetate. The combined results demonstrate that both rates reach a more or less constant level (about 20 g $m^{-2}d^{-1}$ acetate; 13 g $m^{-2}d^{-1}$ oxygen), once certain minimum concentrations have been exceeded (about 30 g m^{-3} acetate; 1.6 g m^{-3} oxygen). However, the measurements of the acetate removal rate scatter significantly more than the respiration rate measurements. Biofilm performance in terms of

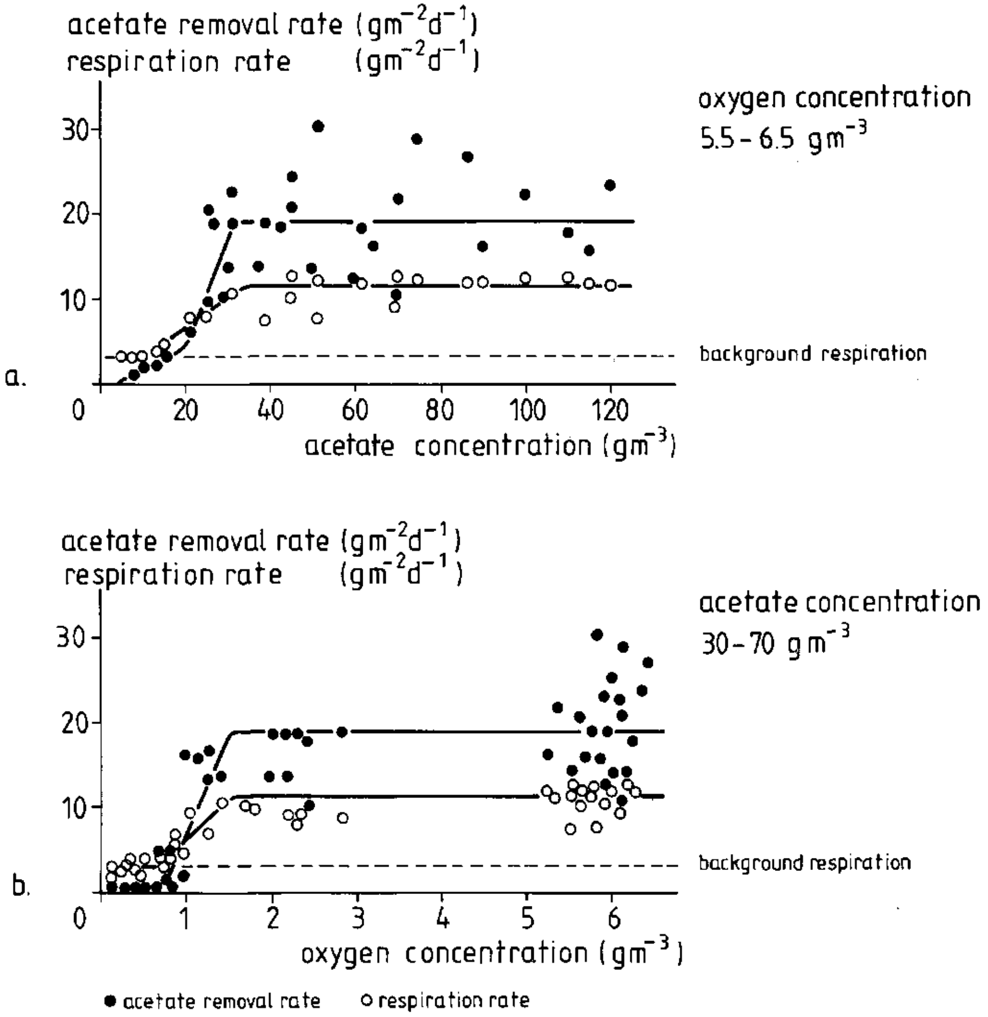

Fig. 5. Effect of the bulk substrate concentration on acetate removal and simultaneous total respiration by the UASB effluent biofilm sample; dissolved substrate: acetate (Fig. 5a) and dissolved oxygen (Fig. 5b)

the background respiration rate related to the bulk concentration of dissolved oxygen is shown for a single experiment, again using the UASB effluent biofilm sample (Fig 6). The result suggests that the constant level of the background respiration rate observed in the biofilm monitoring experiments described, appears for dissolved oxygen levels exceeding 1.0 gm^{-3}, in absence of organic substrate in the bulk liquid.

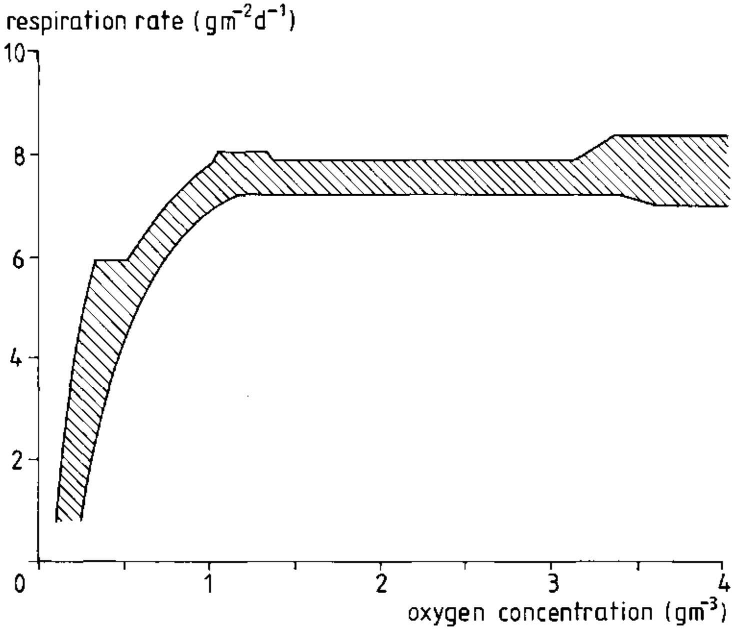

Fig. 6. Background respiration of the UASB effluent biofilm sample in relation to the concentration of dissolved oxygen. The shaded area represents over 300 measurements

DISCUSSION AND CONCLUSIONS

The particular suitability of the biofilm monitoring system for biofilm respiration performance is demonstrated by the results of the acetate-experiments (Fig. 3). The respiration of acetate caused a strongly increased respiration rate untill the acetate concentration decreased to a limiting level. As a result of the observed background respiration, after circa 20 hours the total respiration has reached the equivalent of total acetate removed. This phenomenon indicates that the background respiration is the result of oxidation of some internal organic substrate absorbed by the biofilm in the period prior to the testing period. Consequently, the background respiration may be regarded as the maintenance level for the oxygen consumption. On the basis of net respiration (i.e. total respiration corrected for the background respiration level), about 58% of the acetate removed was calculated to be actually oxidized (Fig. 5). This figure is only valid under circumstances of sufficient availability of both acetate and dissolved oxygen.

The overall relations between the acetate removal rate and the corresponding respiration rate on one hand, and the concentrations of acetate and dissolved oxygen on the other hand (Fig. 5), seem to answer the 0-order kinetic model for substrate concentrations exceeding the corresponding minimum concentration. Although the scattering of the measurements does not allow a full verification of the ½-order/0-order concept for biofilm kinetics (LaMotta, 1976a,b; Harremoës, 1978), it seems reasonable to adopt ½-order kinetics for substrate concentrations lower than the transition concentration. Moreover, the characteristic relation between the biochemical oxidation rate for internal substrate (background respiration) and the dissolved oxygen concentration (Fig. 6) strongly favours the ½-order/ 0-order kinetic model.

Based on the discussed biofilm removal kinetics for acetate and the corresponding respiration, illustrated by Fig. 3,5 and 6, it seems reasonable to adopt the ½-order/0-order kinetic model for both the removal and respiration of any dissolved organic substrate. The schematic course of the reaction rates for a hypothetical substrate are given in relation to the dissolved organic substrate concentration, in presence of sufficiently available oxygen (Fig. 7a), and also in relation to the concentration of dissolved oxygen in presence of sufficiently available organic substrate (Fig. 7b). Accounting the background respiration for oxidation of internal substrate already present in the biofilm, the constructed graphs indicate that the difference between the total respiration and the background respiration may be regarded as the result of oxidation involved with the external substrate removed. Further, the difference between the maximum levels of substrate removal and the total respiration may be regarded as substrate removal involved biomass production. In the case of acetate a biomass yield of

$0.45\ gg^{-1}$ is calculated (Fig. 5), for reactor conditions enabling maximum reaction rates.

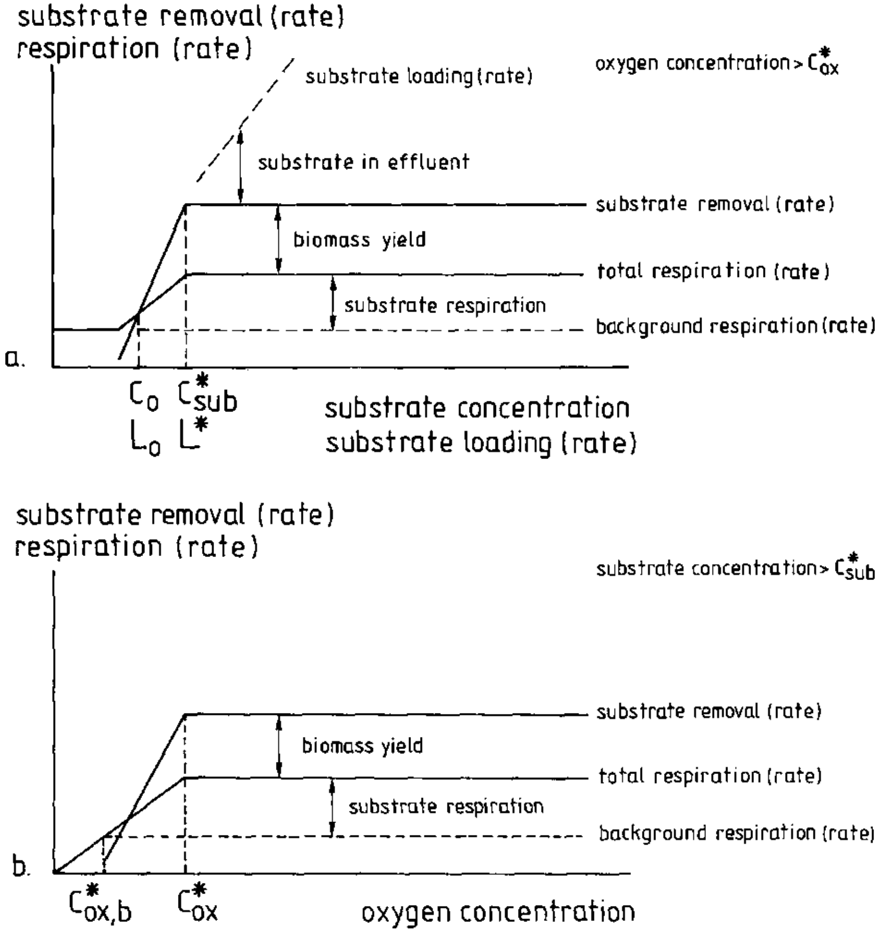

Fig. 7. Schematic presentation of fixed-biofilm (reactor) performance related to substrate concentration in the bulk liquid (Fig. 7a dissolved organic substrate; Fig. 7b dissolved oxygen)

Within certain restrictions, the substrate concentration can be substituted by the biofilm loading rate. The relations shown in Fig. 7a indicate that biofilm growth, defined as positive biomass yield, can only occur for substrate concentrations (or loading rates) exceeding a certain maintenance level c_0 (or loading rate L_0). Maximum biofilm growth or substrate removal is obtained for substrate concentrations exceeding the transition concentration c_{sub}^* (or loading rate L^*). On the other hand, in case these values are exceeded dramatically in continuously operated reactors, it is unavoidable that a substantial part of the substrate or waste is lost by discharge of the reactor effluent. Fig. 7b indicates that substrate oxidation occurs for oxygen concentrations exceeding the transition concentration for the background respiration ($c_{ox,b}^*$), whereas the maximum substrate oxidation is possible only if dissolved oxygen levels are maintained exceeding c_{ox}^*. Since the waste concentration restrictions set for discharge of effluents generally result in values lower than c_{sub}^*, the design of a RBC installation treating dissolved organic waste should be based on a concentration/loading rate couple for the final stage or compartment, causing ½-order removal kinetics organic substrate limitation, viz. c_{sub}^* and L_{sub}^*, and $c_{ox} \geq c_{ox}^*$.

In Fig. 4 it is shown that, in comparison to acetate, the oxidation of UASB effluent COD is of minor importance in the total removal mechanism. After 5 hours the respiration accounts for about 8%, 10% and 20% of the removal of COD_t, COD_f, and COD_d respectively. In Fig. 4 it is also shown that during the first 5 hours of this particular experiment, COD_c is removed very efficiently almost without being oxidized. These results indicate, firstly that removal processes other than biochemical oxidation are accounting for short-term COD removal, and secondly that the background respiration accounts for the bigger part of the total respiration, the net COD oxidation being of minor importance. Spanjers and Klapwijk (1987) also observed this phenomenon for organic matter elimination by non-nitrifying activated sludge, and they suggested a rapid physical process, probably adsorption, followed by biochemical oxidation at a low rate. The rapid removal of COD_c is also reported by Särner (1981), who observed high COD_c removal of rates in the upper compartments of trickling filters.

Since no significant distinction is found for the COD removal characteristics for the measurements in the RBC units, compared to the biofilm monitoring system measurements –in the COD concentration ranges up to 200 g m^{-3} (COD_d), 300 g m^{-3} (COD_s) and 400 g m^{-3} (COD_f)– (Fig. 2), it is demonstrated that short-term complete submergence of the biofilm is no obstacle for biofilm COD removal studies. Furthermore, the difference observed for the removal characteristics of COD_s compared to COD_f and COD_d corresponds to the results of continuously loaded biofilm reactors, presented by Rusten (1984), and Bovendeur and Klapwijk (1986). Therefore, it may be concluded that biofilm performance studies, carried out in the biofilm monitoring system, are significant for attached biofilm performance in general. The extremely high COD_s removal rates observed (Fig. 2), which are still directly proportional to the COD_s concentration or COD_s loading rate, point to biofilm adsorption processes rather than biochemical oxidation.

The estimation of the biomass yield involved with COD_t degradation as demonstrated for acetate, is not allowed because of the COD adsorption processes. However, for demonstration purposes only, COD_f is adopted as COD fraction approximating the removal characteristics of dissolved biodegradable substrate as illustrated in Fig. 7. This assumption is supported by the 0-order COD_f removal rates observed (Fig. 2) and results presented earlier (Bovendeur and Klapwijk, 1986). Based on this assumption, and combining the removal kinetics for the COD_s and COD_f fractions (Fig. 2 and 7), Fig. 8 is constructed, schematically illustrating the proportional contributions by the removal of COD_s and COD_f in COD_t removal, as functions of the corresponding COD concentrations or loading rates. It appears from Fig. 8 that the transition concentration for COD_f ($c^*_{COD,f}$) is also significant for the kinetic order of the resultant COD_t removal and, obviously, the reaction constant for COD_t removal kinetics highly depends on the COD_s/COD_f ratio. Based on the adoption of the ½-order/0-order kinetic model for biofilm removal of COD_f and 1-order kinetics for the removal of COD_s, the possible kinetic orders of the resultant COD_t removal are listed in Table 2.

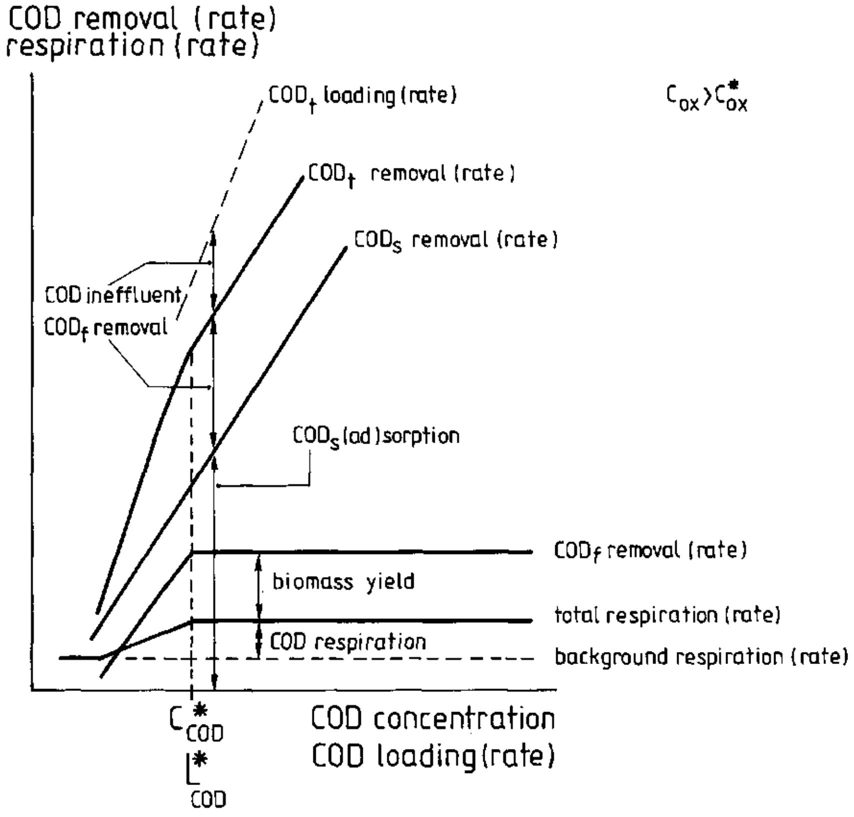

Fig. 8. Schematic presentation of fixed-biofilm (reactor) performance in a mixture of dissolved and suspended COD

Table 2. Kinetic order of COD$_t$ removal for possible COD$_s$/COD$_f$ ratios in relation to the COD$_f$ concentration ($c_{COD,f}$)

	COD$_t$ removal kinetics			
	COD$_f$ = 0 COD$_s$ ≠ 0	COD$_s$>>COD$_f$	COD$_s$<<COD$_f$	COD$_s$ =0 COD$_f$ ≠0
$c_{COD,f} \leq c^*_{COD,f}$	1-order	pseudo 1-order	pseudo ½-order	½-order
$c_{COD,f} \geq c^*_{COD,f}$	-	pseudo 1-order	pseudo 0-order	0-order

The overall result may explain why so many emperical formulas can be found in literature describing COD_t removal by biofilm reactors. Further, the removal of COD_t –with a rather constant COD_s/COD_f ratio as a result of primary sedimentation– described by a hyperbolic function (Rusten, 1984) may also be explained by the possible combination of pseudo 1/2-order and 1-order kinetics for loading rates varying from relative low values up to values exceeding the transition loading rate $L^*_{COD,f}$ in some degree. In addition, the results presented earlier by Bovendeur and Klapwijk (1986) are almostnly explained by the presented combination of COD removal kinetics, based on $c^*_{COD,f}$ 100 ± 20 g m^{-3}, 0-order COD_f removal rate 30-40 g $m^{-2}d^{-1}$, and a strongly varying COD_s/COD_f ratio.

As a consequence of the combined results, illustrated by Fig. 8, the final stage or compartment of a RBC reactor treating waste water composed of COD_s and COD_f mixtures, should be designed and operated for COD_f concentrations not severely exceeding $c^*_{COD,f}$, whereas a certain COD_s is admissible. However, since excess sludge production is the total of COD_s adsorbed and the biomass yield resulting from COD biodegradation, high COD_s loads will lead to dilution of the active biomass in the biofilm by high excess sludge production due to high COD_s adsorption. Therefore, high COD_s loads should be prevented. It should be noted that COD_f in the presented schematic approach should be replaced by COD_d or even better by BOD_d (dissolved Biochemical Oxygen Demand).

The overall conclusion of the results is that a RBC reactor loaded with significant amounts of dissolved suspended organic matter may be described as a reactor in which the biofilm oxidizes only a part of dissolved organic matter and acts as a catalyst in the coagulation and flocculation of fine suspended solids.

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REFERENCES

American Public Health Association (1980). Standard Methods for the examination of waste and waste water. APHA, New York.
Bovendeur, J. and Klapwijk, A. (1986). Aerobic post-treatment of anaerobically digested municipal waste water by a trickling filter and a rotating biological contactor. In: Anaerobic Treatment, a Grown-Up Technology. Proceedings of the Aquatech '86 Water Treatment Conference, Amsterdam, The Netherlands, 15-19 September 1986. NVA, Rijswijk, 385-397.
De Man, A.W.A., Grin, P.C., Roersma, R.E., Grolle, K.C.F. and Lettinga, G. (1986). Anaerobic treatment of municipal waste water at low temperatures. In: Anaerobic Treatment, a Grown-Up Technology. Proceedings of the Aquatech '86 Water Treatment Conference, Amsterdam, The Netherlands, 15-19 September 1986. NVA, Rijswijk, 451-466.
Famularo, J., Mueller, J.A. and Mulligan, T. (1978). Application of mass transfer to rotating biological contactors. J. Water Pollut. Control Fed., 50, 653-671.
Grin, P.C., Roersma, R.E. and Lettinga, G. (1983). Anaerobic treatment of raw sewage at lower temperatures. In: Anaerobic Waste Water Treatment. Proceedings of the European symposium, Noordwijkerhout, The Netherlands, 23 - 25 November 1983, 335-347.
Harremoës, P. (1978). Biofilm Kinetics. In: Water Pollution Microbiology, R. Mitchell (Ed)., Vol.2, Wiley, New York, 71-109.
Klapwijk, A., Spanjers, H. and Van den Akker, D. (1987). Control of an activated sludge plant with a Wazu-respirometer. In: Proceedings of the 7th European Sewage and Refuse Symposium, München, FRG, 19 - 22 May, 1987, 125-139.
LaMotta, E.J. (1976a). Kinetics of growth and substrate uptake in a biological film system. Appl. envir. Microbiol., 31, 286-293
LaMotta, E.J. (1976b). Internal diffusion and reaction in biological films. Envir. Sci. Technol, 10, 765-769.

Lettinga, G., Roersma, R. and Grin, P. (1983). Anaerobic Treatment of Raw Domestic Sewage at Ambient Temperatures Using a Granular Bed UASB Reactor. Biotechnol. Bioengg, XXV, 1701-1723.

Marsh, D., Benefield, L., Bennett, E., Linstedt, D. and Hartman, R. (1981). Coupled trickling filter-rotating biological contactor nitrification process. J. Water Pollut. Control Fed., 53, 1469-1480.

Poon, C.P.C., Chin, H.K., Smith, E.D. and Mikuchi, W.J. (1981a). Upgrading with rotating biological contactors for BOD removal. J. Water Pollut. Control Fed., 53, 474-481.

Poon, C.P.C., Chin, H.K., Smith, E.D. and Mikuchi, W.J. (1981b). Upgrading with rotating biological contactors for ammonia nitrogen removal. J. Water Pollut. Control Fed., 53, 1158-1165.

Roberts, J. (1973). Towards a better understanding of high rate biological film flow reactor theory. Wat. Res., 7, 1561-1588.

Rusten, B. (1984). Wastewater treatment with aerated submerged biological filters. J. Water Pollut. Control Fed., 56, 424-431.

Särner, E. (1980). Removal of dissolved and particulate organic matter in high-rate trickling filters. Wat. Res., 15, 671-678.

Särner, E. (1986). Removal of particulate and dissolved organics in aerobic fixed-film biological processes. J. Water Pollut. Control Fed., 58, 165-172.

Spanjers, H. and Klapwijk, A. (1987). The Wazu respirationmeter - a breakthrough in automation and control for a wastewater treatment plant. In: Proceedings of the 10th Symposium on Wastewater Treatment, Montreal, Quebec, November 1987.

Williamson, K. and McCarty, P.L. (1976a). A model of substrate utilization by bacterial films. J. Water Pollut. Control Fed., 48, 9-24.

Williamson, K. and McCarty, P.L. (1976b). Verification studies on the biofilm model for bacterial substrate utilization. J. Water Pollut. Control Fed., 48, 281-296.

CHAPTER 4

DESIGN AND PERFORMANCE OF A WATER RECIRCULATION SYSTEM FOR HIGH-DENSITY CULTURE OF THE AFRICAN CATFISH, *Clarias gariepinus* (BURCHELL 1822)

Aquaculture, 63 (1987) 329-353

Design and Performance of a Water Recirculation System for High-Density Culture of the African Catfish, *Clarias gariepinus* (Burchell 1822)*

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ABSTRACT

Bovendeur, J., Eding, E.H. and Henken, A.M., 1987. Design and performance of a water recirculation system for high-density culture of the African catfish, *Clarias gariepinus* (Burchell 1822). *Aquaculture*, 63: 329-353

A water recirculation system for the culture of the African catfish has been developed in relation to waste production and waste removal kinetics.

The production of contaminants in terms of organic matter, nitrogen compounds and carbon dioxide, as well as the consumption of oxygen by the fish were calculated for two feeding levels. The loading rates of the separate water treatment units were determined from the distribution of organic matter and nitrogen compounds between dissolved and solid matter. The removal of the contaminants was studied in pilot installations. Suspended solids could be efficiently removed by a lamella separator, while the elimination of ammonium was achieved with either a trickling filter or a submerged upflow filter loaded with settled fish tank effluent. The performance of the biofilm material attached to the filter medium was examined in batch experiments. It was found that ammonium removal by nitrification could be described by either 1/2-order kinetics or 0-order kinetics, depending on the ratio of the concentrations of dissolved oxygen and ammonium.

Based on the observed biofilm kinetics, a design philosophy is presented for a water treatment system consisting of a primary clarifier and an aerobic fixed-film reactor. In this philosophy, four possible types of reactor performance are described in relation to the ambient concentrations of ammonium and dissolved oxygen and to the diurnal variation of the ammonium loading of the reactor. Attention is paid to (a) hydraulic loading of the primary clarifier, (b) dimensions of the biological fixed-film reactor, (c) water recirculation rate and (d) water exchange rate.

A follow-up experiment to test the design methods, applied to primary lamella sedimentation and trickling filtration, gave satisfactory results for a high-density culture of the African catfish.

**Clarias lazera* (Cuvier and Valenciennes 1840) and *Clarias gariepinus* (Burchell 1822) are synonyms. Recently the nomenclature of the African *Clarias* species has been revised (Teugels, 1984).

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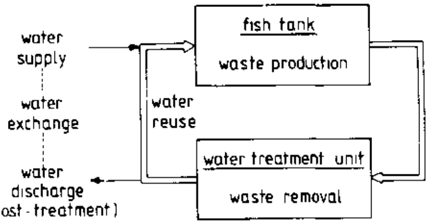

Fig. 1. Flow diagram of a water recirculation system.

INTRODUCTION

The potential of the African catfish for aquaculture has been demonstrated for ponds as well as for intensive culture in tanks under controlled indoor conditions (Hogendoorn and Koops, 1983; Hogendoorn et al., 1983). As a result of the high optimum water temperature and the high culture intensity (Huisman and Richter, 1987), water recirculation systems are preferable to flow-through systems for the intensive culture of the African catfish in areas with moderate climatological conditions.

Water quality control in a recirculation system is achieved by combining treatment and reuse of water with water exchange effected by supply of fresh water. A schematic diagram of the main items of a recirculation system is given in Fig. 1. The degree of water reuse depends on system design and treatment efficiencies (Liao and Mayo, 1972). Recirculation systems including a settling tank and an aerobic biological treatment unit for the removal of suspended solids and biological oxidation of both organic matter (to carbon dioxide) and ammonium (to nitrate; nitrification) allowed a high degree of water reuse (e.g. Liao and Mayo, 1974). Otte and Rosenthal (1979) demonstrated that water exchange could be minimized by introducing the denitrification process (biological reduction of nitrate to gaseous nitrogen) for the elimination of nitrate and ozonation for the elimination of non-biodegradable organic matter.

In general, high culture intensities require high flow rates of both recirculating water and exchange water, to attain sufficiently low waste levels in the fish tank, as Broussard and Simco (1976) demonstrated for high-density culture of channel catfish (*Ictalurus punctatus*). For this reason the general system configuration should support high hydraulic loading rates. Since lamella sedimentation is a compact separation technique for suspended solids (Forsell and Hedström, 1975), its principle may be successfully applied in intensive fish culture for primary clarification (Parker, 1981).

High hydraulic loading rates of a biological water treatment unit require an adequate sludge retention. Because high cell residence times are easily maintained, fixed-film processes are especially attractive for water treatment in high-density fish cultures. Previous results (unpublished) using fluidized bed technology, however, did not support its application in intensive fish culture.

Therefore, the combination of a lamella separator and an aerobic biological reactor containing a bacterial film attached to a fixed matrix (e.g. trickling filter and submerged filter) was judged to be the most appropriate system configuration for intensive fish culture.

This paper presents a study on the development and performance of a recirculation system for African catfish culture, with emphasis on water (and energy) conservation. The study comprises three connected parts, dealing with waste production and waste removal, design philosophy and system performance.

WASTE PRODUCTION AND WASTE REMOVAL

Materials and methods

Experimental facilities and definitions

Pilot installations for the design of a recirculation system were obtained by packing three circular columns, providing effective filter volumes of 0.05, 0.14 and 0.39 m^3, with a light-weight plastic filter medium (Filterpak CR50, Mass Transfer International, Heversham, Cumbria, U.K.), having a specific surface area of 200 $m^2 m^{-3}$. Two reactors were operated as trickling filters (0.05 and 0.39 m^3) and one (0.14 m^3) as a submerged upflow filter. In the columns of the 0.05-m^3 trickling filter and the submerged filter respectively, 0.62 and 0.42 m^2 of the packing material was placed in a basket, enabling easy removal and replacement. Each column was connected to a fish tank (circa 1 m^3) and a lamella separator (plate angle 45°), operated as a primary clarifier. Forced aeration of the water was used only prior to the biological treatment in the submerged filter in between the clarifier and the biological reactor; additional internal aeration of the submerged filter was done during a part of the operational period.

The fish tanks connected to the 0.39-m^3 trickling filter and to the submerged filter were stocked at densities of 90–160 kg m^{-3} and 20–40 kg m^{-3} respectively with African catfish weighing 5–250 g during the period of operation. Trouvit pellets (Trouw & Co., Putten, The Netherlands) were fed at the level recommended by Hogendoorn et al. (1983) in the submerged filter system and about half this level in the trickling filter system. The water temperature of these systems was maintained at 25 ± 1°C; the water temperature of the system including the 0.05-m^3 trickling filter was maintained at 15 ± 1°C in order to quantify the effect of temperature on the waste removal kinetics. This system was stocked with rainbow trout (*Salmo gairdneri*) at 20 kg m^{-3}. In all systems the fish performed as waste producers.

The relevant unit operations of the pilot installations for the African catfish systems are expressed in units independent of the actual system dimensions and are presented in round figures in Table 1. Note that the unit operations

TABLE 1

Average unit operations of the pilot installations for water treatment in recirculation systems for the African catfish

Unit operation	Water treatment system	
	lamella separator submerged filter	lamella separator trickling filter
Feed loading rate (g $m^{-2}d^{-1}$)	12	18
Water recirculation rate (m^3 $kg^{-1}d^{-1}$)	52	20
Water exchange rate (m^3 $kg^{-1}d^{-1}$)	0.60	0.45
Hydraulic loading rate (m^3 $m^{-2}d^{-1}$)		
lamella separator	9	15
biological reactor	450	210

refer to the feed supply which is the origin of waste production. Since attached growth of bacteria is found for any underwater surface including pipe and tank walls (Wickins, 1981), the introduced quantity "feed loading rate" (g $m^{-2}d^{-1}$) is defined as the ratio of the daily feed supply and the total wet surface of the system. The "water recirculation rate" or "water reuse rate" (m^3 $kg^{-1}d^{-1}$) is the volume of recirculated water per kg feed per day and the "water exchange rate" (m^3 $kg^{-1}d^{-1}$) the volume of discharged water per kg feed per day. The hydraulic loading rate or superficial water velocity (m^3 $m^{-2}d^{-1}$) is defined as the volume of water treated daily per unit surface area, using the sum of the projections of the plates on the horizontal plane as surface area for the lamella separators (Forsell and Hedström, 1975) and the surface of the cross-sectional area of the columns for the biological reactors.

Experimental procedures

Grab samples or 24-h composite samples were taken daily at several places in the systems and were analysed for COD (chemical oxygen demand) or ammonium, nitrite and nitrate according to Standard Methods. Dissolved oxygen and pH were measured directly in the water cycle; sodium bicarbonate was added to maintain pH 7.

The overall capacities for the removal of COD and ammonium by the lamella separator and the biological reactor respectively, were determined during periods of constant conditions in the complete system. The performance of the lamella separators was examined at varying hydraulic loading rates by changing the number of plates. The lamella separators were loaded with fish tank effluent (African catfish) when the system was operated in the recirculation mode as well as the flow-through mode. Influent and effluent samples were analysed for total COD and dissolved COD. The biodegradability of organic matter was determined occasionally by analyses of initial COD of the fish tank effluent and COD after 5 days of aeration at 20°C.

The ammonium production was determined once during 24 h by analyses of 1-h composite samples of the effluent of a fish tank operated in the flow-through mode.

Direct measurements of the ammonium removal rate of the attached biofilm in the pilot reactors were performed in separate batch experiments using the biofilm samples present in the baskets in the 0.05-m^3 trickling filter and the submerged filter. The submerged filter biofilm was only available for these experiments during the second month after the start-up. The biofilm samples were put in a 15-l reactor tank and submerged in primary clarified fish tank effluent coming from the corresponding recirculation system, under the corresponding conditions of temperature and pH. Initial ammonium concentrations were varied between 0 and 12 g NH_4-N m^{-3}, if necessary by adding ammonium chloride; initial dissolved oxygen concentrations were near saturation as a result of aeration prior to the experiments. At the start of the experiment the reactor tank was closed without enclosure of air and the water phase was stirred continuously. The oxygen consumption rate of the biofilm was determined in relation to the ambient concentration of ammonium by means of a continuous monitoring of the concentration of dissolved oxygen (electrode). The ammonium removal rate was derived from the oxygen consumption rate using the stoichiometric coefficient (4.57 g O_2 g^{-1} NH_4-N) and correcting for oxygen consumption due to oxidation of organic matter present in the fish tank effluent (NH_4-N blank). The biofilm samples were replaced in the reactor column immediately after termination of the measurements.

All calculations of the ammonium removal rate are based on the apparent (specific) surface area of the packing material.

Waste production calculations

Under the experimental conditions all produced waste originates from the feed ration. Uneaten feed not taken into consideration, the quality and quantity of the waste depend on feeding level, feed composition, feed digestibility and utilization of digested feed (Fig. 2). Experimental data on these subjects are reported in detail for the African catfish by Hogendoorn (1983) and Henken et al. (1985). Those data are used for calculations of mass balances concerning dry matter and nitrogen compounds, as well as oxygen consumption and carbon dioxide production for rearing African catfish from 1 to 400 g body weight at 25°C and feeding levels of 8.4 and 16.8 g feed per day per kilogram of metabolic weight (g d^{-1} $kg^{-0.8}$). The latter feeding level is recommended by Hogendoorn et al. (1983).

The analysed feed composition (Trouvit 2) is presented in Table 2. Digested and undigested dietary dry matter and crude protein are calculated using (interpolated) apparent digestibility coefficients based on samples of the con-

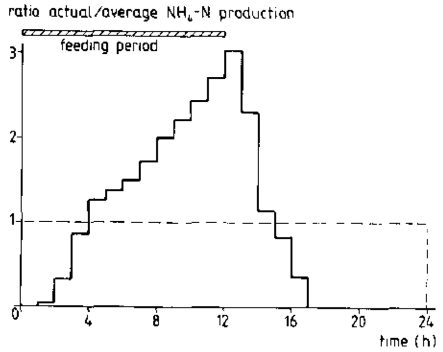

Fig. 3. Diurnal variation of the ammonium production by African catfish fed for 12 h at 25°C. Average production rate (dotted line): assuming a constant release of the total amount of ammonium during 24 h.

showed a relatively high and constant removal rate under circumstances characterized by high concentrations of dissolved oxygen and ammonium. In the submerged filter operated without internal aeration, the concentration of dissolved oxygen generally decreased from about 6 to 2 g m^{-3} during the residence of the water in the reactor. With internal aeration the dissolved oxygen concentration remained rather constant at a value of 6.4 g m^{-3}, showing little, if any, difference between influent and effluent concentrations.

Nitrite concentrations in the fish tanks were low during the experimental periods: 1.24 ± 0.75 g NO_2-N m^{-3} in the trickling filter system and 0.75 ± 0.43 g m^{-3} in the submerged filter system. The concentration of nitrate varied strongly in both systems, but remained low (up to 30 g NO_3-N m^{-3}).

The ammonium removal rate of the biofilm samples from the submerged filter and the 0.05-m^3 trickling filter have been plotted in relation to the concentrations of ammonium and dissolved oxygen in Fig. 5. At a fixed value of

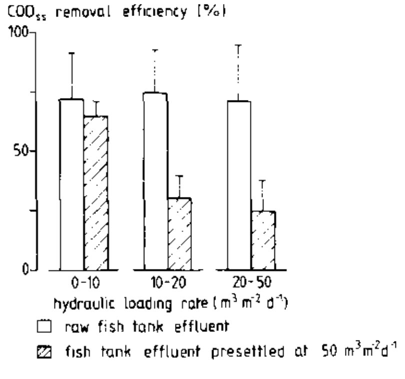

Fig. 4. Removal of suspended solids-COD (COD_{ss}) by lamella sedimentation.

TABLE 4

Ammonium removal rates and ambient concentrations of dissolved oxygen and ammonium ($\mu \pm$ SD (n)), *observed in pilot reactors loaded with primarily clarified fish tank effluent of African catfish* at 25°C and pH 7. Submerged filter without (a) and with (b) internal aeration

	Submerged filter		Trickling filter
	a	b	
NH_4-N removal rate (g $m^{-2}d^{-1}$)	0.36 ± 0.10 (17)	0.42 ± 0.19 (8)	0.55 ± 0.13 (15)
O_2 concentration (g m^{-3})	3.9 ± 1.3 (45)	6.4 ± 0.9 (25)	6.4 ± 0.7 (15)
NH_4-N concentration (g m^{-3})	3.2 ± 3.7 (17)	1.0 ± 0.7 (8)	9.9 ± 6.9 (15)

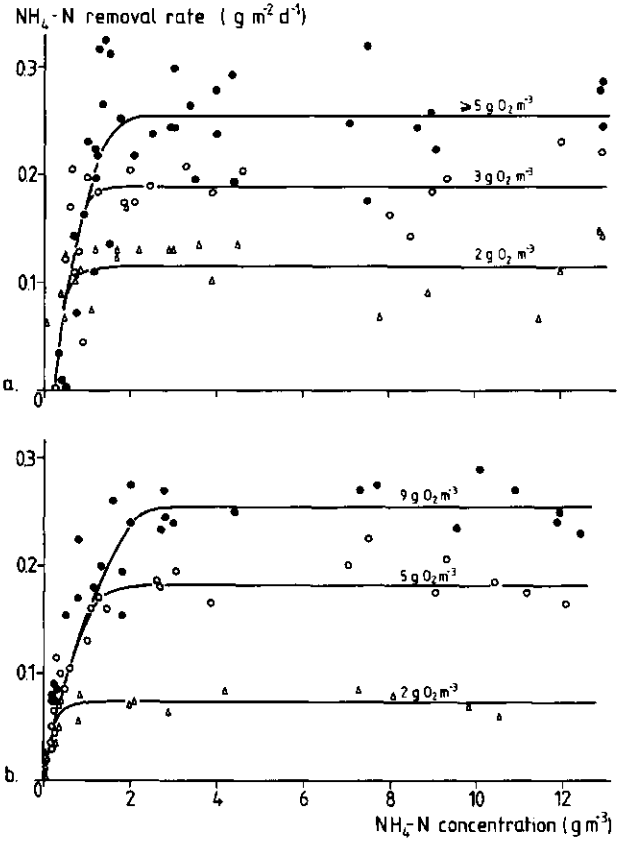

Fig. 5. Ammonium removal rate in relation to the concentrations of ammonium and dissolved oxygen in fixed-film reactors loaded with settled fish tank effluent: a. submerged upflow filter, culture of African catfish, 25°C; b. trickling filter, culture of rainbow trout, 15°C.

the oxygen concentration, the ammonium removal rate increased strongly with increasing concentrations of ammonium, up to a maximum removal rate proportional to the concentration of dissolved oxygen. However, dissolved oxygen concentrations exceeding 5 g m^{-3}, in combination with high ammonium concentrations, did not result in a significantly increased average removal rate of the submerged filter biofilm, but the removal rate did show increased fluctuations (Fig. 5a). This phenomenon was not observed from the trickling filter biofilm operated at 15°C (Fig. 5b).

Discussion

Quantities and distribution of waste products

The amounts of waste determine the loading of the water treatment units and are, therefore, important figures for the dimensions of those units. Considering the production of dry matter to be identical to the faecal loss, and the production of nitrogen compounds to the sum of faecal loss and non-faecal loss, Table 3 demonstrates that, in accordance with the relation between feeding level and feed utilization (Hogendoorn et al., 1983; Henken et al., 1987), the production of wastes is also significantly affected by the feeding level. Respectively 437 and 517 g dry matter kg^{-1} feed, and 50.7 and 55.7 g N kg^{-1} are produced at feeding levels of 8.4 and 16.8 g d^{-1} $kg^{-0.8}$. The shares of these amounts that will be removed by either the primary clarifier or the biological reactor strongly depend on the distribution between dissolved and solid matter.

The observed removal efficiencies of the lamella separator show that 70–75% of the suspended solids settle with an average settling velocity equal to or greater than 50 m d^{-1}. However, high standard deviations, especially for hydraulic loading rates exceeding 20 $m^3m^{-2}d^{-1}$, indicate that the average settling velocity fluctuated widely, due to a varying particle size distribution, probably caused by disintegration of the original faecal material due to water movements in the fish tanks. This phenomenon is also observed in trout culture (Querellou et al., 1982). The total COD removal efficiency of the lamella separator operated in a recirculation system is lower than the removal efficiency for operation in a flow-through system as a result of the accumulation of dissolved non-biodegradable organic matter.

Due to the unintended retention of solid matter in the fish tank outlet construction, there are four items in the mass balance determining the distribution of waste products between the primary clarifier and the biological reactor: dissolved matter (A), solid matter retained in the fish tank outlet (B), solid matter settled in the clarifier (C) and non-settleable solids (D). Relationships between these items (in %) are provided by mass conservation (eqn. 1) and the performance of the primary clarifier (eqns. 2 and 3):

$$\begin{aligned} &A+B+C+D &&= 100 \quad (1)\\ &0.73\,(C+D) &&= C \quad (2)\\ &0.33\,(A+C+D) &&= C \quad (3) \end{aligned}$$

A fourth relationship is based on the difference in feed digestibility resulting from a quantitative sampling method of faecal material after defaecation versus sampling the contents of the lower part of the intestine (Henken et al., 1985). The observed disparity in feed digestibility is assumed to be caused by loss of faecal material due to leaching and wash-out of small particles. The calculated loss of faecal waste strongly increased with decreasing feeding levels. This phenomenon has also been observed for trout by Butz and Vens-Cappell (1982), although the absolute amounts of faecal waste found for African catfish are higher. For average conditions in the experiment of Henken et al., separate relationships are derived for the loss of faecal dry matter and faecal nitrogen compounds at two feeding levels:

$$\text{dry matter}\quad \begin{aligned} &8.4\ \mathrm{g\,d^{-1}\,kg^{-0.8}}: &&A+D=58 \quad (4a)\\ &16.8\ \mathrm{g\,d^{-1}\,kg^{-0.8}}: &&A+D=45 \quad (4b) \end{aligned}$$

$$\text{nitrogen}\quad \begin{aligned} &8.4\ \mathrm{g\,d^{-1}\,kg^{-0.8}}: &&A+D=45 \quad (4c)\\ &16.8\ \mathrm{g\,d^{-1}\,kg^{-0.8}}: &&A+D=25 \quad (4d) \end{aligned}$$

Resolving each set of four equations for the distribution of faecal material allows an estimation of the distribution of the total amounts of dry matter and nitrogen compounds in waste produced at the two feeding levels (Table 5).

On the basis of the presented data, it may be concluded that the feeding level does not only affect the amounts of waste products, but also their distribution

TABLE 5

Estimated distribution of dry matter and nitrogen per kg feed between dissolved material (I), solids retained in the fish tank outlet (II), solids settled in the clarifier (III) and non-settleable solids (IV) for African catfish waste

Feeding level ($\mathrm{g\,d^{-1}\,kg^{-0.8}}$)	Distribution ($\mathrm{g\,kg^{-1}}$)							
	dry matter				nitrogen			
	I	II	III	IV	I	II	III	IV
8.4	210	61	122	44	35	9	5	2
16.8	186	176	114	41	31	17	6	1

between solid matter and dissolved matter, which in its turn affects the loading of the separate water treatment units. In the recirculation systems described, 1 kg feed results in a loading of the biological reactor with approximately 265 g BOD and 37 g N at a feeding level of 8.4 g d^{-1} $kg^{-0.8}$, while a feeding level of 16.8 g d^{-1} $kg^{-0.8}$ results in a lower (!) loading of 240 g BOD and 32 g N per kg feed, due to the smaller proportion of dissolved faecal matter in combination with the application of a primary clarifier. The figures also show that the efficiency of the clarifier can be increased by an improved outlet construction of the fish tank.

Ammonium removal rate

The characteristic ammonium removal performance of the biofilm samples with respect to the concentration in the bulk liquid of the two substrates, viz. ammonium (electron donor) and oxygen (electron acceptor), is completely described by models for biofilm kinetics developed by Williamson and McCarty (1976a,b) and Harremoës (1978, 1982). The substrates have to be transported from the bulk liquid into the biofilm and the reaction products have to be transported out again. The phenomena involved are molecular diffusion and the nitrification reaction in the biofilm. The overall process may be reaction-rate-limited if the biofilm is completely penetrated by both substrates, or diffusion-rate-limited if the biofilm is partly penetrated by one or both substrates. Harremoës (1978) demonstrated that the rate of the overall process is accurately described by 1/2-order kinetics with respect to the bulk concentration of the diffusion-rate-limiting substrate and by 0-order kinetics with respect to the bulk concentration of the substrate that is not rate-limiting or when the overall process is reaction-rate-limited. The results of the kinetic experiments (Fig. 5) demonstrate that the submerged filter biofilm had been reaction-rate-limited for ammonium concentrations exceeding about 2 g NH_4-N m^{-3} and oxygen concentrations higher than approximately 5 g m^{-3}. This can be understood since the biofilm sample was incubated in the submerged filter operated without internal aeration, resulting in an average oxygen concentration lower than 5 g m^{-3} (Table 4). The ammonium removal rate of the 0.05-m^3 trickling filter biofilm observed for concentrations of 9 g O_2m^{-3} and $>$ 2.5 g NH_4-N m^{-3} (0.25 g NH_4-N $m^{-2}d^{-1}$) is similar to the higher removal rates reported by Liao and Mayo (1974) for reactors operated in salmonid cultures and receiving water with low contents of organic matter.

The oxygen/ammonium concentration ratio at which the transition occurs from ammonium limitation to oxygen limitation and vice versa is determined from the kinetic experiments (Fig. 5) and was 3.6 ± 0.8.

Applying the information from the kinetic experiments to the observed performance of the pilot reactors (Table 4), it is clear that the submerged filter without internal aeration was oxygen-limited. The increased oxygen concentration as a result of internal aeration resulted in an increased ammonium removal rate. However, based on the oxygen concentration of 3.9 g m^{-3} without aeration, the theory on biofilm kinetics predicts a removal rate of 0.46 g m^{-2} d^{-1} for the oxygen concentration with aeration. This removal rate was not reached because the new biofilm conditions involved an ammonium-limited biofilm. The difference of the removal rates resulting from the kinetic experiments and the pilot reactor, under comparable conditions (0.26 and 0.36 g $m^{-2}d^{-1}$ respectively), may be the result of difference in age of the biofilm: the kinetic experiments were carried out with biofilm samples after one month of incubation; the removal rate of the pilot reactor was determined for a period of several months. The trickling filter demonstrated a high removal rate during the experimental period, under conditions of either high but rate-limiting oxygen concentrations or high oxygen concentrations and a reaction-rate-limited biofilm. The difference in removal rate between the 0.05-m^3 and the 0.39-m^3 trickling filter biofilm was attributed entirely to the different temperatures during the operational period, according to a temperature-activity coefficient of 1.08, typical for trickling filters (Metcalf & Eddy, 1979).

It follows from comparison of the feed loading rate, the ammonium load per kg feed and the observed ammonium removal rate that the pilot reactors removed 87–100% of the daily ammonium load by nitrification. Denitrification in the lamella separator was found to be a significant nitrate elimination process, accounting for 40–80% of the nitrate produced by the pilot reactors, depending on the frequency of cleaning of the lamella separator. The remaining amounts of both ammonium and nitrate were removed by water exchange.

DESIGN PHILOSOPHY

The African catfish can practice aquatic as well as aerial respiration and therefore has a low aquatic oxygen threshold value. Consequently the general objective of the water treatment is control of the concentrations of ammonium, biodegradable organic matter and suspended solids. Suspended solids are efficiently removed by a primary clarifier resulting in a decreased loading of the sequent biological reactor. The production of excess sludge in the biological reactor is low and a secondary clarifier is superfluous. Generally, the biological oxidation of organic matter is achieved easily (e.g. Liao and Mayo, 1974; Otte and Rosenthal, 1979). Therefore, the accumulation of ammonium may be regarded as the first capacity-limiting factor of a recirculation system for the African catfish, and the design of the recirculation system is focussed on the biological conversion of toxic ammonium into nitrate, which is relatively harmless (Wickins, 1981; Muir, 1982).

Primary clarifier

Hydraulic loading rates $\leqq 20$ m^3 $m^{-2}d^{-1}$ are recommended in order to anticipate changes in particle size distribution, as has been discussed in the first part of this paper. However, although the contribution of small suspended particles to total COD was found to be negligible, hydraulic loading rates of about 10 m^3 $m^{-2}d^{-1}$ may be preferred in order to prevent accumulation of these particles in the recirculation system.

The required settling surface area is to be calculated after determining the required water recirculation rate.

Biological reactor

The biofilm surface area (A, m^2) required for the complete removal of the ammonium load (L, g d^{-1}) is obtained according to

$$A = k\ (L/r), \tag{5}$$

where k is a constant and r denotes the ammonium removal rate of the biofilm (g $m^{-2}d^{-1}$). However, as a result of the diurnal variation of the ammonium production, the ammonium loading of the biological reactor is described by $d \cdot L$, were d represents the diurnal variation of the ammonium load, adopted from Fig. 3. The constant k is a fixed value in the range $1 \leqq k \leqq d_{max}$, depending on the intended type of reactor performance. The biofilm kinetics discussed imply that r may be affected either by the concentration of ammonium ($C_{NH_4\text{-}N}$) or the concentration of dissolved oxygen (C_{O_2}). From an engineering point of view, the concentration ratio at which transition occurs from 1/2-order kinetics to 0-order kinetics ($C^*_{O_2}/C^*_{NH_4\text{-}N}$) and the connected removal rate (r^*) are important figures with respect to reactor design. The maximum admissible ammonium concentration (C_{max}) is determined by the tolerance of the fish for ammonium, while the ambient C_{O_2} in the reactor is the result of reactor type and mode of operation. Based on the possible combinations of $C_{NH_4\text{-}N}$ and C_{O_2}, four types of reactor performance can be distinguished. These types of reactor performance and the connected design philosophy are schematically presented (Fig. 6) in relation to the diurnal variation of the ammonium load:

a. 0-order removal kinetics in relation to $C_{NH_4\text{-}N}$ as a result of either reaction rate limitation or a constant concentration of the rate-limiting substrate (oxygen) in the reactor (Fig. 6a). The required biofilm surface area A is obtained by substituting $k=1$ and $r=r^*$ in eqn. 5. During the period when $d>k$, the excess ammonium will accumulate in the system but it will be removed during the period when $d<k$. Therefore, a minimum water volume of the system is required in order to avoid $C_{NH_4\text{-}N}$ exceeding C_{max} at $t=t_2$. This will be discussed further in relation to the water recirculation rate. System performance based on 0-order kinetics can be characterized by a high constant r, but the system is vulnerable with respect to unexpected ammonium peak loads.

b. 1/2-order removal kinetics in relation to $C_{NH_4\text{-}N}$; $C_{NH_4\text{-}N} \leqq C^*_{NH_4\text{-}N}$ as a result of biofilm activity (Fig. 6b). A is found by substitution of $k=d_{max}$ and

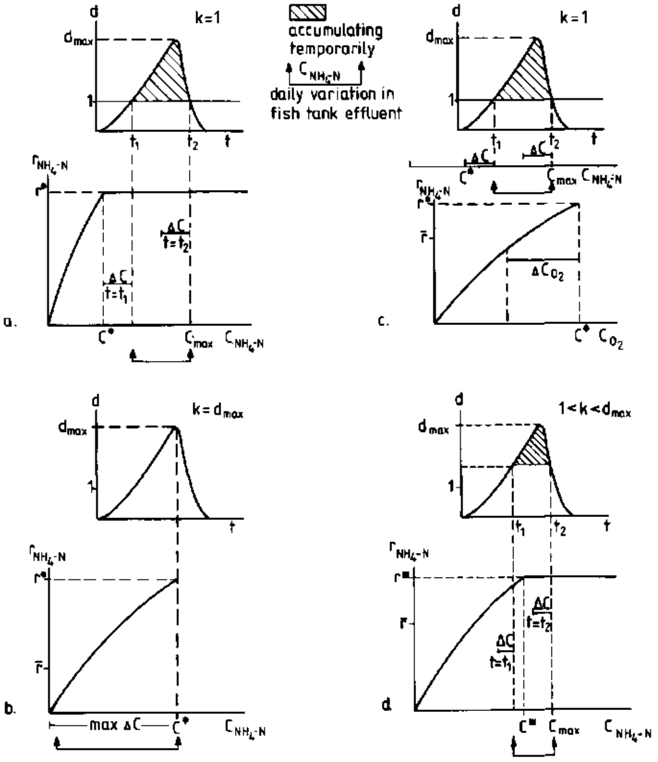

Fig. 6. Reactor performance in relation to the diurnal variation d of the ammonium loading of fixed-biofilm reactors (12-h feeding period), operated at different concentration ratios for dissolved oxygen and ammonium: a. 0-order kinetics in relation to $C_{NH_4\text{-}N}$; b. 1/2-order kinetics in relation to $C_{NH_4\text{-}N}$; c. 1/2-order kinetics in relation to C_{O_2}; d. alternating 0-order and 1/2-order kinetics.

$r=r^*$ (eqn. 5). The system is characterized by a low average ammonium removal rate ($\bar{r}$) and therefore a large reactor, but will show a high stability with respect to $C_{NH_4\text{-}N}$.

c. 1/2-order removal kinetics in relation to C_{O_2}; $C_{O_2} \leqq C^*_{O_2}$ as a result of biofilm activity, e.g., in a submerged filter without internal O_2 supply (Fig. 6c). A is found by substitution of $k=1$ and $r=\bar{r}$ in eqn. 5. With respect to $C_{NH_4\text{-}N}$ the system will show a similar performance as described under a.

d. alternating 0-order and 1/2-order removal kinetics in relation to $C_{NH_4\text{-}N}$ (Fig. 6d). In fact this is a combination of reactor performance type a and b or b and c. The combination of type a and b may be considered to combine the benefits of both types of reactor performance. A is obtained by substituting a fixed value $1<k<d_{max}$ and $r=r^*$ in eqn. 5.

The required biofilm surface area is calculated for each type of reactor performance and is presented in Table 6. As a result of the differences for the

TABLE 6

Review of system characteristics resulting from design based on biofilm kinetics with respect to the removal of ammonium (Fig. 6)

	Type of reactor performance				
	a_1	a_2	b	c	d
Biofilm surface area (m^2)	58	58	175	80	87
Water recirculation rate (m^3d^{-1})	10	50	48	32	24
Total water volume (m^3)	3	1		2	1.3
NH_4-N removal per cycle ($g\ m^{-3}$)	3.1	0.6	$\leqq 2.0$	1.0	$\leqq 2.0$
Min. NH_4-N conc. fish tank ($g\ m^{-3}$)	5.1	2.6	(0.1)	2.0	(2.0)
Max. NH_4-N conc. fish tank ($g\ m^{-3}$)	10.0	17.0	2.0	10.0	10.0

a. 0-order kinetics (a_1, a_2: different combinations of C_{max}, V and Q_r); b. 1/2-order kinetics in relation to $C_{NH_4\text{-}N}$; c. 1/2-order kinetics in relation to C_{O_2}; d. alternating 0-order and 1/2-order kinetics ($k = 1.5$).

All figures refer to a daily feed supply of 1 kg, feeding level 16.8 g d^{-1} $kg^{-0.8}$ and removal kinetics found for the pilot-reactors.

required biofilm surface areas, the loading rates vary between 1.5 and 4.0 g BOD $m^{-2}d^{-1}$ (biochemical oxygen demand) and between 0.18 and 0.55 g NH_4-N $m^{-2}d^{-1}$.

The volume of the filter bed is determined as a result of the specific surface area of the selected filter medium. The final shape of the reactor is determined by the combination of intended hydraulic loading rate and the required water recirculation rate. For a plastic filter medium with a high specific surface area, 100–200 $m^3\ m^{-2}d^{-1}$ was found to be a suitable hydraulic loading rate for trickling filtration. In general trickling filters are attractive for application in fish culture for two reasons. Firstly, high and constant oxygen levels are maintained easily. Secondly, the carbon dioxide produced by the fish as well as by the biofilm is stripped easily from the percolating water.

Water recirculation rate

During each run of the water through the biological reactor, the biofilm removes a certain amount of ammonium, resulting in a change of $C_{NH_4\text{-}N}$ (ΔC). For a proper supply of ammonium to the biological reactor and a proper removal of ammonium from the fish tank, a minimum water recirculation rate (Q_r, m^3 d^{-1}) is required:

$$Q_r = k\ (L/\Delta C) \tag{6}$$

The consequence of the diurnal variation of the ammonium loading rate of the reactor are discussed for the described types of reactor performance:

a. ΔC is a constant (0-order removal kinetics, $k=1$, Fig. 6a). The daily accumulation of NH_4-N is described by

$$NH_4\text{-N accumulation} = C_{max} - C^* - \Delta C = \frac{L}{V}\int_{t_1}^{t_2} (d-k)\,dt \qquad (7)$$

where V denotes the water volume of the system. Eqn. 7 shows that C_{max}, V and ΔC cannot be chosen independent of each other: $\Delta C \leqq C_{max} - C^*$. A low value of ΔC (Q_r high) results in a relatively small water volume of the system. Note that the position of ΔC is related to d and moves along the $C_{NH_4\text{-}N}$ axis between C^* and C_{max}. The daily variation of $C_{NH_4\text{-}N}$ in the fish tank is limited by $C^* + \Delta C$ and C_{max}. Eqn. 7 can be resolved by adopting the daily variation of the ammonium loading of the biological reactor from Fig. 3.

b. ΔC is not constant (1/2-order removal kinetics in relation to $C_{NH_4\text{-}N}$, $k = d_{max}$, Fig. 6b). Q_r is obtained from eqn. 6 with $\Delta C = C^*$, corresponding to the maximum ammonium loading rate. $C_{NH_4\text{-}N}$ in the fish tank will show a maximum value equal to C^*.

c. ΔC is constant (1/2-order removal kinetics in relation to C_{O_2}, $k=1$, Fig. 6c). With respect to Q_r and V the same eqns. may by applied as discussed in relation to reactor performance type a.

d. ΔC is constant when $t_1 < t < t_2$ (alternating 0-order and 1/2-order removal kinetics, $1 < k < d_{max}$, Fig. 6d). Q_r and V are obtained from eqns. 6 and 7 applied to the period with 0-order kinetics with the same restrictions as mentioned under a.

Some possible combinations of Q_r, V, ΔC and the daily variation of $C_{NH_4\text{-}N}$ are calculated for the various types of reactor performance and are presented in Table 6. The daily variation of $C_{NH_4\text{-}N}$ for reactor performance types a, c and d is limited by $C_{max} = 10$ g NH_4-N m^{-3}, corresponding to about 0.05 g NH_3-N m^{-3}, which may be regarded as the maximum admissible concentration (Wickins, 1981; Muir, 1982). However, some combinations of V and Q_r will inevitably result in C_{max} exceeding 10 g NH_4-N m^{-3} as is demonstrated by a_2 in Table 6. Very low values of $C_{NH_4\text{-}N}$ are typical for reactor performance type b. This may be illustrated by figures reported by Rosenthal et al. (1981) and Krüner and Rosenthal (1983) who used trickling filters under conditions inducing 1/2-order ammonium removal kinetics.

The described design philosophy with respect to Q_r results in a constant Q_r during the day. However, another possibility is anticipating the daily variation of the ammonium loading rate by tuning Q_r to the diurnal variation of ammonium production.

Water exchange rate

In addition to the denitrification process in the lamella separator, nitrate is also removed by exchange of water. Assuming that the supply water does not contain any nitrate, the water exchange rate (Q_e, $m^3\,d^{-1}$) is determined by

$$Q_e = N/C_{N,max} \tag{8}$$

where N represents the net nitrate load ($g\ d^{-1}$), i.e. the daily amount of nitrified ammonium minus the amount of daily denitrified nitrate, and $C_{N,max}$ denotes the tolerance of the fish for nitrate. However, no data on $C_{N,max}$ are available. Assuming that 50% of the nitrified ammonium is denitrified and $C_{N,max} = 100$ g NO_3-N m^{-3}, Q_e amounts to 0.16 m^3d^{-1} per kg feed (16.8 g d^{-1} $kg^{-0.8}$).

SYSTEM PERFORMANCE

Facilities and procedures

The follow-up to the pilot installation experiments was a verification study on overall performance and capability of the recirculation system in high-density culture of the African catfish.

The starting points for the system design were a daily feed supply of 2 kg and a minimized size of the water treatment installation. Therefore, the combination of a primary lamella separator and a trickling filter operated in reactor performance type a (Fig. 6a) was selected.

A rectangular fish tank with an effective volume of 0.8 m^3 was stocked with 32 kg African catfish (mean body weight 38 g). The feeding level was maintained at 8.4 g d^{-1} $kg^{-0.8}$ and the feed was supplied during 16 h. Fish growth was checked each month by weighing. The temperature of the water was maintained at 25 ± 0.5 °C.

The realized water recirculation rate was 52 ± 10 m^3d^{-1}. A total effective settling surface area of 3.9 m^2 was placed in the lamella separator (11 plates, 0.5 m^2 each, plate angle 45°), in order to meet a suitable hydraulic loading rate (13 m^3 $m^{-2}d^{-1}$). The trickling filter column had an effective volume of 0.62 m^3, providing 124 m^2 of a plastic packing material.

The cross-sectional surface area of the column was 0.33 m^2, resulting in a hydraulic loading rate of 158 m^3 $m^{-2}d^{-1}$. The total water volume of the system was 1.4 m^3 and the total wet surface area of the complete system was about 150 m^2. A minimum water exchange was effected by the batch-wise sludge discharge from the lamella separator; the value of the overall water exchange rate was not fixed since the determination of an acceptable water exchange rate was one of the objectives of the experiment.

Water quality in the fish tank was checked daily, just before the fish were fed, by determining dissolved oxygen, pH, ammonium, nitrite and nitrate. Sodium bicarbonate was added for pH control (pH 7.0–7.5).

The configuration of the total system in shown in Fig. 7.

Fig. 7. The water recirculation system developed for the culture of African catfish.

Performance

The general performance of the system is illustrated in Fig. 8 by the course of the water quality parameters for a 2-month period during which the 2 kg

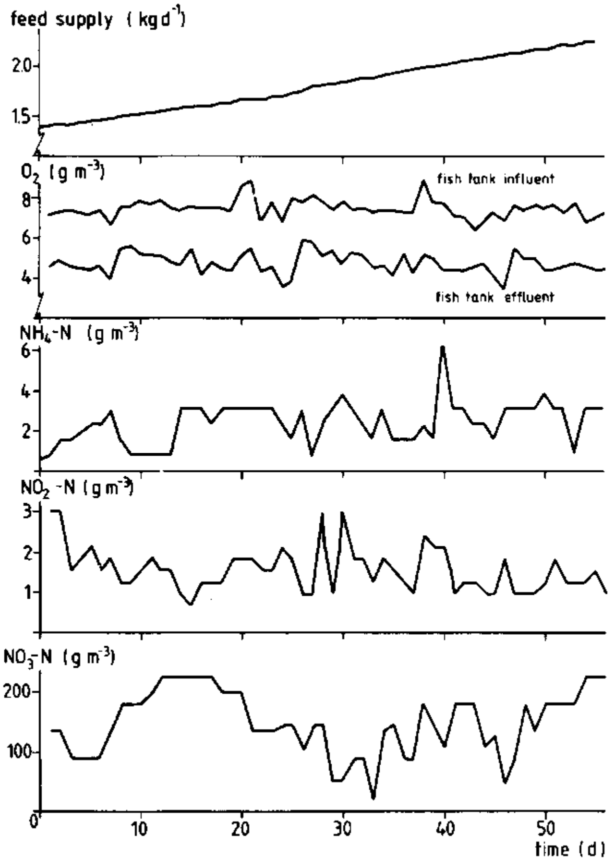

Fig. 8. General performance of the developed recirculation system: water quality parameters in relation to the feed supply for the last 2 months of the experimental period. Water samples were analysed just before feeding.

daily feed supply was reached and even exceeded. After the removal of the suspended solids in the lamella separator the water was clear, but had a dark brownish colour due to the accumulation of dissolved humic matter (Fig. 9).

Trickling filtration resulted in high dissolved oxygen concentrations at the inlet of the fish tank (7.5 ± 0.5 g m^{-3}), while even at the fish tank outlet the concentration was 4.7 ± 0.5 g m^{-3}, as the combined result of a short average residence time of the water in the fish tank (0.3 h) and the partial aerial respiration of the African catfish.

The observed ammonium concentrations in the fish tank (2.5 ± 1.1 g NH_4-N m^{-3}) were comparable to or lower than the expected average concentration based on the design philosophy (3.5 g NH_4-N m^{-3}). The removal of ammonium per cycle of the trickling filter was 1.5 ± 0.5 g NH_4-N m^{-3}, while the

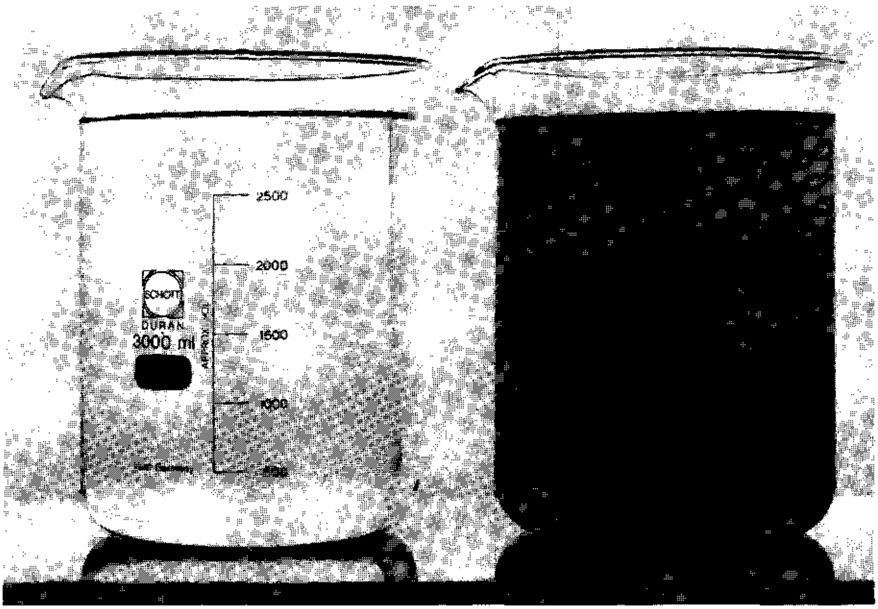

Fig. 9. Illustration of the accumulation of dissolved non-biodegradable organic matter in the recirculation system: drinking water used for water supply and recirculating fish tank water.

expected value was 1.4 g m^{-3}. However, a few measurements of the ammonium concentration just after feeding were lower than the expected maximum concentration: 11.9 ± 0.4 g NH_4-N m^{-3} versus about 20 g m^{-3}. This difference may be caused by a feeding period of 16 h instead of 12 h, affecting the amount of ammonium that will accumulate temporarily in the system. Another possible explanation is that the ammonium removal rate of the trickling filter biofilm exceeded the design removal rate (0.55 g NH_4-N $m^{-2}d^{-1}$).

The *concentration of nitrite measured just before feeding* was 1.5 ± 0.6 g NO_2-N m^{-3}, but it was observed that the nitrite concentration increased several times just after the feeding period. The nitrate concentration was found to fluctuate strongly (20–220 g NO_3-N m^{-3}) without a specific pattern during the day. The observed fluctuations can be explained by the batch-wise discharge of sludge from the lamella separator, resulting in an intermittant water exchange as well as a varying denitrification capacity in the lamella separator. The average water exchange rate during the experimental period was 0.16 m^3d^{-1}, i.e. 0.08 m^3d^{-1} per kg feed. The net nitrate production was about 11 g NO_3-N kg^{-1} feed, indicating that about 65% of the nitrified ammonium had been denitrified. This net nitrate production involves an alkalinity requirement equivalent to about 80 g $NaHCO_3$ per kg feed, which was confirmed in the experiment.

The regular water analyses were terminated at the moment a daily feed supply of 2.3 kg was reached.

At the start of the 2-month period presented in Fig. 8 the total fish biomass was 100 kg (mean body weight 136 g). In one month the biomass increased to 152 kg (mean body weight 188 g, mortality 2 fish) with a feed conversion of 1.0. During the next month the total fish biomass increased to 212 kg (mean body weight 265 g, mortality 8 fish). The feed conversion was as low as 0.97. In this period 4.9 m^3 make-up water had been used to produce 60 kg African catfish. After termination of the water analyses, the recirculation system was operated for several weeks with a daily feed supply of 2.0–2.5 kg resulting in a final stocking density of 436 kg per m^3 effective volume of the fish tank.

The results indicate that the presented design philosophy is an effective tool for designing water recirculation systems for aquaculture purposes. The water quality maintained in the recirculation system described did not have any negative effect on growth of the African catfish. Nevertheless, the daily peak concentrations of nitrite are undesirable. Therefore, nitrite may be regarded as the next capacity-limiting factor in a recirculation system including a biological reactor operated with 0-order ammonium removal kinetics.

SUMMARY AND CONCLUSIONS

The final performance of a water recirculation system is the resultant of waste production by the fish and waste removal by the water treatment installation. The water treatment installation included a primary clarifier and a biological reactor.

With other conditions, such as feed composition and water temperature, set constant, the characteristics of the production of waste materials are strongly affected by the feed supply: the feeding level determines the absolute amounts of waste products as well as the distribution between solid waste and dissolved waste materials. Moreover, the length of the feeding period may affect the diurnal variation in ammonium production, which in turn affects the loading rate of the biological reactor.

Solid waste particles produced by the African catfish are successfully removed by sedimentation processes, applying hydraulic loading rates up to 20 $m^3 m^{-2}d^{-1}$.

The elimination of ammonium by a fixed biofilm loaded with settled fish tank effluent, can be described either by 1/2-order or 0-order kinetics in relation to the ammonium concentration, depending on the ratio of the concentrations of ammonium and dissolved oxygen. The biofilm is ammonium-limited (1/2-order kinetics) when the $C_{O_2}/C_{NH_4\text{-}N}$ ratio exceeds 3.6, and oxygen-limited when the ratio is smaller than this value. Trickling filtration results in a 0-order ammonium removal rate of about 0.6 g NH_4-N $m^{-2}d^{-1}$, under the conditions described.

Based on the observed ammonium removal kinetics, there are several possible types of reactor design, resulting in different performances of the overall system. A recirculation system based on 1/2-order kinetics in the biological reactor is characterized by a large reactor, a high water recirculation rate, and low concentrations of ammonium. A system based on 0-order kinetics in the biological reactor is characterized by a relatively small reactor, a low water recirculation rate, and relatively high concentrations of ammonium with a diurnal variation inversely proportional to the available water volume of the system.

A water recirculation system including a primary lamella separator and a trickling filter, operated under conditions inducing 0-order kinetics is an appropriate system for high-density culture of the African catfish. The major conclusions concerning the production and fate of waste materials in the recirculation system presented are summarized schematically in Fig. 10.

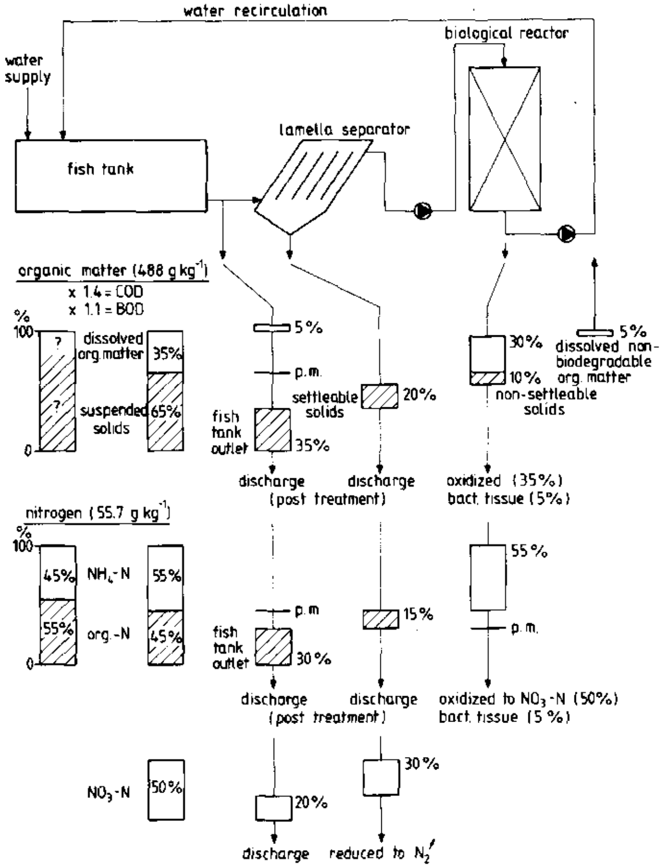

Fig. 10. Production and fate of organic matter and nitrogen compounds in the recirculation system presented for African catfish fed at a level of 16.8 g d^{-1} $kg^{-0.8}$ at 25 °C.

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REFERENCES

Broussard, M.C. and Simco, B.A., 1976. High-density culture of channel catfish in a recirculation system. Prog. Fish Cult., 38: 138-141.

Butz, I. and Vens-Cappell, B., 1982. Organic load from the metabolic products of rainbow trout fed with dry food. In: J.S. Alabaster (Editor), Report of the EIFAC Workshop on Fish-Farm Effluents. Silkeborg, Denmark, 26-28 May 1981. EIFAC Tech. Pap., 41: 73-82.

Forsell, B. and Hedström, B., 1975. Lamella sedimentation: a compact separation technique. J. Water Pollut. Control Fed., 47: 834-842.

Harremoës, P., 1978. Biofilm kinetics. In: R. Mitchell (Editor), Water Pollution Microbiology, Vol. 2. Wiley, New York, NY, pp. 71-109.

Harremoës, P., 1982. Criteria for nitrification in fixed film reactors. Water Sci. Technol., 14: 167-187.

Henken, A.M., Kleingeld, D.W. and Tijssen, P.A.T., 1985. The effect of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish *Clarias gariepinus* (Burchell, 1822). Aquaculture, 51: 1-11.

Henken, A.M., Boon, J.B., Cattel, B.C. and Lobeé, H.W.J., 1987. Differences in growth rate and feed utilization between male and female African catfish, *Clarias gariepinus* (Burchell 1822). Aquaculture, 63: 221-232.

Hogendoorn, H., 1983. Growth and production of the African catfish, *Clarias lazera* (C. & V.). III. Bioenergetic relations of body weight and feeding level. Aquaculture, 35: 1-17.

Hogendoorn, H. and Koops, W.J., 1983. Growth and production of the African catfish, *Clarias lazera* (C. & V.). I. Effects of stocking density, pond size and mixed culture with tilapia (*Sarotherodon niloticus* L.) under extensive field conditions. Aquaculture, 34: 253-263.

Hogendoorn, H., Jansen, J.A.J., Koops, W.J., Machiels, M.A.M., Van Ewijk, P.H. and Van Hees, J.P., 1983. Growth and production of the African catfish, *Clarias lazera* (C. & V.). II. Effects of body weight, temperature and feeding level in intensive tank culture. Aquaculture, 34: 265-285.

Huisman, E.A. and Richter, C.J.J., 1987. Reproduction, growth, health control and aquacultural potential of the African catfish, *Clarias gariepinus* (Burchell 1822). Aquaculture, 63: 1-14.

Krüner, G. and Rosenthal, H., 1983. Efficiency of nitrification in trickling filters using different substrates. Aquacult. Eng., 2: 49-67.

Liao, P.B. and Mayo, R.D., 1972. Salmonid hatchery water reuse systems. Aquaculture, 1: 317-335.

Liao, P.B. and Mayo, R.D., 1974. Intensified fish culture combining water reconditioning with pollution abatement. Aquaculture, 3: 61-85.

Metcalf & Eddy, Inc., 1979. Waste Water Engineering: Treatment, Disposal, Reuse, 2nd edn. McGraw-Hill, New York, NY, 920 pp.

Muir, J.F., 1982. Recirculated water systems in aquaculture. In: J.F. Muir and R.J. Roberts (Editors), Recent Advances in Aquaculture. Westview Press, Boulder, CO, pp. 358-453.

Otte, G. and Rosenthal, H., 1979. Management of a closed brackish water system for high-density fish culture by biological and chemical water treatment. Aquaculture, 18: 169-181.

Parker, N.C., 1981. An air-operated fish culture system with water reuse and subsurface silos. In: L.J. Allen and E.C. Kinney (Editors), Proceedings of the Bio-Engineering Symposium for Fish Culture. Traverse City, MI, 16-18 October 1979. American Fisheries Society, FCS Publ. No. 1, pp. 131-137.

Querellou, J., Fauré, A. and Fauré, C., 1982. Pollution loads from rainbow trout farms in Brittany, France. In: J.S. Alabaster (Editor), Report of the EIFAC Workshop on Fish-Farm Effluents. Silkeborg, Denmark, 26-28 May 1981. EIFAC Tech. Pap., 41: 87-97.

Rosenthal, H., Andjus, R. and Krüner, G., 1981. Daily variations of water quality parameters under intensive culture conditions in a recycling system. In: K. Tiews (Editor), Aquaculture in Heated Effluents and Recirculation Systems, Vol. 1. Heenemann, Berlin. Proceedings of the EIFAC-ICES Symposium, Stavanger, Norway, 28-30 May 1980, pp. 113-120.

Teugels, G.G., 1984. The nomenclature of African *Clarias* species used in aquaculture. Aquaculture, 38: 373-374.

Wickins, J.F., 1981. Water quality requirements for intensive aquaculture: a review. In: K. Tiews (Editor), Aquaculture in Heated Effluents and Recirculating Systems, Vol. 1. Heenemann, Berlin. Proceedings of the EIFAC-ICES Symposium, Stavanger, Norway, 28-30 May 1980, pp. 17-37.

Williamson, K. and McCarty, P.L., 1976a. A model of substrate utilization by bacterial films. J. Water Pollut. Control Fed., 48: 9-24.

Williamson, K. and McCarty, P.L., 1976b. Verification studies of the biofilm model for bacterial substrate utilization. J. Water Pollut. Control Fed., 48: 281-296.

CHAPTER 5

FIXED-BIOFILM REACTORS IN AQUACULTURAL WATER RECYCLE SYSTEMS: EFFECT OF ORGANIC MATTER ELIMINATION ON NITRIFICATION KINETICS

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FIXED-BIOFILM REACTORS IN AQUACULTURAL WATER RECYCLE SYSTEMS: EFFECT OF ORGANIC MATTER ELIMINATION ON NITRIFICATION KINETICS

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ABSTRACT

A special biofilm adaptation system and a biofilm monitoring system has been developed. By means of the adaptation system loaded with simulated fish tank effluent, artificial biofilm samples were available without having a neccessity of stocking a certain fish biomass. The good resemblance of biofilm performance of these samples and of samples taken out of an operative trickling filter showed that the used methods are suitable tools for research on fixed-biofilm processes.

The research is focussed on the effect of simultaneous removal of organic matter on the 0-order biofilm nitrification rate and this effect is discussed in relation to the production of faecal organic matter and its 24h fluctuation. The biofilm removed organic matter at rates directly proportional to the COD loading rate, showing efficiencies around 85%. Biofilm respiration experiments showed that only a small fraction (circa 10%) of the COD removed has actually been oxidized simultaneously. The simultaneous oxygen consumption involved was only 0.065 g O_2 per g COD removed. As a result of the observed partial oxidation of the removed organic matter, the reduction of the oxygen penetration of the biofilm was restricted in degree. The corresponding reduction factor for the simultaneous nitrification process could be quantified: -0.015 g $m^{-2}d^{-1}$ NH_4-N nitrified per g $m^{-2}d^{-1}$ COD removed. The reduction of the nitrification rate by simultaneous COD oxidation indicates that under the applied conditions the nitrification process has been limited by the transfer of oxygen into the biofilm layer.

Keywords –aquaculture, biological water treatment,
fixed-biofilm, monitoring, biofilm performance,
nitrification, COD removal, respiration

INTRODUCTION

Water quality control in a water recycle system in aquaculture is achieved by water treatment and water exchange.

The availability of dissolved oxygen in the fish tank generally can be controlled independently of the water recycle flow rate by additional aeration or supply of oxygen gas. Therefore, the general objective of the water treatment is control of the concentrations of faecal organic matter, either dissolved and suspended, and total ammonia. Suspended solids can be largely removed by (primary) sedimentation or other physical methods (Metcalf & Eddy, 1979; Muir, 1982), resulting in loading the sequential biological reactor with non-settleable and dissolved organic matter, and total ammonia. Since the biological elimination of organic matter is achieved easily, the accumulation of total ammonia may be regarded as the first capacity limiting factor of a water recycle system in aquaculture (Liao and Mayo, 1974; Otte and Rosenthal, 1979). Consequently, the nitrification process may be regarded as the key in designing water treatment installations. Nitrification reactors based on a bacterial film attached to a fixed bed matrix, e.g. trickling filters and submerged filters, are especially attractive for water treatment in intensive aquaculture systems, since high cell residence times are maintained easily, even at high hydraulic loading rates of the reactor.

Previous research concerning the fixed-biofilm nitrification process applied to aquaculture (Bovendeur et al., 1987) demonstrated reaction kinetics corresponding with the ½-order/0-order kinetic model for fixed-biofilms introduced by the research group of Harremoës (1978): fixed-biofilm samples from water recycle pilot-plants showed a transition of the nitrification rate per unit area between ½-order kinetics and 0-order kinetics (both in relation to the bulk concentration of total ammonia), respectively for relatively low and high concentrations of total ammonia and a constant concentration of dissolved oxygen. According to the kinetic model ½-order kinetics result from ammonia diffusion limitation, while 0-order kinetics result from oxygen diffusion limitation or metabolism rate limitation.

Based on the observed fixed-biofilm nitrification kinetics, Bovendeur et al. (1987) presented a design philosophy for fixed-biofilm reactors operated in water recycle systems in aquaculture. In this philosophy the required reactor dimensions and the flow rate of the recycling water anticipate the 24h variation of the production of total ammonia, which is also reported and related to feeding strategy by Poxton and Lloyd (1987). Since the design is based on the nitrification capacity of the fixed-biofilm reactor, information is needed concerning the influence of the organic matter loading rate on nitrification kinetics of fixed-biofilms adapted to fish tank effluents. Therefore, a lab-scale fixed-biofilm adaptation system and a biofilm monitoring system have been developed; the present study is focussed on (a) 24h fluctuation of faecal organic matter production, (b) removal of (artificial) faecal organic matter, and (c) 0-order nitrification rates under simultaneous organic matter removal and the biofilm oxygen consumption rates involved.

MATERIAL AND METHODS

Biofilm material

Fixed-biofilm material was obtained from an operative trickling filter recycle system (African catfish, Clarias gariepinus, fed with Trouvit pellets, Trouw & Co., Putten, The Netherlands), designed according to and described by Bovendeur et al. (1987), and from a biofilm adaptation system (loaded with artificial fish tank effluent) to be described in this paper. All biofilm material was attached to a light-weight plastic filter medium (Filterpak CR50, Mass Transfer International, Heversham, Cumbria, U.K.), having a specific surface area of 200 m^2m^{-3}. Biofilm samples attached to 1.1 m^2 filter medium have been tested, grown for several months under average conditions inducing 0-order nitrification kinetics: hydraulic loading rate (superficial water velocity, 150-200 m^3 $m^{-2}d^{-1}$), organic loading rate per unit area (circa 5 g $m^{-2}d^{-1}$ COD, Chemical Oxygen Demand), nitrogen loading rate per unit area (circa 0.6 g $m^{-2}d^{-1}$ NH_4-N, total ammonia), dissolved oxygen concentration (circa 7 g m^{-3}), water temperature (25 ± 1°C), and pH (7.0-7.5). The attached biofilm samples were placed in a basket of wire-netting, enabling easy removal and replacement of the biofilm samples.

Biofilm adaptation system

The biofilm adaptation system is composed of a biofilm reactor unit and an organic substrate supply unit (Fig. 1). In the reactor unit two biofilm samples are loaded in the trickling filter mode (150-200 m^3 $m^{-2}d^{-1}$) by recirculation. The total liquid volume is 0.025 m^3 and the water temperature 25°C.

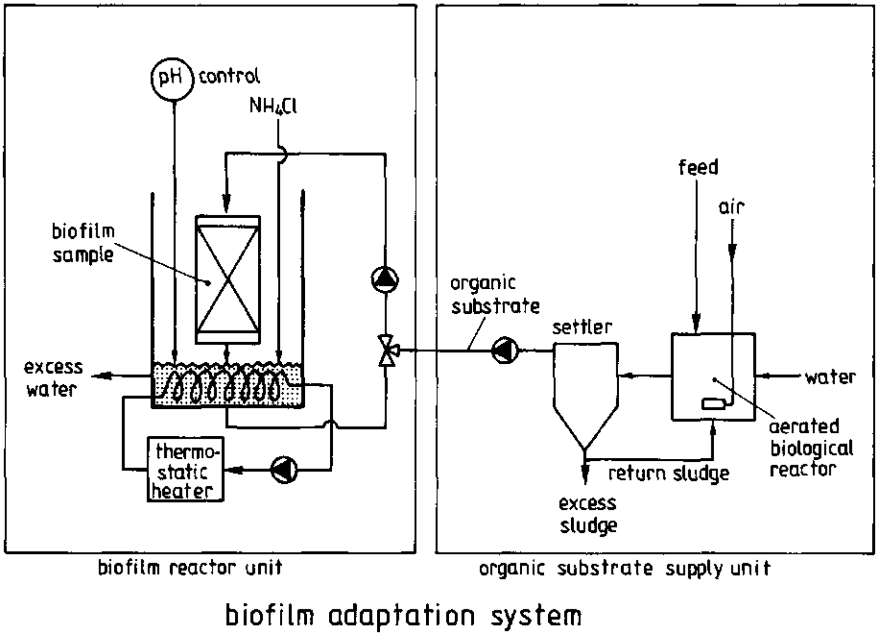

Fig. 1. Schematic diagram of the biofilm adaptation system

The reactor unit is loaded with ammonia by adding NH_4Cl (4-5 g d^{-1}), resulting in a loading rate of about 0.5 g $m^{-2}d^{-1}$ NH_4-N; nitrate produced is removed from the reactor unit by water exchange, pH 7 is controlled by adding a $NaHCO_3$ solution, and dissolved oxygen is maintained at about 7 g m^{-3} by diffusion of oxygen from the air phase into the waterfilm in the packed bed.

Faecal waste production by fish is imitated in the organic substrate supply unit by batch-wise loading an aerated activated sludge reactor (0.007 m^3) with 10-15 g d^{-1} ground Trouvit feed (Trouw & Co., Putten, The Netherlands). The supplied feed is partly dissolved and digested in the aerated reactor, resulting in organic substrate with a BOD/COD ratio of about 0.8, comparable to faecal organic waste. After settling of the coarse suspended solids, the organic substrate suspension is pumped to the biofilm reactor unit at a rate meeting the desired organic loading of the biofilm samples (5 g $m^{-2}d^{-1}$ COD). Both for COD and NH_4-N a 24h fluctuation was achieved with maximum loading rates about 2-3 times the average loading rates.

The two 'artifical' biofilm samples were alternately used for experiments in the biofilm monitoring system.

Biofilm monitoring system

The biofilm monitoring system is composed of a biofilm reactor unit (total water volume 0.012 m^3, water recycle flow rate 0.26 m^3h^{-1}), which can be connected to a respirationmeter unit (Fig. 2). Dissolved oxygen in the reactor unit is controlled by supply of air, or pure oxygen, or a mixture, in the aerator. The attached biofilm sample is monitored in the reactor unit, in the submerged filter mode during several hours under constant conditions in terms of dissolved oxygen level, concentration (or loading rate) of total ammonia and COD, water temperature, and pH. Standard monitoring conditions are: 7 g m^{-3} O_2, 10 g m^{-3} NH_4-N, pH 7, 25°C. Two experiments are conducted monitoring the artifical biofilm samples in the trickling filter mode by

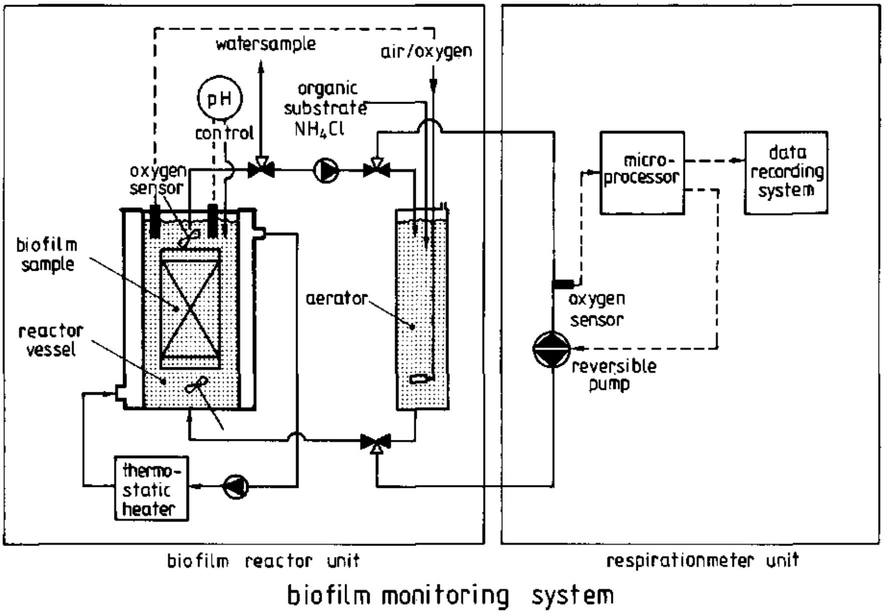

Fig. 2. Schematic diagram of the biofilm monitoring system

changing the direction of the water flow in the biofilm reactor unit and reducing the bulk liquid volume. If added, organic substrate is produced as described for the biofilm adaptation system. The biofilm waste removal capacity is determined from water samples taken at constant time intervals (0.5 or 1.0 h), analysed for the parameters involved (COD, total ammonia, nitrite, and nitrate).

In some experiments the overall oxygen consumption rate of the biofilm sample is monitored simultaneously with the removal of COD and total ammonia by a respirationmeter system (Fig. 2). Dissolved oxygen is measured periodically in the inflow and in the outflow of the reactor vessel of the biofilm reactor unit with one oxygen sensor. This is realised in alternately changing the flow direction in the bypass using a reversible pump. The oxygen sensor is connected to a modular microprocessor system, calculating the respiration rate and controlling the reversible pump. The used respirationmeter unit (WAZU respirationmeter, patent pended) and its possibilities are described in more detail by Klapwijk et al. (1987), and Spanjers and Klapwijk (1987). Combining the overall oxygen consumption rate and the N-balance, the biofilm consumption rate of nitrification involved oxygen (NO_x-O, i.e. NO_2-O + NO_3-O) and of oxygen involved with COD removal (COD-O) can be distinguished. NO_x-O is calculated on the basis of 4.57 g O_2 per g NH_4-N for complete nitrification.

The attached biofilm samples were replaced either in the operative trickling filter or in the biofilm adaptation system, immediately after termination of the measurements. Before each test the walls of the complete monitoring system are cleaned, disinfected, and rinsed with tap water.

Faecal organic matter production

The course of the production of non-settleable and dissolved faecal material over 24 h was determined by COD analyses of 1h composite samples of the (presettled) effluent of a fish tank operated in the flow-through mode. The fish tank (0.8 m^3) was stocked with African catfish of 50 g body weight, fed during 12 h per day (Trouvit pellets, automatic feeder) at a feeding level of 3% body weight per day (recommended feeding level for 25°C by Hogendoorn et al. (1983)). The actual COD production was calculated from concentration increase over time multiplied by the waterflow. The 24h fluctuation of the production is defined as the ratio actual/average production rate, in which the average production rate is calculated from assuming a constant release of the total amount of faecal material during 24 h. Total ammonia has also been determined in the water samples.

Water analyses

The water samples were analysed for COD, total ammonia (NH_3 + NH_4, further denoted as NH_4), nitrite, and nitrate according to Standard Methods (American Public Health Association, 1975). The concentrations of all nitrogen compounds are expressed as g m^{-3} N. Dissolved oxygen and pH were measured directly in the water cycle using electrodes and a WTW OXY 91 meter and a Knick pH/mV meter respectively.

RESULTS

Biofilm waste removal capacity

The biofilm nitrification rate per unit area observed in introductory experiments monitoring the two types of attached biofilm samples under varying conditions are presented in Fig. 3.

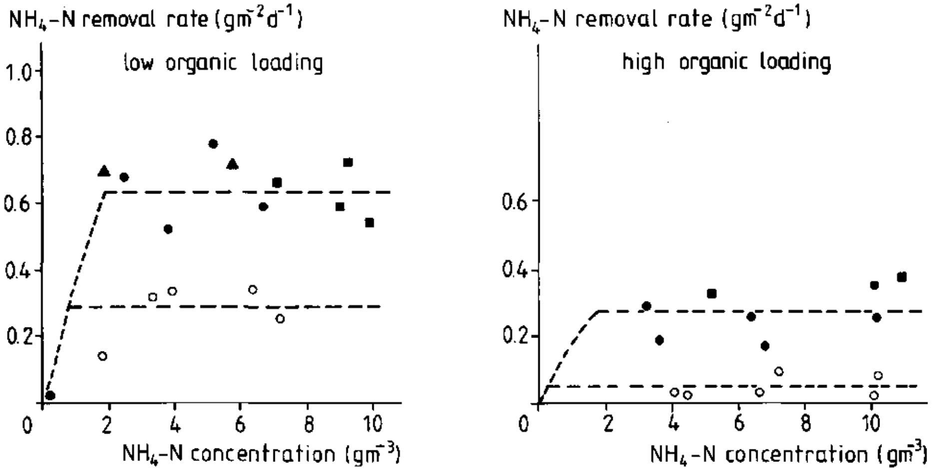

Fig. 3. Biofilm nitrification rates in relation to the total ammonia concentration under varying monitoring conditions: dissolved oxygen level (● ■ ▲ 7 g m^{-3}, ○ 3 g m^{-3}), short-term (3-4 h) organic matter loading rate (low: 1 g $m^{-2}d^{-1}$ COD; high: 20 g $m^{-2}d^{-1}$), origin of the biofilm sample (■ ▲ artificial biofilm; ● ○ operative biofilm), mode of hydraulic loading (● ○ ■ submerged mode; ▲ trickling mode)

Irrespective the mode of hydraulic loading and origin of the attached biofilm sample, comparable nitrification rates were found. The biofilm samples showed unchanged nitrification rates for total ammonia concentrations in the range 2-10 g m^{-3} NH_4-N (0-order kinetics), and constant dissolved oxygen levels.

However, significantly decreased nitrification rates were observed for decreased levels of dissolved oxygen and/or increased organic substrate loading rates.

The organic substrate removal rate of the nitrifying biofilm samples was found to be high and constant in relation to the loading rates investigated (Fig. 4). The COD removal efficiency at a dissolved oxygen level of 3 g m^{-3} was not found to be decreased compared to the efficiency observed for the 7 g m^{-3} dissolved oxygen level.

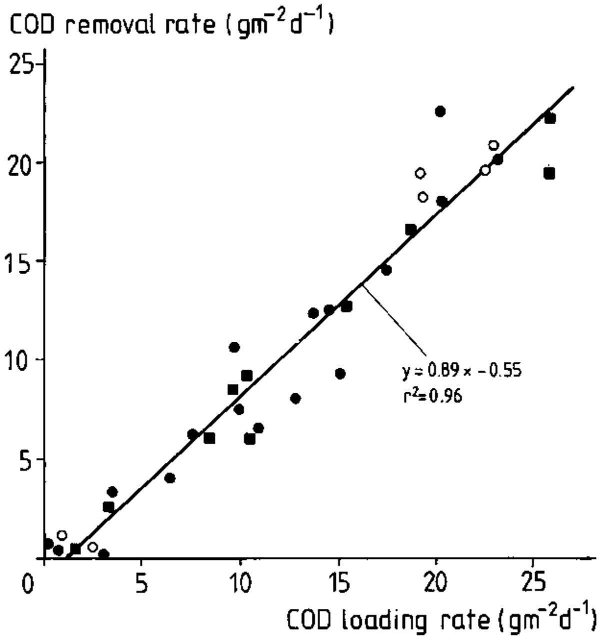

Fig. 4. Relation between the biofilm removal rate and loading rate of organic matter (legend as in Fig. 3)

The influence of simultaneous COD removal on the 0-order nitrification rate of the biofilm samples is demonstrated in Fig. 5 for dissolved oxygen levels of 7 and 3 g m^{-3}. Starting from a nitrification rate of 0.65 g $m^{-2}d^{-1}$ NH_4-N removed, a gradual decrease was found for increasing COD removal rates. A 45% reduction of the 0-order nitrification rate was found at a COD loading rate of 20 g $m^{-2}d^{-1}$. During the monitoring experiments the removed NH_4-N has been nitrified almost completely into NO_3-N, according to the observed ratio of 4.53 ± 0.06 g g^{-1} O_2 per NH_4-N for the 7 g m^{-3} dissolved oxygen level. No distinction has been observed between the two types of attached biofilm samples, either in these experiments or in the COD removal experiments (Fig. 4).

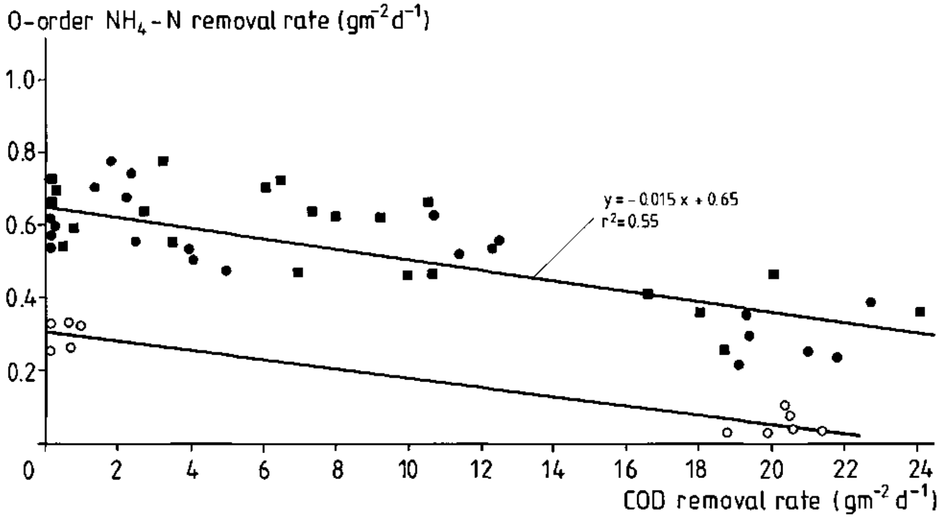

Fig. 5. Effect of simultaneous organic matter removal induced by short term loading on the 0-order biofilm nitrification rate (legend as in Fig. 3)

Connecting the reactor unit to the respirationmeter unit in the biofilm monitoring system, a rather constant value was found for the overall biofilm respiration rate (2.84 ± 0.22 g $m^{-2}d^{-1}$ O_2), composed of the complementary quantities NO_x-O and COD-O for different COD removal rates and corresponding nitrification rates (Table 1). The observed COD-O was very low in comparison with COD removed.

Table 1. Relation between biofilm removal rates of organic matter and total ammonia (0-order nitrification rate), and oxygen consumption rates involved; artificial biofilm monitored under standard conditions and varying COD loading rates in a series of independent experiments

Removal rate (g $m^{-2}d^{-1}$)		Oxygen consumption rate (g $m^{-2}d^{-1}$)		
COD	NH_4-N	Overall respiration	NO_x-O	COD-O
0	0.575	2.75	2.49	0.268
9.89	0.475	2.54	1.89	0.655
10.74	0.446	2.96	1.82	1.14
11.36	0.536	3.11	1.99	1.12

Faecal COD production over 24 h

The COD concentration in the settled tank effluent rose quite strongly starting 1 h after the feeding began, and peaked about 10 h later. The actual COD production rate reached a maximum exceeding the average production rate about two times (Fig. 6). Thereafter, the COD production rate decreased more gradually than the increase untill background levels were reached about 20 h after the feeding had started. The coarse of the total ammonia production is also shown in Fig. 6.

DISCUSSION

The faecal COD production by African catfish fluctuated less over 24 h than the total ammonia production (Fig. 6). The NH_4-N production and its 24h fluctuation are the main decisive factors for the dimensions of the fixed-biofilm reactor and, by that, also the average organic matter loading of the reactor is determined (Bovendeur et al., 1987). However, the fluctuation of the COD production over 24 h will result in varying loading rates of the reactor over the day. As an example, the COD loading rate of a reactor based on 0-order nitrification kinetics may vary between 2 and 12 g $m^{-2}d^{-1}$ with an average loading rate of 5 g $m^{-2}d^{-1}$. Moreover, assuming a relation between faecal waste production and feeding strategy, as has been observed for ammonia production by eel, <u>Anguilla anguilla</u> (Poxton and Lloyd, 1987), and considering the different amounts of waste produced by different species (Heinsbroek, 1987), it is clear that a significant variation of short-term COD loading rates may be generally expected in aquacultural recycle systems.

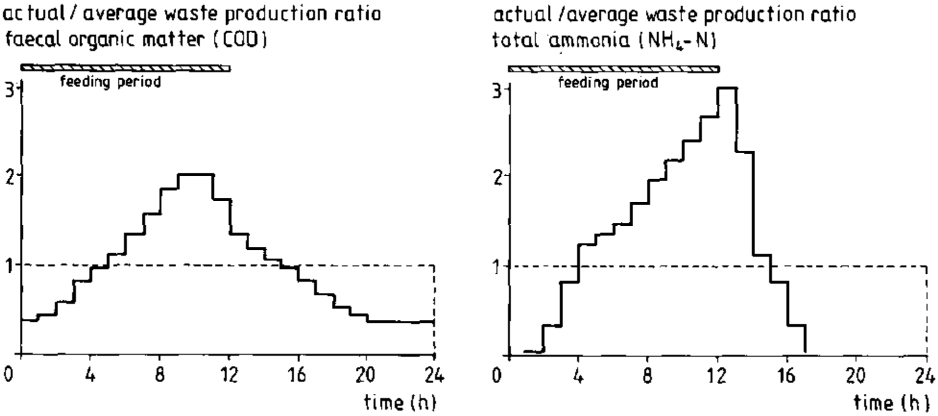

Fig. 6. Fluctuation over 24 h of the production of waste material by African catfish. Average production rate (dotted line): assuming a constant release of the total amount of waste during 24 h

The comparison between the biofilm samples coming from the operative trickling filter system and from the biofilm adaptation system can be made by reference to Fig. 3,4 and 5. The resemblance of performance between the two types of biofilm samples indicate that the presented biofilm adaptation method provides artifical biofilm samples suitable for research on biofilm performance in operative water treatment systems.

By this, controlled biofilm samples are available at any time, not being affected by the factor fish.

The observed levels for the biofilm nitrification rate (Fig. 3) confirm the 0-order biofilm kinetics postulated by Harremoës (1978, 1982). Biofilm nitrification rates under standard monitoring conditions agreed with biofilm nitrification rates in operative fixed-biofilm reactors obtained from N-balances (Bovendeur et al., 1987), indicating that the used biofilm monitoring method adequately simulated the conditions in practice.

Otte and Rosenthal (1979) already reported that the trickling filter capacity for removal of organic matter increased with gradually increasing organic loads due to increased fish biomass by growth. Fig. 4 shows that the biofilm capacity for organic matter removal is also able to cope with short-term increasing COD loading rates in the relevant range, without any reduction of efficiency. This means that the 24h accumulation of organic matter in the recycle system will not be as serious as the NH_4-N accumulation, after the maximum (0-order) nitrification rate in the reactor is reached (Bovendeur et al., 1987). However, some reservations have to be made since the attached biofilm samples were not monitored in the trickling filter mode during these experiments.

The effect of the short-term COD peak loads and the corresponding COD removal rates on the 0-order nitrification rate is rather small: approximately -0.015 g $m^{-2}d^{-1}$ NH_4-N nitrified per g $m^{-2}d^{-1}$ COD removed (Fig. 5). It should be emphasized that removal of COD from the water phase is not neccessarily equivalent to COD oxidation. Table 1 shows that the overall respiration rate of the biofilm samples remained at a more or less constant level, apparently unaffected by, and considerably lower than the COD removal rates realised. This indicates that only a very small part of the removed COD is simultaneously oxidized and that the actual removal of organic matter from the bulk liquid must be the result of some other process. Spanjers and Klapwijk (1987) also observed this phenomenon for organic matter elimination in the activated sludge process, and suggested a rapid physical process followed by biological oxidation at a low rate, while Bovendeur et al. (1989) discussed such adsorption and respiration processes for a fixed-biofilm sample grown in a rotating biological contactor. Low COD oxidation rates may explain the absence of a perceptible effect of decreasing the dissolved oxygen level from 7 to 3 g m^{-3} on the overall COD removal rate (Fig. 4). Moreover, low COD oxidation rates also explain the relatively small effect of COD removal on the biofilm nitrification rate: low COD oxidation rates correspond to low oxygen consumption rates, resulting in a relatively small effect on the oxygen penetration depth of the biofilm, and thereby also in a small effect on the biofilm nitrification rate. On the other hand, the short-term character of the COD load restricted the increase of non-nitrifying biofilm material –i.e. adsorbed COD and heterotrophic biomass– resulting in sufficient residence time of the nitrifying bacteria in the aerobic zone of a growing biofilm. These criteria for fixed-biofilm nitrification are modelled analytically by Harremoës (1982).

In Table 1 it is shown that a biofilm sample monitored under standard conditions (i.e. without COD load) also consumes some oxygen additional to the amount of oxygen needed for the nitrification process. Such a background oxygen consumption is probably the result of endogenous respiration and oxidation of organic matter absorbed by the biofilm during the residence in the adaptation system foregoing to the respiration experiment. This phenomenon has also been observed by Bovendeur et al. (1989) for a non-nitrifying biofilm in a rotating biological contactor. Furthermore, it is shown in Table 1 that the amount of oxygen involved with COD oxidation is deducted from the amount of oxygen available for nitrification. This may be confirmed by comparison of the proportionality factors of the corresponding oxygen equivalents for both the reduction of the nitrification (NO_x-O), and the oxygen involved with simultaneous oxidation of COD (COD-O). The average reduction of the nitrification is about 0.015 g $m^{-2}d^{-1}$ NH_4-N per g $m^{-2}d^{-1}$ COD removed (Fig. 5). Based on the observed stoichiometric coefficient (4.53 g O_2 g^{-1} NH_4-N) the corresponding reduction of NO_x-O is 0.068 g $m^{-2}d^{-1}$ COD removed. On the other hand, the proportionality factor for COD-O can be estimated from Table 1 according to

$$\frac{\text{COD-O consumption rate} - 0.268}{\text{COD removal rate}} .$$

COD-O values per g $m^{-2}d^{-1}$ COD removed are found varying as 0.039, 0.081 and 0.075 g $m^{-2}d^{-1}$. The average value, i.e. 0.065 g $m^{-2}d^{-1}$ COD-O per g $m^{-2}d^{-1}$ COD removed, closely resembles the observed average reduction of NO_x-O per g $m^{-2}d^{-1}$ COD removed (0.068 g $m^{-2}d^{-1}$), confirming that the reduction of the oxygen is caused only by the small fraction of COD removed from the water phase that is simultaneously oxidized in the biofilm.

Although the temporal reduction of the nitrification capacity is low, it should be accounted for in calculating the required dimensions of the fixed biofilm reactor. This is especially true in those cases where a considerable amplitude of the 24h fluctuation of the organic matter production may be expected –e.g. as a result of some extreme feeding strategy– or when a submerged filter is planned without internal aeration or supply of pure oxygen, resulting in low dissolved oxygen levels in the biological reactor. It should be emphasized that the discussed effect is related to short-term exposure to relatively high organic matter loading rates. Structural high loading rates cause more dramatically reduced nitrification capacities (e.g. Metcalf and Eddy, 1979). Therefore, an absent or poorly functioning primary clarifier in intensive cultures will result in very low nitrification rates per unit area, and therefore very large (uneconomic) biological reactors are needed.

CONCLUSIONS

Under conditions of high organic matter loading rates, fixed-biofilm nitrification is reduced by reduced oxygen penetration of the biofilm, caused by oxidation of organic matter by heterotrophic bacteria. Therefore, under conditions of high organic matter loading rates, the biofilm nitrification process is limited by transfer of oxygen into the biofilm layer.

In aquacultural recycle systems the reduction of biofilm nitrification due to organic waste production is restricted in time and degree by the short-term character of the increased COD loads and by the low degree of biological oxidation of organic matter: only about 10% of COD removed from the water phase was found to be simultaneously oxidized.

The course over 24 h of the 0-order biofilm nitrification rate in aquacultural recycle systems (25°C) can be estimated from the 24h course of the organic matter production, the biofilm COD removal efficiency (circa 85%), and the reduction factor for the nitrification (-0.015 g $m^{-2}d^{-1}$ NH_4-N nitrified per g $m^{-2}d^{-1}$ COD removed).

The biofilm adaptation method and the biofilm monitoring method used in this research are suitable tools for research on fixed-biofilm performance, enabling rational design of biological reactors in aquaculture.

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REFERENCES

American Public Health Association. (1975) Standard methods for the examination of waste and wastewater. APHA, New York.

Bovendeur, J., Eding, E.H. and Henken, A.M. (1987) Design and performance of a water recirculation system for high-density culture of the African catfish, Clarias gariepinus (Burchell 1822). Aquaculture, 63, 329-353.

Bovendeur, J., Spuij, F., Hermans, P.G.B. and Klapwijk, A. (1989) Mechanisms and kinetics of COD removal and simultaneous oxygen consumption in a rotating biological contactor biofilm. In: Proceedings of the EWPCA-IAWPRC Conference "Technical advances in biofilm reactors", Nice, France, 4-6 April 1989, 91-103.

Harremoës, P. (1978) Biofilm kinetics. In: R. Mitchell (Editor), Water Pollution Microbiology, Vol. 2. Wiley, New York, NY, 71-109.

Harremoës, P. (1982) Criteria for nitrification in fixed film reactors. Water Sci. Technol., 14, 167-187.

Heinsbroek, L.T.N. (1987) Waste production and discharge of intensive culture of African catfish, Clarias gariepinus, European eel, Anguilla anguilla, and rainbow trout, Salmo gairdneri, in recirculation and flow-through systems in The Netherlands. Report of the EIFAC Workshop on Fish Farm Effluents. The Hague, The Netherlands, 29 May-1 June 1987. EIFAC Techn. Pap., in press.

Hogendoorn, H., Jansen, J.A.J., Koops, W.J., Machiels, M.A.M., Van Eijk, P.H. and Van Hees, J.P. (1983) Growth and production of the African catfish, Clarias lazera (C.&V.). II. Effects of body weight, temperature and feeding level in intensive tank culture. Aquaculture, 34, 265-185.

Klapwijk, A., Spanjers, H. and Van den Akker, D. (1987) Control of an activated sludge plant with a WAZU-respirometer. In: Proceedings of the 7th European Sewage and Refuse Symposium. München, FRD, 19-22 May 1987, 125-139.

Liao, P.B. and Mayo, R.D. (1974) Intensified fish culture combining water reconditioning with pollution abatement. Aquaculture, 1, 317-335.

Metcalf & Eddy, Inc. (1979) Waste Water Engineering: Treatment, Disposal, Reuse, 2nd edn. Mcgraw-Hill, New York, NY, 920 p.

Muir, J.F. (1982) Recirculated water systems in aquaculture. In: J.F. Muir and R.J. Roberts (Editors), Recent Advances in Aquaculture. Westview Press, Boulder, CO, 358-543.

Otte, G. and Rosenthal, H. (1979) Management of a closed brackish water system fir high-density fish culture by biological and chemical water treatment. Aquaculture, 18, 169-181.

Poxton, M.G. and Lloyd, N.J. (1987) Fluctuations in ammonia production by eels (Anguilla anguillal) as a result of feeding strategy. In: Proceedings of the International Aquaculture Conference "Aquaculture Europe '87', Amsterdam, The Netherlands, 2-5 June 1987. In press.

Spanjers, H. and Klapwijk, A. (1987) The WAZU-respirationmeter- a breakthrough in automation and control for as wastewater treatment plant. In: Proceedings of the 10th Symposium on Wastewater Treatment, Montreal Québec, November 1987.

CHAPTER 6

EFFECTS OF SUBSTRATE LOADING REGIME, DISSOLVED OXYGEN AND pH ON FIXED-BIOFILM NITRIFICATION IN AQUACULTURAL WATER RECIRCULATING SYSTEMS

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EFFECTS OF SUBSTRATE LOADING REGIME, DISSOLVED OXYGEN AND pH ON FIXED-BIOFILM NITRIFICATION IN AQUACULTURAL WATER RECIRCULATING SYSTEMS

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ABSTRACT

A study is made on biofilm nitrification and its sensitivenesses to factors related to (aquacultural) waste water treatment. A special biofilm adaptation system is used to grow "artificial" biofilm samples, the performance of which is studied under well controlled laboratory conditions in a biofilm monitoring system. Distinction is made between oxidation capacities of ammonia and nitrite by feeding the biofilm with either ammonia or nitrite or a mixture of both nitrogen sources. Removal capacities are presented for a developing biofilm sample and a full-grown biofilm sample; the full-grown biofilm sample is used for the experiments on the effects of organic loading regime, dissolved oxygen levels and pH on the nitrification capacity. For this full-grown biofilm sample the oxidation of ammonia was found to be oxygen diffusion limited, while on its turn the removal rate for nitrite was controlled by the ammonia oxidation. The effects of the organic matter loading rate –long-term and short-term loadings– and the effects of a strongly increased dissolved oxygen level are discussed in relation to the observed oxygen diffusion limitation of the ammonia removal capacity. The effect of low pH levels in the bulk liquid –strongly reduced removal rates both for ammonia and nitrite– is explained for ammonia removal in terms of transition of the limiting factor (dissolved oxygen/alkalinity), and for nitrite removal in terms of substrate inhibition by unionized nitrous acid. It is concluded that any serious accumulation of nitrite in an aquaculture recycle system is not likely to be the result of incomplete nitrification induced by the factors discussed under the conditions indicated.

Keywords –aquaculture, biological water treatment, biofilm monitoring, biofilm performance, nitrification kinetics

INTRODUCTION

Water quality control in aquacultural water recycle systems is achieved by water treatment and water exchange, where the degree of water reuse depends on system design and treatment efficiencies. Since the accumulation of ammonia may be regarded as the first capacity limiting factor of a water recycle system (Liao and Mayo, 1974; Otte and Rosenthal, 1979), the nitrification process may be regarded as the key to adequate system design. Bovendeur et al. (1987) showed the suitability of biofilm nitrification reactors based on a fixed support matrix, emphazising the need for information on reaction mechanisms and kinetics; a design philosophy has been presented in which ammonia production dynamics are combined with nitrification kinetics in terms of ammonia removal. In this design philosophy the ½-order/0-order kinetic concept is adopted, presented by LaMotta (1976a, b) and Harremoës (1978). This conceptual biofilm model mathematically associates diffusional mass transport with biofilm thickness and intrinsic reaction kinetics, and its relevancy is recently reviewed for several processes by Arvin and Harremoës (1989).

Any factor, however, that reduces the number or the activity of the nitrifying bacteria in the biofilm will interfere with the intended balance between ammonia production and the removal of ammonia or nitrite as intermediate product of the nitrification. Such a factor may be the use of a parasiticide or antibacterial agent treating diseases of fish. The effects of such chemicals on nitrification have been reported by Collins et al. (1976a,b), indicating that therapeutic levels of several agents may be fatal for the total system. However, apart from the use of external chemicals, the nitrification process is affected even more easily by several system-bound properties: nitrification is an oxygen consuming and acidity producing process, therefore the recirculating water needs constant control of dissolved oxygen (DO) and pH. Moreover, nitrification is also affected by simultaneous loading of organic matter (e.g. Sharma and Ahlert, 1977), and since a fixed-biofilm grown under standard aquacultural conditions tends to a oxygen diffusion limited capacity (Bovendeur et al., in press), the effect of the simultaneous presence of more than one oxygen consuming substrate on nitrification kinetics requires special attention. Consequently, a malfunctioning control of pH and DO, a poor retention of suspended solids (SS), or leaching of organic matter from SS may interfere with optimal system performance in practice. Such failures in operation easily result in accumulation of ammonia or nitrite up to toxic levels.

Regarding the dominant role of the nitrification capacity of the fixed film reactor in total system design and operation, information concerning the effects of accumulating biofilm substrate, DO and pH on the nitrification kinetics is generated and discussed in terms of changes in both ammonia and nitrite removal rates per unit area of attached biofilm. These characteristics have been studied for different phases of biofilm development, enabling a description of the final stage of biofilm nitrification capacity and its sensitivenesses to the factors introduced.

MATERIALS AND METHODS

Biofilm samples attached to 1.1 m^2 filter medium were grown under controlled conditions in a biofilm adaptation system. The supporting matrix material used was a light-weight plastic trickling filter medium (Filterpak CR 50, Mass Transfer International, Heversham, Cumbria, U.K.) having a specific surface area of 200 m^2m^{-3}. The attached biofilm samples were placed in laboratory-scale trickling filters made of wire-netting, enabling easy removal and replacement without affecting the attached biomass. Biofilm performance is studied in a separate biofilm monitoring system. The suitability of both the biofilm adaptation and monitoring system is illustrated by Bovendeur et al. (in press); a description of the systems and the experimental set-up is given below.

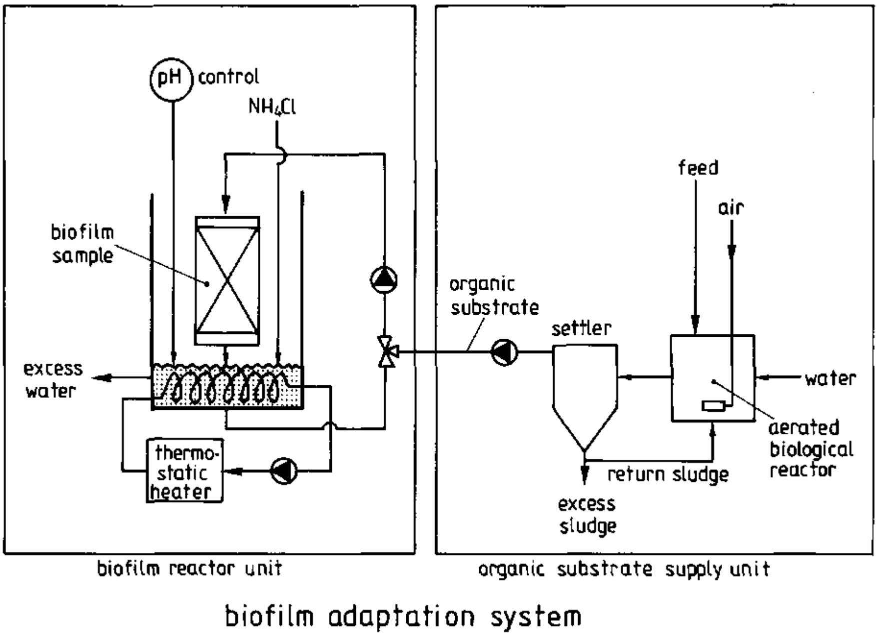

Fig. 1. Schematic diagram of the biofilm adaptation system. Note that only one of the two biofilm samples is shown

Biofilm adaptiation system

The biofilm adaptation system is composed of a biofilm reactor unit and an organic substrate supply unit (Fig. 1). In the reactor unit two biofilm samples are loaded in the trickling filter mode (superficial velocity 150-200 $m^3m^{-2}d^{-1}$) by water recirculation. The total liquid volume is 0.025 m^3 and the water temperature 24°C. The reactor unit is loaded with ammonia by adding NH_4Cl (4-5 g d^{-1}), resulting in a loading rate of about 0.5 g $m^{-2}d^{-1}$ NH_4-N; nitrate produced is removed from the reactor unit by water exchange; pH 7 is controlled by adding a $NaHCO_3$ solution, and DO levels are maintained at about 7 g m^{-3} by diffusion of oxygen from the air phase into the water film in the packed bed.

Faecal waste production by fish is simulated in the organic substrate supply unit by batch-wise loading an aerated activated sludge reactor (0.007 m^3) with ground Trouvit feed (Trouw & Co., Putten, The Netherlands). The supplied feed is partly dissolved and digested in the aerated reactor, resulting in organic substrate with a BOD/COD ratio of 0.8, comparable to faecal organic waste of fish. After settling of the coarse suspended solids, the organic substrate suspension is pumped to the biofilm reactor unit. The amount of daily supplied feed (10-20 g d^{-1}) and the pumping rate of the substrate suspension are adjusted to meet the desired average organic matter loading of the biofilm samples (5 or 10 g $m^{-2}d^{-1}$), allowing some diurnal fluctuation. The combination of DO 7 g m^{-3}, pH 7, 0.5 g $m^{-2}d^{-1}$ NH_4-N and 5 g $m^{-2}d^{-1}$ COD is indicated as "standard adaptation conditions".

The two "artificial" biofilm samples were alternately used for duplicate experiments in the biofilm monitoring system.

Biofilm monitoring system

The biofilm monitoring system is a semi-closed water recycle system (Fig. 2), composed of a biofilm reactor vessel and an aerator vessel (total water volume 0.012 m^3, water recycle flow rate 0.26 m^3h^{-1}). DO in the system is controlled by supply of air, or pure oxygen, or a mixture, in the aerator. The attached biofilm sample is monitored in the submerged filter mode during several hours under constant conditions in terms of DO level (7 or 30 g m^{-3}), COD loading rate (various constant values), water temperature (24°C), and pH (7). Some experiments were executed under conditions of various constant NH_4-N levels inducing 0-order removal kinetics according to Bovendeur et al. (in press), while most experiments have been performed according to Bovendeur et al. (1987) under conditions of various nitrogen batch loads: NH_4-N and/or NO_2-N; initial bulk concentrations 10 g m^{-3}. If added, organic substrate is produced as described for the biofilm adaptation system.

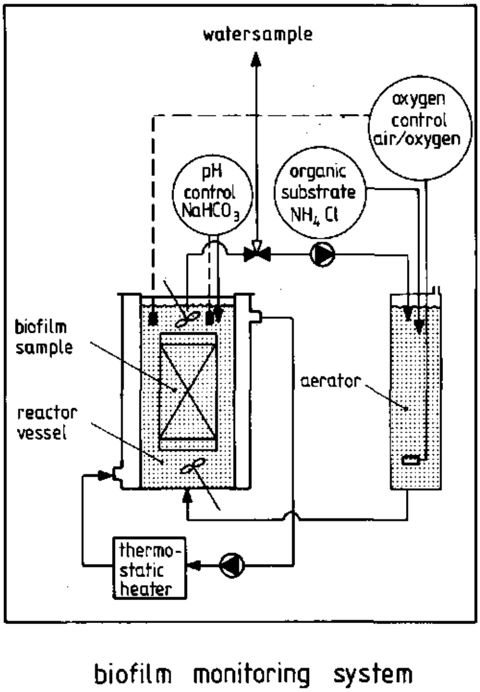

Fig. 2. Schematic diagram of the biofilm monitoring system

Experimental set-up

Two types of biofilm samples have been used in this research, differing in the adaptation COD loading rate. Applied loading rates were about 5 g $m^{-2}d^{-1}$, comparable to operative trickling filters in aquaculture practice (Bovendeur et al., 1987), and about 10 g $m^{-2}d^{-1}$ (average values long-term COD load). The performance of both types of biofilm samples in terms of ammonia removal rate, nitrite removal rate and COD removal rate (all in g $m^{-2}d^{-1}$) is tested under monitoring conditions with various nitrogen sources (NH_4-N or NO_2-N or both simultaneously) and short-term COD loading rate, DO level or pH. The biofilm waste removal capacities are determined from water samples taken at constant time intervals (0.5 or 1.0 h), analysed for the parameters involved (COD, ammonia, nitrite and nitrate). Unless otherwise is indicated, results are presented for "full-grown" biofilm samples, i.e. biofilm samples that show the highest and constant removal rates for ammonia and nitrite under standard monitoring conditions (DO 7 g m^{-3}, pH 7, absence of COD).

Before each test-run the walls of the complete monitoring system were cleaned, disinfected, and rinsed with tap water. Immediately after termination of the measurements, the attached biofilm samples were replaced in the biofilm adaptation system, and left there for adaptation during several days under the original loading conditions, before a new test is performed.

Analyses

Water samples were analysed for COD, (total) ammonia (NH_3 + NH_4, further denoted as NH_4), nitrite and nitrate according to Standard Methods (American Public Health Association, 1975). The amounts of all nitrogen compounds are expressed on the basis of the N-weight fraction of those compounds. DO and pH were measured directly in the water cycle using electrodes connected to a WTW OXY 91 and a Knick pH/mV meter respectively.

RESULTS

Development of biofilm nitrification

Some biofilm nitrification activity, resulting in ammonia removal and nitrite accumulation, was observed within about two weeks after the packed columns were loaded in the biofilm adaptation system. After such a start-up period of about one month nitrite accumulation did not longer occur. Biofilm monitoring experiments resulted in equal removal rates for ammonia and nitrite both showing 0-order kinetics for substrate concentrations exceeding a certain minimum value. Note that separate experiments were executed for NH_4-N and NO_2-N loads. In Fig. 3a results are shown

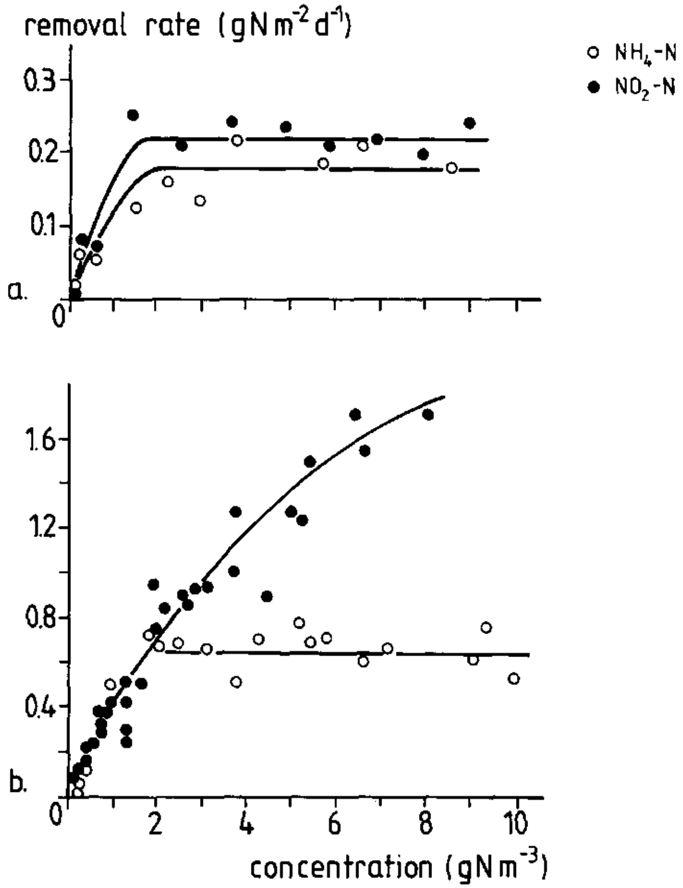

Fig. 3. Biofilm development: NH_4-N and NO_2-N removal rates at two different phases of development, measured in separate experiments per N-source; a. developing biofilm; b. full-grown biofilm

for a still developing biofilm sample after about two month of adaptation to standard adaptation conditions; Fig. 3b illustrates the final removal rates for NH_4-N and NO_2-N, where the NO_2-N removal rate shows a certain positive correlation with the NO_2-N bulk concentration in the range examined, in contrast to the developing biofilm sample (Fig. 3a). The relation between removal rate and bulk concentration up to 8 g m^{-3} NO_2-N for NO_2-N oxidation by a full-grown biofilm sample can be described by ½-order kinetics: $y = 0.69\ x - 0.24$ ($r^2 = 0.97$), where y represents the NO_2-N removal rate (g $m^{-2}d^{-1}$), and x represents $\sqrt{c}$, where c is the NO_2-N bulk concentration (g m^{-3}), and r the correlation coefficient for linear regression.

Biofilm monitoring experiments in which the biofilm sample was continuously loaded with NH_4-N, no serious NO_2-N accumulation was observed (max. concentration 0.4 g m^{-3}), neither for full-grown samples nor for developing biofilm samples after the start-up phase.

Table 1. NH_4-N and NO_2-N removal performance of full-grown biofilm samples, grown under adaptation conditions differing in COD load, monitored under conditions of various nitrogen sources (NH_4-N and/or NO_2-N). Results are presented as μ ± SD (n) for (apparent) 0-order removal rates, or as $y = a\sqrt{c} + b$ (r^2; n) for ½-order removal rates, where y is the NO_2-N removal rate and c is the NO_2-N bulk concentration (g m^{-3}); a and b are constants

Performance			
	Monitoring conditions	Adaptation conditions	
	7 g m^{-3} DO, pH 7, 24°C no COD load	7 g m^{-3} DO, pH 7, 24°C NH_4-N load circa 0.5 g $m^{-2}d^{-1}$	
	nitrogen source (*concentration range*)	5 g $m^{-2}d^{-1}$ COD	10 g $m^{-2}d^{-1}$ COD
NH_4-N removal rate (g $m^{-2}d^{-1}$)	NH_4-N (2-10 g m^{-3})	0.60 ± 0.10 (13) 0-order	0.39 ± 0.12 (38) 0-order
	NH_4-N + NO_2-N (2-10 ; 1-5 g m^{-3})	0.55 ± 0.17 (20) 0-order	0.33 ± 0.09 (9) 0-order
NO_2-N removal rate (g $m^{-2}d^{-1}$)	NH_4-N (2-10 g m^{-3})	≈NH_4-N removal rate apparent 0-order	0.37 ± 0.13 (18) apparent 0-order
	NO_2-N (0-8 g m^{-3})	$0.69\sqrt{C} - 0.24$ (0.97; 34) ½-order	$0.53\sqrt{C} - 0.18$ (0.94; 30) ½-order
	NH_4-N + NO_2-N (2-10 ; 1-5 g m^{-3})	0.76 ± 0.24 (20) apparent 0-order	0.40 ± 0.12 (10) apparent 0-order

Effects of substrate loads, DO and pH

Distinction should be made in effects of substrate loading: short-term load (monitoring conditions) on one hand, and long-term loads (adaptation conditions) on the other hand. In Table 1 effects are presented for short-term loads of single NH_4-N or NO_2-N and simultaneous loads of NH_4-N and NO_2-N, on full-grown biofilm samples differing in long-term COD loads in the adaptation system. An increased long-term COD load resulted in significantly decreased NH_4-N and NO_2-N removal rates.

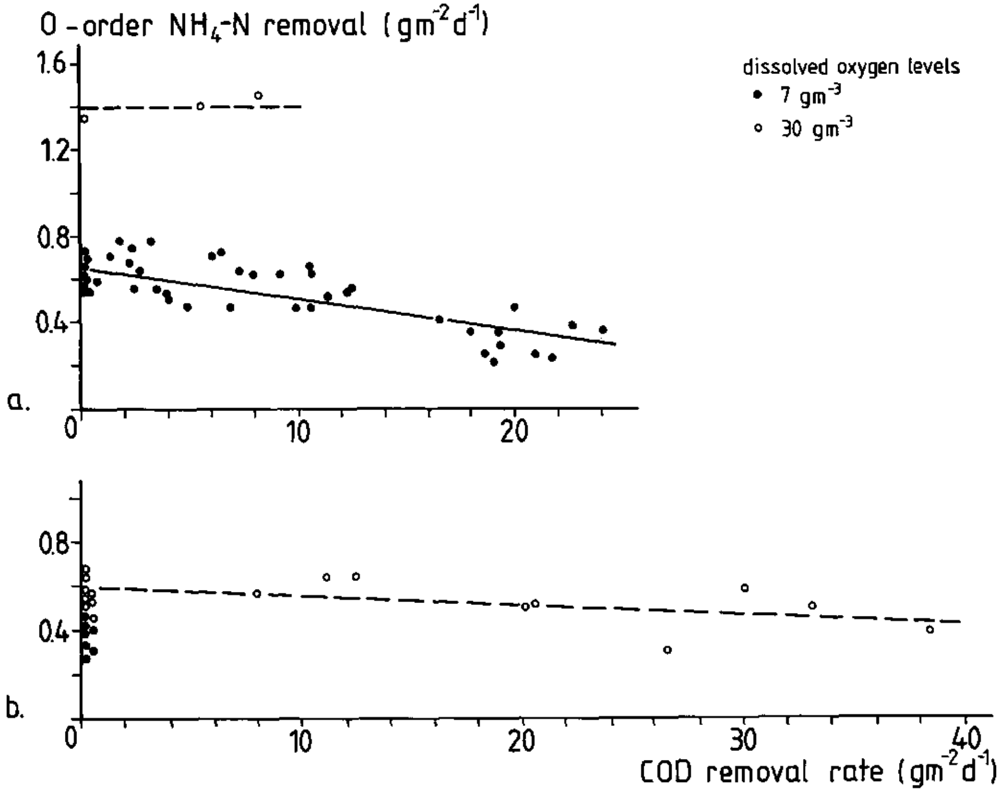

Fig. 4. Effect of increased dissolved oxygen level: 0-order NH_4-N removal rates monitored under standard conditions (pH 7, 7 g m^{-3} O_2, absence of COD); for full-grown biofilm samples; a. adapted to standard adaptation conditions (5 g $m^{-2}d^{-1}$; measurements for DO 7 g m^{-3} after Bovendeur et al., in press); b. adapted to increased COD load (circa 10 g $m^{-2}d^{-1}$)

On the other hand, for both types of biofilm samples the NH_4-N removal rate as well as the NO_2-N removal was lower in case the biofilm was loaded with a mixture of NH_4-N and NO_2-N, than in case of loading the biofilm with the single substrate only. Moreover, the NO_2-N removal rate showed an apparent 0-order removal rate rather than a ½-order removal rate in cases of simultaneous presence of NH_4-N and NO_2-N in the bulk liquid. Such apparent 0-order removal rates were observed over a wide range of NO_2-N concentrations while NH_4-N was present.

The effect of simultaneous COD removal –resulting from short-term COD loading– on the 0-order NH_4-N removal rate is demonstrated in Fig. 4 for full-grown biofilm samples monitored under DO levels of 7 and 30 g m^{-3} (Fig. 4a and 4b: biofilm adapted to long-term COD loads of respectively 5 and 10 g $m^{-2}d^{-1}$). An increased DO level resulted in higher NH_4-N removal rates, but this effect was rather small for the biofilm adapted to 10 g $m^{-2}d^{-1}$ COD loading.

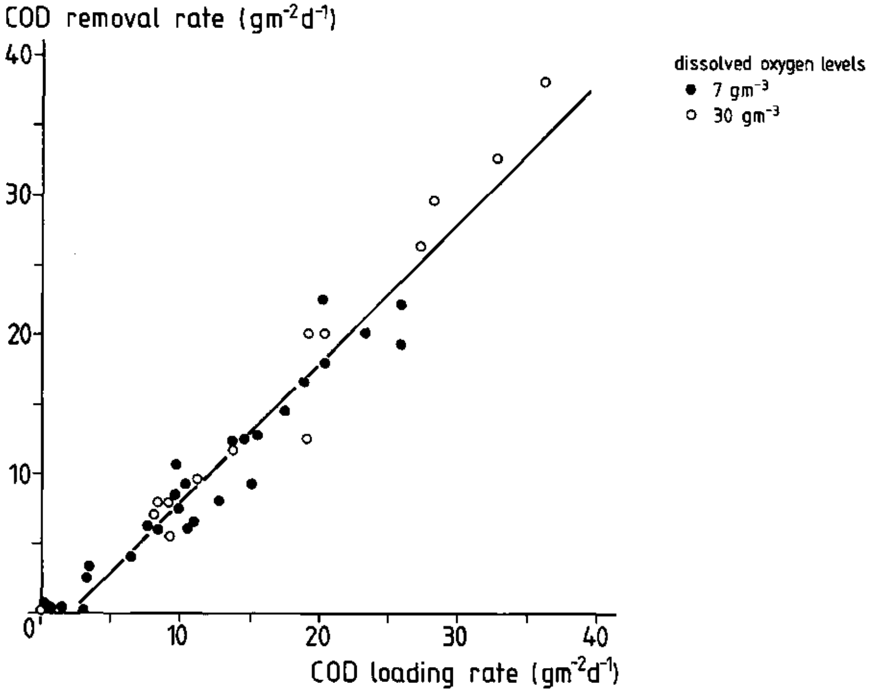

Fig. 5. Effect of increased dissolved oxygen level: COD removal rate versus short-term COD loading rate (measurements for DO 7 g m^{-3} after Bovendeur et al., in press)

The relation between the short-term COD loading rate and the corresponding COD removal rate showed a direct proportionality and was found not to be significantly affected, neither by the DO level (Fig. 5), nor by the long-term COD adaptation load.

The results of the experiments concerning effects of pH in the bulk liquid on the nitrification process are subdivided in effects on the 0-order NH_4-N removal rate (Table 2), and effects on the NO_2-N removal rate (Fig. 6), for full-grown biofilm samples adapted to standard adaptation conditions (pH 7). The biofilm samples showed a considerable decrease in activity for pH 6, both for the removal of ammonia as well as nitrite.

Table 2. Effect of pH on 0-order NH_4-N removal rate (μ ± SD (n)) of a full-grown biofilm sample grown under standard adaptation conditions and standard monitoring conditions

pH	0-order NH_4-N removal rate ($g\ m^{-2}d^{-1}$)
8	0.71 ± 0.06 (8)
7	0.56 ± 0.09 (8)
6	0.20 ± 0.10 (8)

In experiments on ammonia removal no significant nitrite accumulation has been observed. The removal of nitrite seemed to be pH-inhibited at pH 6 for NO_2-N concentrations exceeding a level of 3-4 $g\ m^{-3}$ with bulk liquid. For higher concentrations apparent 0-order removal kinetics has been observed, in stead of the original ½-order kinetics at pH 7 (Fig. 6).

DISCUSSION

Biofilm nitrification development

The particular suitability of the biofilm adaptation system and the biofilm monitoring system has been demonstrated earlier (Bovendeur et al., in press). Based on the observed development of the NH_4-N and NO_2-N removal rate as illustrated in Fig. 3,

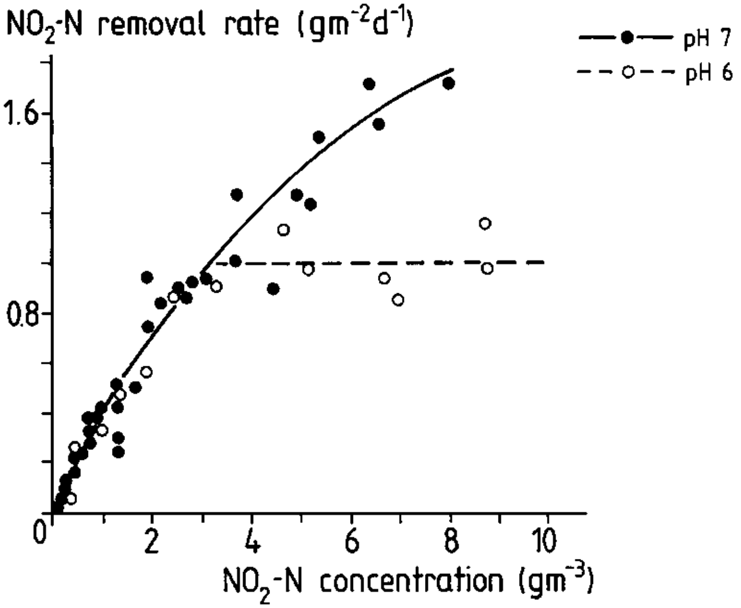

Fig. 6. Effect of pH in the bulk liquid on NO$_2$-N removal rate of a full-grown biofilm sample

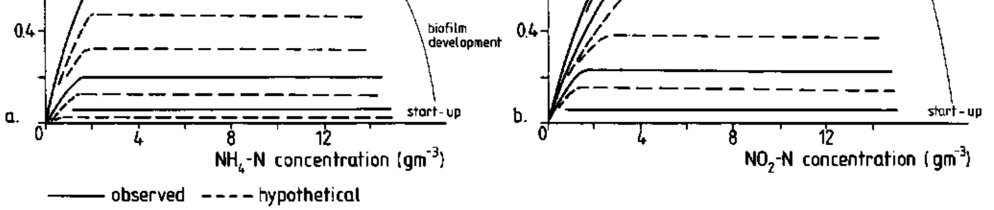

Fig. 7. Schematic biofilm development: observed and hypothetical removal rates for NH$_4$-N and NO$_2$-N

it is obvious that comparing and discussing biofilm performance is not possible without a proper characterization of the phase of development of the biofilm samples to be compared. The hypothetical course of the development of the two nitrification processes under standard adaptation conditions is shown in Fig. 7. After a short start-up phase, in which nitrite accumulated due to a lack of nitrite oxidation capacity, a "developing biofilm" shows more or less equal removal capacities for ammonia and nitrite. The observed 0-order removal rates towards the substrate bulk concentration (Fig. 3a, 7) are the result of metabolism limitation. During the next phase of biofilm development both removal capacities increase untill the maximum capacities have been reached (Fig. 3b, 7). Complete development of the biofilm samples was noticed after about 3 months. However, some reservations have to be made about the time-scale of development, since biofilm growth and development is highly dependent on conditions of loading rates and presence of seed material during the start-up phase. Recently, a special study on biofilm growth is presented by Capdeville and Nguyen (1989), distinguishing six phases of biofilm development and two types of steady state with regards to the biofilm mass, reached in the stabilization phase. The characterization full-grown biofilm used in our study may be related to one of the phases distinguished by Capdeville and Nguyen (1989) showing steady-state performance in relation to removal capacities: phases of linear growth, deceleration and stabilization. In these phases the amount of total biomass shows dynamic characteristics caused by changes of the amount of inert or deactivated organic matter, while the amount of active biomass is constant.

The final NO_2-N removal rate observed under standard monitoring conditions is significantly higher than the NH_4-N removal rate towards comparable N bulk concentrations exceeding the transition concentration for $\frac{1}{2}$-order/0-order kinetics for NH_4-N removal (Fig. 3b, 7). The $\frac{1}{2}$-order kinetics for NO_2-N removal by a full-grown biofilm, even for high NO_2-N concentrations, is the result of apparent NO_2-N diffusion limitation, indicating that the total nitrification process is not limited by the NO_2-N oxidation capacity, but by the 0-order NH_4-N oxidation capacity. However, it should be emphasized that such high-rate NO_2-N removal characteristics provides information on the potential NO_2-N removal, related to bulk concentrations only. Under normal operational conditions in aquacultural systems NO_2-N is produced in the biofilm layer itself at a rate equal to the NH_4-N removal rate. As a result of the high NO_2-N removal capacity the nitrite produced is oxidized almost instantaneously, which is confirmed by the maximum NO_2-N levels of 0.4 g m^{-3} observed during the biofilm monitoring experiments with continuous NH_4-N loading. Such concentrations in the bulk liquid represent less than 4% of the amount NH_4-N applied. A possible explanation for this small NO_2-N leak of a biofilm with a potential overkill of NO_2-N removal capacity may be partial outward-diffusion of NO_2-N that is produced in the outmost part of the biofilm layer, close to the interface of biofilm and liquid phase with a relatively low NO_2-N concentration.

Effects of substrate loads, DO and pH

Previous research (Bovendeur et al., in press) suggested that the 0-order NH_4-N removal rate of a full-grown biofilm sample adapted to standard conditions, is controlled by the availability of DO in the biofilm. According to reduced NH_4-N removal rates, the availability of DO in the biofilm for nitrification, with a DO level in the bulk liquid of 7 g m^{-3}, was found to decrease with increasing rates of simultaneous COD removal by the biofilm. This phenomenon could be explained by the reduced oxygen penetration depth of the biofilm (Harremoës, 1982), caused by the simultaneous consumption of oxygen by the heterophic bacteria in the biofilm. The availibility of oxygen in the biofilm is also the key to the explanation of the results presented in Table 1 and Fig. 4.

According to the observed ½-order kinetics of the full-grown biofilm sample adapted to standard conditions for NO_2-N removal with single NO_2-N substrate (Fig. 3b, Table 1), a NO_2-N removal rate of about 1.3 g $m^{-2}d^{-1}$ corresponds to a NO_2-N bulk concentration of 5 g m^{-3}, where the same biofilm sample converts only 0.6 g $m^{-2}d^{-1}$ NH_4-N when loaded with single NH_4-N substrate. The difference may be explained by the ratio of the stoichiometric oxygen requirements for oxidation of NH_4-N to NO_3-N (4.57 g O g^{-1} N) and oxidation of NO_2-N to NO_3-N (1.14 g O g^{-1} N), showing a four times higher oxygen requirement for complete NH_4-N oxidation. Consequently, at a comparable DO level of 7 g m^{-3}, NO_2-N removal rates even higher than 1 g $m^{-2}d^{-1}$ for a biofilm sample fed with NO_2-N only, are still not limited by diffusion of oxygen into the biofilm, but by the diffusion of NO_2-N which explains the observed ½-order kinetic relationship with the NO_2-N bulk concentration. In case the biofilm is fed with NH_4-N only, the NO_2-N removal rate is following the NH_4-N removal rate, producing the intermediate NO_2-N at a constant process controlling rate. In case of simultaneous loading with NH_4-N and NO_2-N, the overall NO_2-N removal is composed of a) oxidation of NO_2-N produced in the biofilm as intermediate of the NH_4-N oxidation at a rate equal to the NH_4-N removal rate, and b) additional oxidation of NO_2-N diffusing from the bulk liquid into the biofilm, at a constant rate limited by diffusion of DO into the biofilm. The combined result is an apparant 0-order rate for NO_2-N (Table 1), while the NH_4-N removal seems to be affected by the additional NO_2-N oxidation, resulting in a somewhat decreased 0-order removal rate (Table 1). Oxygen diffusion limitation is also confirmed by the apparant 0-order NO_2-N oxidation at a lower rate in presence of both NH_4-N and NO_2-N (0.76 g $m^{-2}d^{-1}$), compared to the removal rate in presence of NO_2-N only (1.3 g $m^{-2}d^{-1}$), both corresponding to NO_2-N bulk concentrations of 5 g m^{-3}. Moreover, the following calculations may be used as a further confirmation of the effects of oxygen diffusion limitation: the additional NO_2-N oxidation (b) represents (0.76 - 0.55)*1.14=0.24 g $m^{-2}d^{-1}$ DO consumption, fairly corresponding to the gain of oxygen availability resulting from the decreased 0-order NH_4-N removal rate: (0.60 - 0.55)*4.57=0.23 g $m^{-2}d^{-1}$.

The same phenomena are presented in Table 1 for the biofilm sample adapted to high COD loading rates (10 g $m^{-2}d^{-1}$). As a result of the high long-term COD load a higher production of non-nitrifying biofilm material –i.e. adsorbed organic matter and heterothrophic bacteria (Bovendeur et al., in press)– has taken place, resulting in a shorter residence time of the nitrifying bacteria in the aerobic zone of the biofilm (Harremoës, 1982). This effect of the high long-term COD load is illustrated by the figures in Table 1, which are lower than the corresponding removal rates for the biofilm sample adapted to 5 g $m^{-2}d^{-1}$ COD load. The oxygen diffusion limitation for the NH_4-N removal, already discussed for simultaneous oxygen consumption by heterotrophic bacteria, can be abolished by strongly increased DO levels, illustrated in Fig. 4. However, the short-term removal of COD itself was found not to be affected, neither by the long-term COD load, nor by the increased DO level (Fig. 5), probably the result of the extremely small fraction of COD that is oxidized simultaneously with removal from the liquid phase (Bovendeur et al., in press).

The observed effect of adverse pH levels in the bulk liquid (Table 2, Fig. 6) shows fair agreement with results reported in literature, e.g. reviewed for several systems by Sharma and Ahlert (1976), or discussed by Wild et al. (1971) for suspended activated sludge, or nitrifying bacteria immobilized on alginate (Tramper et al., 1985). Optimum pH levels of 7.5-8.0 are commonly presented in combination with a dramatic decrease of activity with decreasing pH unto 6. However, Haug and McCarty (1972) reported complete adaptation to low pH levels after 10 days, indicating that distinction should be made between effects of short-term ans long-term adverse pH levels. Szwerinski et al. (1986) demonstrated that the pH in the interior of the biofilm can be much lower than in the bulk liquid due to alkalinity consumption by the nitrification process. In bulk waters of low alkalinity, limitation of the NH_4-N removal rate can occur, caused by depletion of alkalinity in the rear of the biofilm combined with diffusional resistance to the inward diffusion of bicarbonate. This mechanism may be the reason for the observed pH effect on the 0-order NH_4-N removal rate presented in Table 2: transition of DO diffusion limitation to alkalinity diffusion limitation. Transition of limiting substrate is no explanation of the apparent 0-order NO_2-N removal rate observed for pH 6 (Fig. 6), since NO_2-N oxidation is not a alkalinity consuming process like NH_4-N oxidation, and the other substrates, DO and NO_2-N, being sufficiently available. A possible explanation of this phenomenon may be substrate inhibition by unionized nitrous acid, postulated by Anthonisen et al. (1976), although the reported critical bulk concentrations (>0.22 g m^{-3} HNO_2) have not been exceeded in our experiments. On the other hand, the pH effect is related to a certain transition concentration (Fig. 6), below which no effect has been observed. However, the "pH induced metabolism limited" potential NO_2-N removal capacity at pH 6 is still higher than the NH_4-N removal capacity at pH 6, indicating that accumulation of NO_2-N in the fixed-film reactor system is not likely to be induced by decreased pH levels. As a matter of fact, water quality measurements in full-scale installations in practice show slightly increased nitrite levels in the recirculating water after the water has passed the sedimentation tank. This phenomenom suggests that possible nitrite accumulation is the result of incomplete denitrification in the sedimentation tank of the system.

CONCLUSIONS

Comparison of fixed-biofilm performance should be combined with a proper characterization of the potentials for removal processes of the biofilm concerned, measured under standarized conditions.

Generally, oxidation of NH_4-N to NO_2-N by Nitrosomonas is the rate limiting step in fixed-biofilm nitrification, rather than oxidation of NO_2-N to NO_3-N by Nitrobacter.

A full-grown nitrifying biofilm may produce some NO_2-N as a result of outward diffusion of NO_2-N produced in the outmost part of the biofilm, but this process will not result in serious NO_2-N accumulation in a recycle system, since a full-grown biofilm has an extremely high potential NO_2-N removal capacity.

Oxidation of NH_4-N is rate limited by oxygen diffusion into the biofilm, resulting in decreasing 0-order NH_4-N removal rates under conditions of simultaneous oxygen consuming processes in the biofilm. The other way around, NH_4-N or NO_2-N accumulation in aquacultural recycle systems by short-term peak-loads of organic matter may be anticipated by increased DO levels in the nitrification reactor.

Short-term COD loads result in a gradually decrease of the biofilm nitrification capacity with increasing COD loads, which decrease can be abolished for the greater part by increased DO levels (oxygen diffusion limited nitrification). High long-term COD loads result in more dramatically increased nitrification capacities in comparison to short-term COD loads of the same extend, while increased DO levels have a reduced increasing effect on the nitrification capacity in comparism to a biofilm adapted to a low long-term COD load.

Decreased pH levels may lead to transition of DO diffusion limitation to alkalinity diffusion limitation for the NH_4-N removal capacity, while the pH induced metabolism limited potential NO_2-N removal capacity may be caused by unionized HNO_2 inhibition. Decreased NO_2-N removal rates were observed for higher NO_2-N concentrations only, and the effect was less dramatic than for removal of NH_4-N. However, a well-adjusted pH control is still necessary for maintaining the carrying capacity of the aquacultural recycle system by an unaffected NH_4-N removal capacity.

Serious accumulation of nitrite in an aquaculture recycle system –frequently reported in practice– is more likely to be caused by other nitrite involving processes like denitrification, rather than nitrification affected by the factors studied.

ACKNOWLEDGEMENTS

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REFERENCES

American Public Health Association. (1975) Standard methods for the examination of waste and wastewater. APHA, New York.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S. and Srinath, E.G. (1976) Inhibition of nitrification by ammonia and nitrous acid. J. Water Pollut. Control Fed., 48, 835-852.

Arvin, E. and Harremoës, P. (1989) Concepts and models for biofilm reactor performance. In: Proceedings of the Conference "Technical advances in biofilm reactors", Nice, France, 4-6 April 1989, 191-212.

Bovendeur, J., Eding, E.H. and Henken, A.M. (1987) Design and performance of a water recirculation system for high-density culture of the African catfish, Clarias gariepinus (Burchell 1822). Aquaculture, 63, 329-353.

Bovendeur, J., Zwaga, A.B., Lobee, B.G.J. and Blom, J.H. (in press) Fixed-biofilm reactors in aquacultural water recycle systems: effect of organic matter elimination on nitrification kinetics (chapter 5 of this thesis).

Capdeville, B. and Nguyen, K.M. (1989) Kinetics and modelling of aerobic and anaerobic film growth. In: Proceedings of the Conference "Technical advances in biofilm reactors", Nice, France, 4-6 April 1989, 169-190.

Collins, M.T., Gratzek, J.B., Dawe, D.L. and Nemetz, T.G. (1976a) Effects of parasiticides on nitrification. J. Fish. Res. Board Can., 32, 2033-2037.

Collins, M.T., Gratzek, J.B., Dawe, D.L. and Nemetz, T.G. (1976b) Effects of antibacterial agents on nitrification in an aquatic recirculating system. J. Fish. Res. Board Can., 33, 215-218.

Harremoës, P. (1978) Biofilm kinetics. In: R. Mitchell (Editor), Water Pollution Microbiology, Vol. 2. Wiley, New York, NY, 71-109.

Harremoës, P. (1982) Criteria for nitrification in fixed film reactors. Water Sci. Technol., 14, 167-187.

Haug, R.T. and McCarty, P.L. (1972) Nitrification with submerged filters. J. Water Pollut. Control Fed., 44, 206-2102.

LaMotta, E.J. (1976a) Kinetics of growth and substrate uptake in a biological film system. Appl. envir. Microbiol., 31, 286-293.
LaMotta, E.J. (1976b) Internal diffusion and reaction in biological films. Envir. Sci. Technol., 10, 765-769.
Liao, P.B. and Mayo, R.D. (1974) Intensified fish culture combining water reconditioning with pollution abatement. Aquaculture, 1, 317-335.
Otte, G. and Rosenthal, H. (1979) Management of a closed brackish water system for high-density fish culture by biological and chemical water treatment. Aquaculture, 18, 169-181.
Sharma, B. and Ahlert, R.C. (1977) Nitrification and nitrogen removal. Wat. Res., 11, 897-925.
Szwerinski, H., Arvin, E. and Harremoës, P. (1986) pH-decrease in nitrifying biofilms. Wat. Res., 20, 971-976.
Tramper, J. Suwinska-Borowiec, G. and Klapwijk, A. (1985) Characterization of nitrifying bacteria immobilized in calcium alginate. Enzyme Microb. Techn., 7, 155-160.
Wild jr., H.E., Sawyer, C.N. and McMahon, T.C. (1971) Factors affecting nitrification kinetics. J. Water Pollut. Control Fed., 43, 1845-1854.

CHAPTER 7

FIXED FILM NITRIFICATION CHARACTERISTICS IN SEA WATER RECIRCULATING FISH CULTURE SYSTEMS

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FIXED FILM NITRIFICATION CHARACTERISTICS IN SEA WATER RECIRCULATING FISH CULTURE SYSTEMS

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ABSTRACT

Biofilm nitrification characteristics in sea water recirculation systems are measured and compared to those in fresh water systems. As in fresh water systems, biofilm kinetics could accurately be described by the ½-order/0-order kinetic model. However, sea water systems need a much longer start-up period in which an accumulation of nitrite occur. Thereby, the maximum nitrification capacity is considerably lower than in fresh water systems; at 24°C a maximum 0-order ammonia removal rate of 0.28 g $m^{-2}d^{-1}$ NH_4-N is observed, versus 0.69 g $m^{-2}d^{-1}$ in comparable fresh water systems. Adaptation of a fresh water biofilter to higher salinities is found to be possible, providing an effective tool for a shorter start-up period of a sea water system. Nitrification rates are obtained which allow a rational design of intensive recirculating fish culture systems.

Key words –aquaculture, sea water recirculation systems,
biological sea water treatment, fixed-biofilm
reactors, biofilm performance, nitrification

INTRODUCTION

Presently, the cultivation of luxury sea fish seems to be a promising form of aquaculture in Europe. However, for optimum farming results many of these fish species, e.g. turbot, dover sole, seabass, and seabream demand a considerable higher water temperature than the ambient sea water temperature along most of the European coasts. Combining the fish production unit with a water treatment installation may be a possible procedure to reuse warm water. Moreover, such a system strongly reduces the output of organic wastes, which is also a merit from many other points of view.

For the culture of fish in fresh water a few systems are reported which are designed on a rational confrontation of waste production dynamics on one hand, and the kinetics of biological waste removal processes on the other hand, using fixed biofilm reactors (Bovendeur et al., 1987). In these studies the carrying capacity of the water treatment installation appears to be limited by the nitrification capacity, and therefore this characteristic turns out to be one of the most important keys in designing such systems. Fixed biofilm reactors are especially suitable, because high residence times of the bacterial biomass are easily maintained at high hydraulic loading rates. The kinetics of fixed biofilm nitrification processes could be described by the $\frac{1}{2}$-order/0-order kinetic model introduced by LaMotta (1976a,b) and Harremoës (1978, 1982). In sea water systems however, no experiments have been carried out to obtain nitrification rates which can be used for design purposes. A preliminary literature study about this topic reveals that transition of nitrification rates obtained from fresh water experiments to sea water systems is hazardous; in some studies the ammonia oxidation was almost completely inhibited as a result of full strength sea water chlorinity (Hill and Gellman, 1977), whereas the final conversion of nitrite into nitrate did not occur at all. However, despite such negative experiences, complete nitrification in sea water filter systems at a certain rate has been reported in many studies before (Forster, 1974;

Hirayama, 1974; Bower and Turner, 1981; Poxton et al., 1981; Wheatherly, 1984). Nevertheless, these studies hardly give any information about nitrification rates applicable for engineering purposes. The techniques used in these studies (sand or gravel beds) were meant for experimental facilities and not for large scale intensive fish culture systems. Moreover, in many studies the nitrification capacity of the system is tested under ammonia concentrations probably far below the lowest concentrations encountered in operative commercial production plants.

The objective of the present study is to quantify nitrification characteristics of fixed biofilm reactors loaded with sea water fish tank effluent, enabling a rational design of sea water recirculating fish culture systems, according to the design philosophy proposed by Bovendeur et al., 1987.

MATERIALS AND METHODS

Nitrifying biofilm material attached to a supporting medium was produced in biofilm adaptation systems. A widely used plastic supporting medium (Filterpak CR50, Mass Transfer Int., Heversham, Cumbria, U.K.), having a specific surface area of 200 m^2m^{-3}, was used in laboratory scale trickling filters (total effective surface area 0.82 m^2). The filter medium was put in a steel mesh basket enabling easy removal and replacement. The nitrification characteristics of these biofilm samples grown under various conditions were examined during periods of several hours in a biofilm monitoring system.

Biofilm adaptiation system

The biofilm adaptation system (Fig. 1) consists of biofilm samples placed above a reservoir containing water closely resembling intensive fish culture effluent with the desired salinity. This "artificial fish tank effluent" was obtained by digesting commercial fish food (Trouvit, Trouw & Co., Putten,

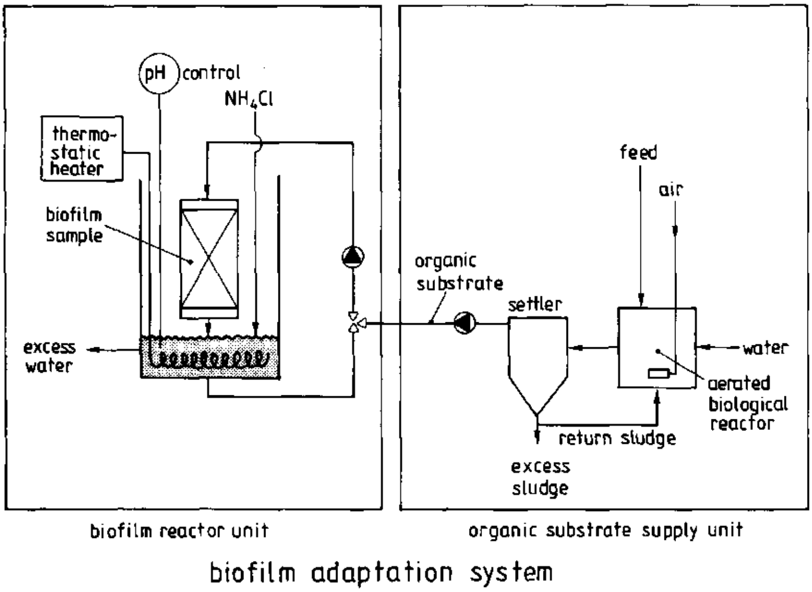

Fig. 1. Schematic presentation of the biofilm adaptation system

The Netherlands) in an activated sludge system; after settling the solids, a certain amount of the supernatant was added daily to the reservoirs in order to maintain a BOD concentration of approximately 25 g m^{-3}. Additional ammonia was added as chloride or nitrate salts maintaining an averaged total ammonia concentration of circa 5 g N m^{-3}. Desired salinities were obtained by using either natural sea water (salinity 33-34 pro mille), or tap water, or mixtures of both. The water temperature was set and maintained by a thermostatic heater at 24°C. Removal of the nitrate produced, and maintainance of the pH was achieved by water exchange. The prepared effluent was trickling continuously trough the filter bed at a hydraulic loading rate of 150 $m^3m^{-2}d^{-1}$ achieved by recirculation.

Biofilm monitoring system

The biofilm monitoring system (Fig. 2) is composed of a closed plexiglass container in which the complete biofilm samples were placed and examined batch-wise in the trickling filter mode, under controlled conditions corresponding to the conditions in the previous period. The container was filled with 0.0035 m^3 water which was recirculated through the biofilm sample by a centrifugal pump. Ammonia was added to the water to obtain an initial total ammonia concentration of circa 7 g m^{-3} NH_4-N.

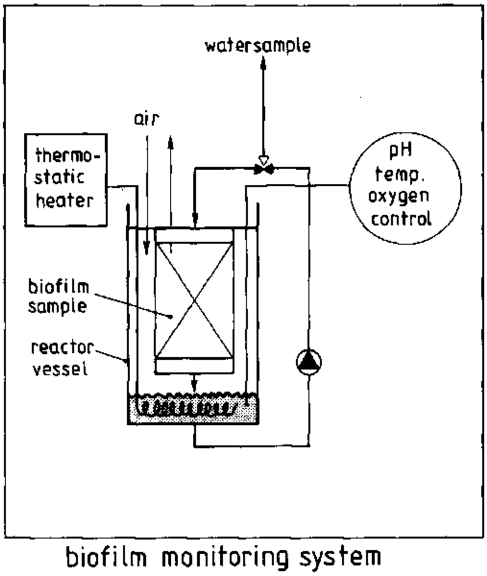

Fig. 2. Schematic presentation of the biofilm monitoring system

Experimental set up

Several attached biofilm samples were kept in biofilm adaptation systems under conditions enabling growth and maintenance. For reference purposes, measurements were made with full-grown biofilm samples obtained from trickling filters in operative systems for the culture of European eel.

The nitrification capacities examined in this study are characterized as the maximum nitrification rate as a function of the total ammonia concentration and the duration of biofilm development to reach this rate. The outcome of these experiments has led to additional experiments in which the effect of salinity was studied in more detail. This effect was demonstrated by increasing the salinity in a fresh water system at a rate of 3.5 pro mille per day. In an additional experiment, the salinity was increased by exposing a fresh water biofilm sample promptly to a salinity of 17 and 34 pro mille. All experiments were conducted at 24°C.

Water samples were taken at regular time intervals varying from 0.25-1.0 h depending on the expected biofilm performance. Total ammonia, referring to the sum of NH_3-N and NH_4-N, was measured as NH_4-N according to the method of Solórzano (1969), modified by heating the reaction products at 75°C during 0.3 h to achieve fast and complete colour formation. Nitrite was measured according to Bendschneider and Robinson (1952). Total nitrate + nitrite nitrogen was measured according to the same method after reduction of the nitrate into nitrite by cadmium-copper reductors according to Wood et al. (1967).

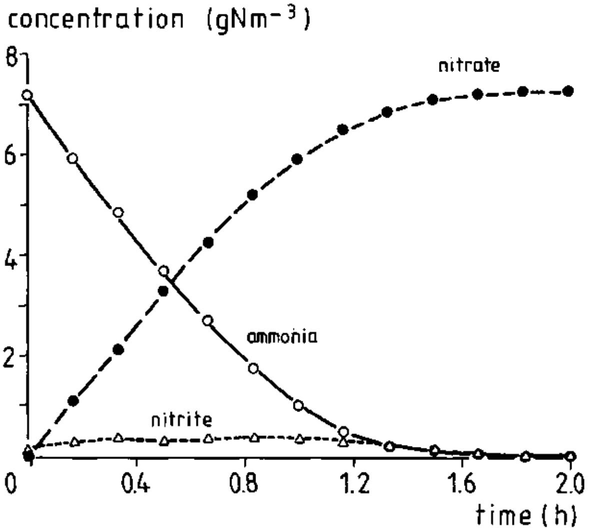

Fig. 3. Course of ammonia, nitrite and nitrate concentrations during a biofilm monitoring experiment (full-grown fresh water biofilm sample)

Immediately after termination of the measurements the biofilm samples were placed again in the biofilm adaptation system and the monitoring system was disinfected to prevent biofilm formation on the internal walls of the system itself.

RESULTS

The distinctive course of the different nitrogen compound concentrations during a nitrification experiment is shown in Fig. 3. During the experiments a slight accumulation of the intermediate product nitrite could occur.

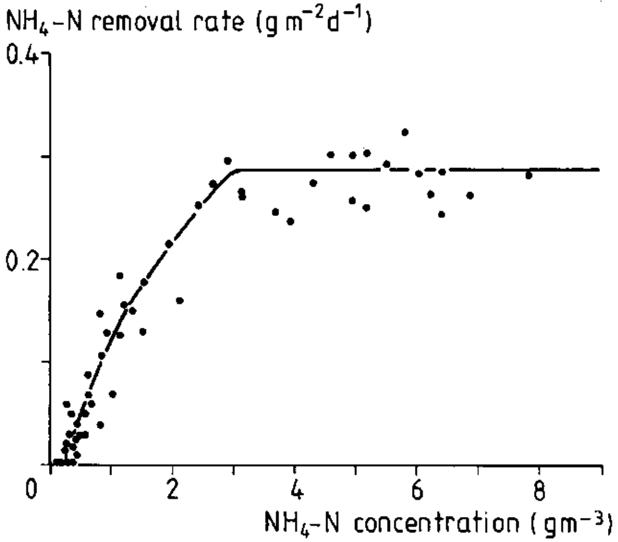

Fig. 4. Ammonia removal rate of a full-grown sea water biofilm at various ammonia concentrations (24°C, pH 8.3, 7 g m^{-3} O_2)

The ammonia removal rates of a full-grown sea water biofilm obtained from the results of seven monitoring experiments are given in Fig. 4 for the corresponding ammonia concentrations. During these experiments no significant nitrite accumulation was observed, indicating complete nitrification. The fitted relation between concentration and removal rate is based on the ½-order/ 0-order model, showing that the nitrification characteristics could accurately be described by ½-order/0-order kinetics for the sea water biofilm as well. The relations involved are mathematically described in Table 1.

Table 1. Characteristics of the ½-order and 0-order ammonia removal rates in full-grown sea water and fresh water biofilms at 24°C; C is NH_4-N concentration (g m^{-3})

	Sea water	Fresh water
½-order ammonia removal rate (g $m^{-2}d^{-1}$ NH_4-N)	$0.23\sqrt{C} - 0.11$	$0.55\sqrt{C} - 0.12$
0-order ammonia removal rate (g $m^{-2}d^{-1}$ NH_4-N)	0.28	0.69
transition concentration, C^* (g m^{-3} NH_4-N)	3.0	2.2

Comparison of the 0-order ammonia removal rates during the development of a fresh water and a sea water nitrifying biofilm reveals a much longer start-up period for sea water biofilters (Fig. 5). In addition, it is shown that a sea water biofilm reaches a significantly lower maximum 0-order ammonia removal rate than a fresh water biofilm. Moreover, at all ammonia concentrations the ammonia removal rate of a sea water biofilm is significantly lower than a fresh water biofilm, whereas the transition concentration, denoted as C^*, is somewhat higher (Fig. 6, Table 1).

The course of the ammonia and nitrite concentrations during experiments in which the salinity is increased daily, is

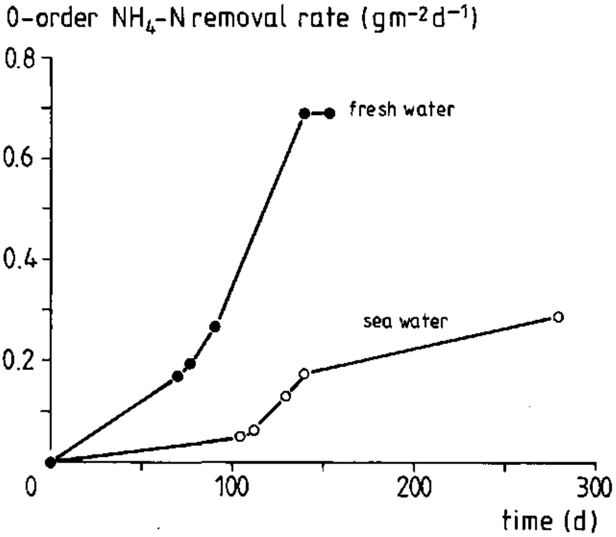

Fig. 5. Increase of 0-order ammonia removal rate during the development of a sea water and a fresh water biofilm

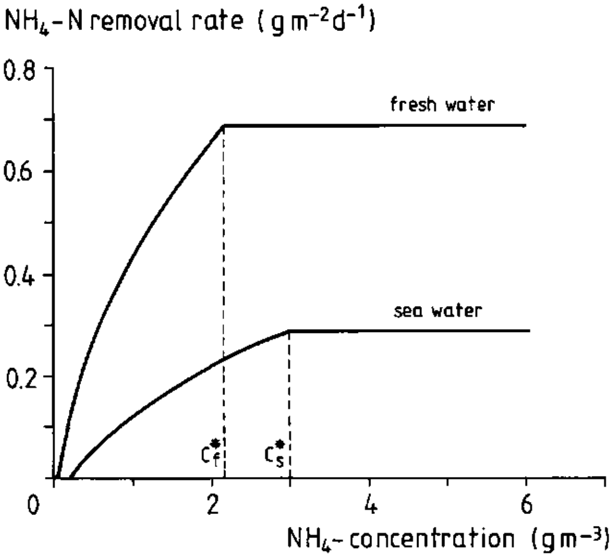

Fig. 6. Ammonia removal rate versus ammonia concentration for fresh water and sea water biofilms at 24°C (sea water: pH 8.3, 7 g m^{-3} O_2; fresh water: pH 8.2, 8.4 g m^{-3} O_2); C_s^* and C_f^* represent the transition concentration in sea water and fresh water, respectively

displayed in Fig. 7. In a certain salinity range (0-14 pro mille) the ammonia removal rate remains unaffected, whereas the accumulation of nitrite is strongly increased. Further increased salinities also affected the ammonia oxidation process, as is shown in Fig. 7 for a salinity of 17 pro mille. However, the effect of higher salinities on the ammonia oxidation process, in terms of reduction of the 0-order removal rate of a fresh water biofilm, is found to be temporary: after about 10 days the 0-order removal rate has restored to the original level of 0.27 g $m^{-2}d^{-1}$, both for 17 and 34 pro mille salinities (Fig. 8).

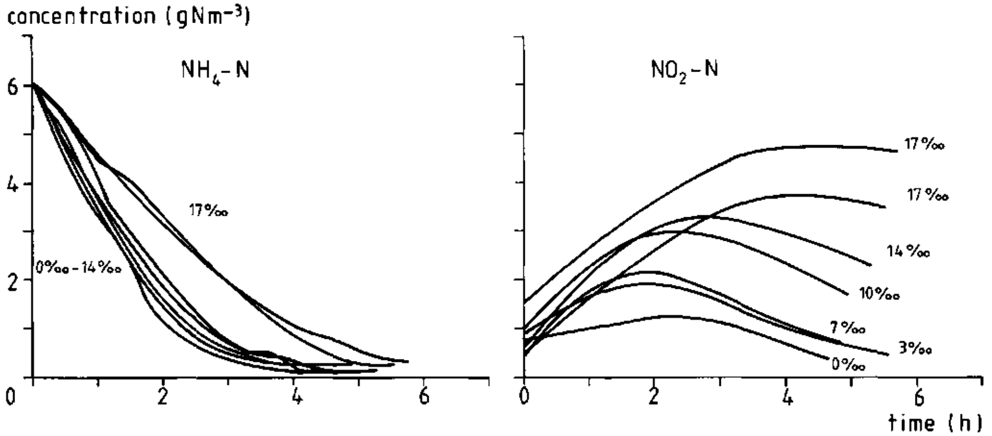

Fig. 7. Ammonia and nitrite concentration plots obtained by exposing a fresh water biofilm sample to a daily increased salinity of the recirculating water

DISCUSSION

The curves in Fig. 1 demonstrate that the decrease of the total ammonia concentration is the result of the nitrification process only, since all NH_4-N removed is finally recovered as NO_3-N. Therefore, it can be concluded that NH_3-N stripping does not occur during the measurements.

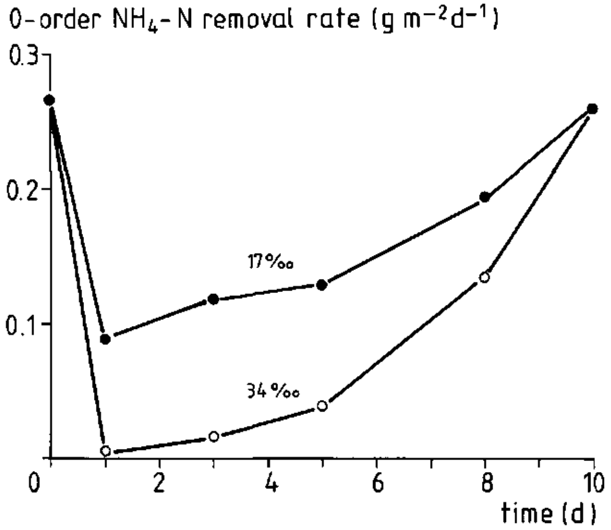

Fig. 8. Course of 0-order ammonia removal rate of fresh water biofilm samples after exposure to salinities of 17 and 34 pro mille

Nitrification in sea water systems using attached biofilms is possible at rates of about 0.28 g $m^{-2}d^{-1}$ NH_4-N (Fig. 4), under the conditions described. The nitrification process could accurately be described be the ½-order/0-order kinetic model. Briefly, this model is based on diffusional transport of substrates in the biofilm and biological substrate utilization processes. Fig. 3 shows that in presence of relatively high ammonia concentrations a constant decrease of the ammonia concentration is observed, indicating a reaction rate independent of the ammonia concentration (0-order kinetics). As the ammonia concentration declines below a certain transition concentration (C^*), the declining ammonia removal rate suggests dependence on the ammonia concentration. According to the model, concentrations lower than C^* cause incomplete penetration in the biofilm resulting in a decreased removal rate per unit area, related to the square root of the ammonia concentration (½-order kinetics).

To achieve maximum ammonia removal rates in sea water systems, a longer start-up period is required (Fig. 5), in which larger accumulations of nitrite occur than in fresh water systems. Also the maximum nitrification capicity is considerably lower in these systems (Fig. 5 and 6). The explanation for the relatively low ammonia removal rate in sea water systems can probably be found in the inhibiting effect of chloride on the nitrification, which is reported to occur at chloride concentrations exeeding 10 g m^{-3} (Richardson, 1985).

It should be emphasized that the ammonia removal rates in Fig. 5 do not necessarily represent complete nitrification rates into nitrate, but only ammonia oxidation rates. During this study the biofilm nitrite oxidation rate, in comparison to the ammonia oxidation rate, developed significantly slower in sea water than in fresh water. As a result, a considerable accumulation of nitrite was observed during the first months of operating these filters until complete nitrification was achieved. The curves shown in Fig. 4 and 6 are based on complete nitrification rates into nitrate by full-grown biofilm samples.

Fig. 7 suggests a higher sensitiveness to salinity of the nitrite oxidation process than the ammonia oxidation process. This may account for the slow development of the nitrite oxidation capacity during the start-up, and therefore also for the high accumulations of nitrite. Although nitrite is a highly toxic compound in fresh water, its toxicity is greatly reduced in sea water (Poxton and Allouse, 1982). Therefore, the high concentrations of nitrite during the first months of operating sea water recirculations systems are not restricting the application of this technique in practice.

In contradiction with other studies (Bower and Turner, 1981), a very effective procedure in overcoming the long start-up period of a sea water biofilm is starting the system in fresh water and adapting it to higher salinities (Fig. 8). This procedure is successfully applied in practice at the Netherlands Institute for Fishery Investigations. A biofilm is grown in fresh water until the filter showed some nitrification capacity (approx. 0.3 g $m^{-2}d^{-1}$ NH_4-N). Then the fresh water is replaced by 17 pro mille sea water (obtained by adding tap water to natural sea water), and after the ammonia removal has restored again, the biofilm was exposed to full strenght sea water with a salinity of 34 pro mille. It is judged unnecessary to grow a fresh water biofilm with a higher initial nitrification rate than about 0.3 g $m^{-2}d^{-1}$, since higher nitrification rates were not achieved in sea water (Fig. 4 and 6). A period of exposure to intermediate salinity is strongly recommended for reasons of safety. In practice, it is found that the presented procedure may lead to a shorter start-up period than suggested in Fig. 5 and 8.

The use of the nitrification rates for engineering purposes is obvious; for instance, 1 kg of pelleted feed, fed to eel in a recirculation system, induces an ammonia production of about 40 g NH_4-N (Heinsbroek, 1987). If the system is to be designed to maintain an averaged ammonia concentration just lower than the transition concentration, an ammonia removal rate of about 0.25 g $m^{-2}d^{-1}$ NH_4-N (less than half of the removal rate in a comparable fresh water system) can be expected. This means that 160 m^2 biofilm surface area (0.8 m^3 of the filter medium used) should be regarded as the absolute minimum amount of surface area to be installed per kg daily feed supply. In practice the recommended amount will be highly dependent on the effectiveness of the remaining engineering and management of the system.

CONCLUSIONS

Complete nitrification of anmmonia into nitrate is possible in sea water systems, although the obtained nitrification rates per unit area are considerably lower than in comparable fresh water systems.

Like in fresh water systems, the biofilm nitrification process in sea water can also be described by the $\frac{1}{2}$-order/0-order kinetic model.

The development of nitrification, particularly the nitrite oxidation capacity, is considerably slower in sea water than in fresh water.

The effect of the salinity on the nitrification process is more pronounced for the nitrite oxidation step than for the ammonia oxidation step.

The start-up period of a sea water recirculating fish culture system can be shortened by adapting a fresh water biofilm reactor to higher salinities.

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REFERENCES

Bendschneider, K., Robinson, R.J. (1952). A new spectrofometric method for the determination of nitrite in sea water. J. Mar. Res., 11:87

Bovendeur, J., Eding, E.H., Henken, A.M. (1987). Design and performance of a water recirculation system for high density culture of the African catfish, Clarias gariepinus (Burchell 1822). Aquaculture, 63:329-353.

Bower, C.E., Turner, D.T. (1981). Accelerated nitrification in new sea water culture systems: effectiveness of commercial additives and seed media from established systems. Aquaculture, 24:1-9.

Forster, J.R.M. (1974). Studies on Nitrification in Marine Biological Filters. Aquaculture, 4:387-397.

Harremoës, P., 1978. Biofilm Kinetics. In: R. Mitchell, Editor, Water Pollution Microbiology, Vol. 2, Wiley and Sons, New York, NY, 71-109.

Harremoës, P., 1982. Criteria for Nitrification in Fixed Film Reactors. Water Science and Technology, 14: 167-187.

Heinsbroek, L.T.N., (1987). Waste production and discharge of intensive culture of African catfish, European eel and rainbouw trout in recirculation and flow-through systems in the Netherlands. EIFAC working party on fish farm effluents, The Hague, 29 May - 1 June 1987, 8 p.

Hill, D.O., Gelman, S.R. (1977). Effects of chloride on nitrification rates in activated sludge systems. Proc. of Industrial Waste Conference, Purdue University ext. 808-813.

Hirayama, K. (1974). Water control by filtration in closed culture systems. Aquaculture, 4:369-385.

LaMotta, E.J. (1976a). Kinetics of growth and substrate uptake in a biological film system. Appl. envir. Microbiol., 31: 286-293.

LaMotta, E.J. (1976b). Internal diffusion and reaction in biological films. Envir. Sci. Technol., 10: 765-769.

Poxton, M.G., Allouse, S.B. (1982). Water quality criteria for marine fisheries. Aquaculture Engineering 1:153-191.

Poxton, M.G., Murray, K.R., Linfoot, B.T., Pooley, A.B.W. (1981). The Design and Performance of Biological Filters in an experimental Mariculture Facility. Proceedings World Symposium on Aquaculture in Heated Effluents and Recirculation Systems, Stavanger 28-30 May, 1980. Vol I. Heeneman, Berlin.

Richardson, M. (1985). Nitrification inhibitors in the treatment of sewage. C. Biol., M. Biol., M.I.W.E.S. Thames water, Reading, U.K.

Solórzano, L. (1969). Determination of ammonia in natural waters by the phenol hypochlorite method. Limnol. Oceanogr. 14: 799-801.

Weatherly, L.R. (1984). Rate models for a Marine Biological Filter. Aquacultural Engineering 3: 15-29.

Wood, E.D., Amstrong, F.A.J., Richards, F.A. (1967). Determination of nitrate in sea water by cadmium-copper Reduction to nitrite. J. Mar. Biol. Ass. U.K., 47:23-31.

CHAPTER 8

GENERAL DISCUSSION

GENERAL DISCUSSION

In the constituent chapters of this thesis it is shown that in general fixed-biofilm performance is the resultant of several processes, which are affected in different ways by various factors, thus making connecting fixed-biofilm performance with external conditions a very complex matter. Although the results of fixed-biofilm experiments on COD removal and simultaneous nitrification have been discussed in detail in the relevant chapters, it is useful to consider some observed phenomena in a wider context, linking the experimental results of the various chapters and the applications concerned.

Kinetic model

First of all it is appropriate to emphazise the suitability of the $\frac{1}{2}$-order/0-order kinetic model for explaining fixed-biofilm performance in relation to dissolved substrates. Consequently, the model is found useful for design and operation purposes for water treatment systems including fixed-biofilm reactors, especially in combination with information on the mechanisms of suspended organic matter removal. This combination is covered recently in a general biofilm model as presented by Gujer and Boller (1989) for rotating biological contacting.

Trickling filter and rotating biological contactor performance

Regarding the overall results, the trickling filter showed fair resemblance in nitrification capacities per unit biofilm area both for application in aquaculture (Chapter 4, 5 and 6) and post-treatment of anaerobic effluent (Chapter 2). The observed capacities also agreed with the capacities found for direct treatment of domestic sewage (Boller and Gujer, 1986). Some differences may be explained by different waste water composition, water temperature and COD loading rate. In comparison to trickling filter performance, the rotating biological contactor showed a greater ability to handle high loading rates, resulting in significantly higher removal, c.q. oxidation capacities for COD and NH_4-N (Chapter 2 and 3). The nitrification capacities observed agree very well with the results reported by Gönenc and Harremoës (1985) and Boller et al. (1989). The lower capacities of the trickling filter, as compared to the rotating biological contactor may be accounted for at least the greater part by the hydrodynamica involved with trickling filtration (Harremoës, 1978). The predominating hydrodynamic effect is related to the wetted area of the packing material, and by that, to the effective surface area, while the removal rates are based on the specific surface area. Moreover, within the wetted area significant differences may be expected in the hydraulic and substrate loading, resulting in a strongly varying biofilm thickness, liquid film diffusion resistance, and also varying mixtures of active and inactive biofilm material. In contrast to these phenomena, much more uniform process conditions are maintained in a rotating biological contactor, in combination with a complete utilization of the provided surface area.

Another complicating factor in trickling filtration is the possible oxygen exhaustion of the air phase in full-scale reactors without forced aeration; it is not likely that this phenomena has occurred in the experimental reactors used in this research. Nevertheless, the alternating submersion/emersion cycle of the rotating biological contactor may be more beneficial in relation to the oxygen/substrate distribution (Paolini, 1986), while this mode of operation may also be regarded as a series of short-term loads, of which is found that a biofilm sample is able to handle very well (Chapter 2, 5 and 6).

Aquacultural water recirculation systems

The design philosophy presented in Chapter 4 covers the need for rational design methods in aquacultural water reuse systems, fully utilizing the specific properties of attached biofilms for removal of waste products. The developed design philosophy, based on tuning the waste removal kinetics to the waste production dynamics, is a new and effective approach in designing aquacultural water reuse systems. A significant difference with municipal waste water treatment systems is that there is no need to remove the total amount of waste products in one passage of the reactor, but the complete removal of the waste may take many passages of the reactor, as long as certain maximum concentration levels are not exceeded. Refinements of the design formulas of Chapter 4 can be found in anticipating the effects of system-specific factors on the nitrification kinetics, as discussed in Chapter 5, 6 and 7; the final result is a completely controlled water recirculation system with regards to the original waste products.

The presented design method has already been applied in practice: in 1988 the new experimental accommondation for fish culture of the Wageningen Agricultural University has been put into use, including several recirculation systems, designed according to the presented philosophy. These systems work satisfactory since, while also several commercial full-scale installations in the Netherlands have been evaluated succesfully for improvements on the basis of this design philosophy by Heinsbroek and Kamstra (in prep.). However, since the design philosophy presented is based on the production and elimination of the original waste products, i.e. faecal organic matter and ammonia nitrogen, additional attention should be paid to the behaviour of fish, in terms of feed intake and growth, in the resulting recirculating water. Besides high levels of nitrate and other dissolved salts, the recirculating water usually contains high concentrations of dissolved non-biodegradable organic matter (Chapter 4), which probably are humic substances resulting from the biological breakdown of organic matter. Also the effects of specific external "message" substances produced by the fish, and the fate of these substances in the water treatment installation, deserves further elaboration.

With respect to the objectives of recirculation systems in fish culture –water and energy conservation– estimating calculations showed combined cost savings up to 80% for the experimental facility at the Wageningen Agricultural University, depending on the adopted starting points (e.g. initial water temperature, required water quality). Comparison between flow-through systems and recirculation systems in full-scale practice is not possible, since full-scale flow-through systems were never build; the cost saving percentages mentioned above for recirculation systems explain why! However, it should be noted that higher investments are involved with the installation of water recirculation systems. Besides the water and energy cost savings, the recirculation system also has a significant advantage from an environmental protection point of view: on the basis of oxygen demands, including the oxidation of organic matter and ammonia nitrogen, the amount of waste products discharged into the receiving surface water is reduced to less than 5% of the total amount of waste produced; 45-50% is oxidized in the fixed-film reactor and an equal amount of waste products is collected in the sedimentation tank (Chapter 4; Heinsbroek, 1988).

As a consequence of the high removal capacities of the rotating biological contactor applied to domestic waste water post-treatment, application of such a reactor in aquaculture should be considered. However, a final treatment of the water in a trickling filter before the water enters the fish tank, might be useful in any recirculation system, because in trickling filtration biological water conditioning is combined with stripping of excessive carbondioxide and nitrogen gas, and supply of oxygen up to the saturation level. Moreover, the simple reactor construction and the absence of moving parts in the installation reduce the risks of technical failures which may be catastrophic for the living stock.

Post-treatment of anaerobic effluents

For the post-treatment of anaerobic effluents no complete design proposal has been worked out, like has been done for the aquaculture water recycle systems. However, the main ingredients are provided in Chapter 2 and 3. Based on the demonstrated features it seems reasonable to choose rotating biological contactors in a two stage installation for the duties of removal of dissolved and suspended organic matter as well as nitrification. A special application of the rotating biological contactor may enable a high degree of integration of anaerobic pre-treatment by the UASB process and the aerobic post-treatment, by placing the first stage rotating contactor in the settling compartment of the UASB reactor. In this way the rotating contactor acts as a flocculator of organic matter that is returned via the settler to the anaerobic sludge blanket. On account of such combination an optimal utilization is reached of the observation by Marsh et al. (1981) that "the solids entering the RBC system are not the same solids leaving the system, even though the influent and effluent suspended solids concentrations are essentially the same": the settler/rotating biological contactor unit may be regarded as an intensified by-pass of suspended organic matter. The advantage of the intended integration is clear: no additional land area is required for the additional removal of (suspended) organics from the effluent, thus meeting part of the initial objectives of the post-treatment. Adopting the starting points for the loading rate of the first stage rotating contactor discussed in Chapter 3, in combination with a reasonable average ratio for suspended/ dissolved COD, the dimensions of the settling compartment need only minor modifications to realize this option. The resulting effluent should be nitrified in a separate reactor which may produce that small contents of suspended solids in the final effluent, that a post-settling tank may even be superfluous. This nitrification reactor can be optimized according to the results of Chapter 2, 5 and 6. The proposed integration needs further elaboration, with regards to design and operation as well as cost-effectiveness, which factors are judged to be very specific for each application/location/effluent quality combination (e.g. Hitdlebaugh and Miller, 1981; Fujie et al., 1983; Gilbert et al., 1986). However, it is concluded that the general concept of this integrated anaerobic/aerobic system has very attractive features.

Overall conclusion

Fixed-biofilm performance is found to be very complex indeed, however, the information generated in this reseach may be regarded as a valuable contribution to a more rational approach in design and operation of aerobic fixed-film reactors in the field of water treatment, as compared to the conventional, largly "folklore-based" approaches.

References

Boller, M. and Gujer, W., 1986. Nitrification in tertiary trickling filters followed by deep-bed fiters. Wat. Res., 20, 1363-1373.

Boller, M., Gujer, W. and Nyhuis, G., 1989. Tertiary rotating biological contactors for nitrification. In: Technical advances in biofilm reactors, International EWPCA-IAWPRC Conference, Nice, France, 4-6 April 1989, 105-117.

Fujie, K., Bravo, H.E. and Kubota, H., 1983. Operational design and power economy of a rotating biological contactor. Wat. Res., 17, 1153-1162.

Gilbert, W.G., Wheeler, J.F. and MacGregor, A., 1986. Energy usage of rotating biological contactor facilities. J. Water Pollut. Control Fed., 58, 47-51.

Gönenc, E. and Harremoës, P., 1985. Nitrification in rotating disc systems –I, Criteria for transition from oxygen to ammonia rate limitation. Wat. Res., 19, 1119-1127.

Gujer, W. and Boller, M., 1989. A mathematical model for rotating biological contactors. In: Technical advances in biofilm reactors, International EWPCA-IAWPRC Conference, Nice, France, 4-6 April 1989, 69-89.

Harremoës, P., 1978. Biofilm kinetics. In: R. Mitchell (Editor), Water Pollution Microbiology, Vol. 2. Wiley, New York, NY, 71-109.

Heinsbroek, L.T.N., 1988. Waste pproduction and discharge of intensive culture of African catfish, Clarias gariepinus, European eel, Anquilla anquilla, and rainbow trout, Salmo gairdneri, in recirculation and flow-trough systems in the Netherlands. In: M. Pursiainen (Editor), National contributions on suspended solids from land based fish farms. Papers presented at the first session of the EIFAC working party on fish farm effluents, The Hague, The Netherlands, 29-30 May and 1 June 1987, 55-61.

Heinsbroek, L.T.N. and Kamstra, A., in preparation. Design and performance of water recirculation systems for eel culture.

Hitdlebaugh, J.A. and Miller, R.D., 1981. Operational problems with rotating biological contactors. J. Water Pollut. Control Fed., 53, 1283-1293.

Hogendoorn, H., 1983. The African catfish (Clarias Lazera C.&V., 1840) –a new species for aquaculture. Thesis. Wageningen Agricultural University, Wageningen, The Netherlands, 133 pp.

Jones, A., 1986. Historical background, present status, and future perspectives of the aquaculture industry on a worldwide basis. In: J.G. Balchen and A. Tyssö, Preprints of the EIFAC Symposium on automation and data processing in aquaculture, Trondheim, Norway, August 1986, 1-9.

Marsh, D., Benefield, L., Bennet, E., Linstedt, D. and Hartman, R., 1981. Coupled trickling filter-rotating biological contactor nitrification process. J. Water Pollut. Control Fed., 1469-1480.

Paolini, A.E., 1986. Effect of biomass on oxygen transfer in RBC systems. J. Water Pollut. Control Fed., 58, 306-311.

CHAPTER 9

SUMMARY

SAMENVATTING

SUMMARY

Fixed-biofilm reactors have been applied in waste water treatment for about one century. With the recent introduction of modern packing materials, this type of reactor has received a renewed impuls for the classic applications in waste water treatment as well as increased interest for implementation in new fields of water treatment. In this thesis the possibilities are presented for fixed-biofilm post-treatment of anaerobically digested domestic sewage and water reconditioning in aquacultural recirculation systems. Crucial points for these applications are the biofilm characteristics for removal of organic matter and nitrification of ammonia nitrogen.

In an introductory study on post-treatment of anaerobically pretreated sewage, trickling filtration and rotating biological contacting were employed. In comparison to the trickling filter performance, the rotating biological contactor demonstrated a greater ability to handle high loading rates, resulting in remarkably higher removal rates per unit biofilm surface area. Under relevant loading conditions main removal rates were found of about 35 g $m^{-2}d^{-1}$ COD for the rotating biological contactor and circa 5 g $m^{-2}d^{-1}$ COD for the trickling filter, while the figures for ammonia removal are about 2.4 g $m^{-2}d^{-1}$ NH_4-N and 0.35 g $m^{-2}d^{-1}$ respectively. Additional to this difference in capacity under normal operational conditions, it was concluded that the rotating biological contactor is able to handle peak loads of suspended solids COD. The observed differences in reactor performande are mainly attributed to the different hydrodynamics in the two types of reactor.

With regard to COD removal by the rotating biological contactor, however, it was found that removal efficiency is highly dependent on the content of suspended organics, indicating that different mechanisms are responsable for removal of suspended and dissolved organic matter from the water phase. Therefore, a further study is made on aerobic biofilm COD removal and simultaneous biofilm respiration using a Wazu respirationmeter

unit: in a closed recirculating system completely submerged biofilm samples were examined in short-term batch experiments, in which the biofilm samples were loaded with waste water or an acetate solution as model for dissolved organic substrate. The removal of dissolved organic substrate and the biofilm respiration were found to answer the ½-order/0-order kinetic model, whereas the removal of suspended solids COD showed 1-order kinetics. Under conditions usually met in practice the 0-order rates for dissolved COD removal and biofilm respiration were 30-40 g $m^{-2}d^{-1}$ and 10-16 g $m^{-2}d^{-1}$ respectively. In absence of organic substrate in the bulk liquid a certain background respiration was observed (3.5-9.0 g $m^{-2}d^{-1}$ O_2). As a result of the 1-order kinetics observed for a wide concentration range, extremely high suspended solids COD removal rates were observed, even exceeding 300 g $m^{-2}d^{-1}$. The difference between the maximum respiration rate and the maximum COD removal rate is attributed to physical biofilm adsorption processes, rather than to biodegradation processes. Biofilm COD removal kinetics for waste water containing a mixture of suspended and dissolved COD are discussed, illustrating that the apparent kinetic order of the combined process and the reaction constant strongly depends on the ratio of suspended and dissolved COD. With regard to design of fixed-film reactors for organic matter removal it is demonstrated that the concentration of dissolved organic substrate at which transition between ½-order and 0-order kinetics occurs, is also significant for the kinetic order of the resultant total COD removal. The overall conclusion of this part of the study is that a rotating biological contactor is very suitable for removal of mixed suspended and dissolved organics and may be described as a reactor in which biochemical oxidation of dissolved organic substrate is combined with adsorption/flocculation processes of suspended organic matter up to extreme ratios.

The suitability of fixed-film reactors for application in aquacultural recirculation systems is demonstrated by the development of a pilot-scale recirculation system for the culture of the African catfish. The heart of the water treatment installation was a trickling filter designed for nitrification. Since the nitrification was also found to follow the ½-order/0-order kinetic model, the nitrification kinetics were used to tune the reactor removal performance to the diurnal fluctuation of the production of ammonia by the fish. An average 0-order ammonia removal rate of about 0.55 g $m^{-2}d^{-1}$ NH_4-N could be satisfactory used as a key in the design formulas for reactors treating presettled fish culture water at 25°C, pH 7 and dissolved oxygen levels of about 7 g m^{-3}. Based on the observed biofilm kinetics and the waste production dynamics a complete design philosophy is presented for a water treatment system, consisting of a primary clarifier and an aerobic fixed-film reactor. In this philosophy, the possible types of reactor performance are described in relation to the ambient concentrations of ammonia and dissolved oxygen and to the diurnal variation of the ammonia loading rate of the reactor. Attention is paid to a) hydraulic loading of the primary clarifier, b) dimensions of the fixed-film reactor, c) water recirculation rate, and d) water exchange rate.

Since the 0-order nitrification rate may be regarded as the key in design approaches for fixed-film reactor implementation in aquacultural recirculation systems, several follow-up studies were made on the sensitivenesses of the fixed-film nitrification process to several factors related to the operation of such recirculation systems. The factors concerned are the simultaneous COD loading rate, dissolved oxygen level, pH and salinity. Monitoring experiments on biofilm nitrification development showed that after the initial start-up periods, characterized by accumulation of nitrite, the nitrite oxidation capacity is developing faster than the ammonia oxidation capacity. Finally this faster development resulted in a higher nitrite removal capacity than the corresponding ammonia removal capacity in a full-grown biofilm sample. The impacts of the factors introduced above were studied for full-grown biofilm samples. A special biofilm adaptation system is used in these studies to grow "artificial" biofilm samples, the performance of which is studied under well-controlled laboratory conditions in a biofilm monitoring system.

In the effects of the organic matter loading of the biofilm nitrification, distinction is made between long-term loading and short-term peak loads of organic matter. For short-term loads of organic matter reduced 0-order nitrification rates were found, inversely proportional to the corresponding COD removal rate. The reduction factor is quantified as -0.015 g $m^{-2}d^{-1}$ NH_4-N nitrified per g $m^{-2}d^{-1}$ COD removed. This reduction factor indicated that the nitrification process is limited by transfer of oxygen into the biofilm layer during short-term peak loads of organic matter. The reduction of the nitrification is restricted in degree as a result of the partial oxidation of the organic matter (circa 10%), immediately after removal from the water phase. Long-term background loads of organic matter showed a more dramatic decrease of the 0-order nitrification rate: under simultaneous loading conditions of 5 g $m^{-2}d^{-1}$ COD about 0.6 g $m^{-2}d^{-1}$ NH_4-N was nitrified versus about 0.4 g $m^{-2}d^{-1}$ NH_4-N for 10 g $m^{-2}d^{-1}$ COD loading (pH 7, 7 g m^{-3} O_2, 25°C). This decrease of the nitrification rate is attributed to the higher production of non-nitrifying biofilm material, resulting in shorter residence times of the nitrifying bacteria in the aerobic zone of the biofilm.

The oxygen diffusion limited 0-order nitrification rate under normal operational conditions is confirmed by the effect of increased and decreased dissolved oxygen levels in the bulk liquid. An extremely high level (30 g m^{-3} O_2) resulted in metabolism limited nitrification rates, differing for the long-term background COD loading rate: circa 1.4 g $m^{-2}d^{-1}$ NH_4-N and 0.6 g $m^{-2}d^{-1}$ NH_4-N for 5 and 10 g $m^{-2}d^{-1}$ COD, respectively (pH 7, 25°C, absence of COD in the monitoring experiments). The observed reduction factor for the nitrification caused by short-term simultaneous COD removal was found to be absent or extremely low for such high dissolved oxygen levels. A decreased dissolved oxygen level (3 g m^{-3} O_2) resulted in a decreased 0-order nitrification rate of about 0.3 g $m^{-2}d^{-1}$ NH_4-N for the biofilm grown under COD loading conditions of 5 g $m^{-2}d^{-1}$, while the reduction factor for the nitrification rate as function of the simultaneous COD removal rate was essentially the same as found for a dissolved oxygen level of 7 g m^{-3}. Independent of the dissolved oxygen level, the biofilm samples removed organic matter, expressed as COD, at rates directly proportional to the COD loading rate, showing efficiencies of about 85%. The independence on the dissolved oxygen level is explained as consequence of the very small fraction of COD oxidized, immediately after removal from the water phase.

Low pH levels in the bulk liquid resulted in a considerable decrease in biofilm activity, both for the removal of ammonia and nitrite. However, no accumulation of nitrite was observed in experiments where the biofilm sample was fed with ammonia only. When fed with nitrite only, the biofilm showed an apparent 0-order nitrite removal rate at pH 6, where ½-order kinetics and higher removal rates were observed for pH 7. The reduction of the ammonia oxidation capacity at low pH levels is explained in terms of transition of the limiting factor (dissolved oxygen/alkalinity), while the reduction of the nitrite removal capacity is considered to be the result of possible substrate inhibition by unionized nitrous acid.

Finally, the performance of fixed-biofilm samples in seawater is studied in relation to the desired development of marine recirculation system for aquaculture. Biofilm samples grown in seawater showed a much longer start-up period, in which nitrite accumulation occurred. Thereby, the maximum ammonia removal rate was considerably lower than in the fresh water systems: at 24°C the 0-order ammonia removal rate was about 0.3 g $m^{-2}d^{-1}$ NH4-N versus about 0.6 g $m^{-2}d^{-1}$ NH_4-N (pH 7 in fresh water, pH 8.3 in seawater, both systems 7 g m^{-3} O_2). Although the nitrite oxidation capacity of a fresh water biofilm sample was more fulnerable to increased salinities than the corresponding ammonia oxidation capacity, adaptation of a fresh water biofilm sample to increased salinities was found to be possible, providing an effective tool to shorten the start-up period of a marine recirculation system. The possible increased production of nitrite in a marine system is not as serious as in fresh water systems, since nitrite is much less toxic to aquatic organisms when in presence of high salinities.

It is demonstrated in this thesis that the $\frac{1}{2}$-order/0-order kinetic model for dissolved substrate removal, in combination with 1-order kinetics for the removal of suspended organics, is a suitable tool in explaining fixed-biofilm performance. The fixed-biofilm performance is found to be very complex, however, the specific information generated in this research is judged as a valuable contribution to a more rational approach in design and operation of aerobic fixed-biofilm reactors in the field of water treatment, as compared to the conventional, largely "folklore-based" approaches.

SAMENVATTING

Bovendeur, J. (1989). Fixed-biofilm reactoren toegepast in de afvalwaterzuivering en water recirculatiesystemen in de aquacultuur. Dissertatie, Landbouwuniversiteit Wageningen, Wageningen, Nederland, 168 p.

Fixed-biofilm (slib-op-drager) reactoren worden al gedurende ongeveer een eeuw toegepast in de afvalwaterbehandeling. Door de introductie van moderne pakking materialen kreeg dit type reactor een nieuwe impuls met betrekking tot de toepassing in de klassieke afvalwaterbehandeling, alsmede ontstond er een verhoogde interesse voor het inpassen ervan in nieuwe aandachtsvelden in waterbehandeling. In dit proefschrift worden de mogelijkheden geschetst voor fixed-film systemen bij de nabehandeling van anaeroob vergist huishoudelijk afvalwater en de waterbehandeling in recirculatiesystemen voor de aquacultuur. Kernpunten voor deze toepassingen zijn de biofilm karakteristieken voor de verwijdering van organische stof en de nitrificatie van ammoniumstikstof.

In een inleidende studie werden oxidatiebedden en biorotoren toegepast met betrekking tot de nabehandeling van anaeroob voorbehandeld rioolwater. In vergelijking met de prestaties van het oxidatiebed werd bij de biorotor een groter vermogen geconstateerd om hoge belastingen te verwerken, hetgeen resulteerde in aanmerkelijk hogere verwijderingssnelheden per eenheid biofilm oppervlak. Onder relevante belastingscondities werden gemiddelde verwijderingssnelheden waargenomen van circa 35 g $m^{-2}d^{-1}$ CZV voor de biorotor en ongeveer 5 g $m^{-2}d^{-1}$ voor het oxidatiebed, terwijl de cijfers voor ammoniumverwijdering respectievelijk ongeveer 2,4 en 0,35 g $m^{-2}d^{-1}$ NH_4-N bedroegen. Naast dit verschil in capaciteit onder normale bedrijfsomstandigheden werd vastgesteld dat de biorotor tevens in staat was hoge piek-belastingen van gesuspendeerde organische stof op te vangen. De waargenomen verschillen in het gedrag van de reactoren worden vooral toegeschreven aan de verschillende hydrodynamische omstandigheden in de twee reactortypen.

Met betrekking tot de eliminatie van CZV door de biorotor werd echter waargenomen dat de verwijderingsefficientie in hoge mate afhankelijk is van het gehalte aan gesuspendeerd organisch materiaal, daarmee aangevend dat verschillende mechanismen verantwoordelijk zijn voor de verwijdering uit de waterfase van gesuspendeerde en opgeloste organische stof. In verband hiermee werd een vervolgstudie gemaakt van CZV verwijdering door een aerobe biofilm, gerelateerd aan het gelijktijdige zuurstofverbruik door de biofilm. Hierbij werd gebruik gemaakt van de Wazu respiratiemeter: in een gesloten recirculerend systeem werden geheel ondergedompelde biofilmmonsters onderzocht in kortdurende batch-experimenten, waarbij het biofilmmateriaal werd belast met afvalwater of met een acetaatoplossing als model opgelost organisch substraat. Hierbij werd vastgesteld dat zowel de verwijdering van opgelost organisch substraat als de respiratie beantwoorden aan het ½-orde/0-orde kinetiek model, terwijl de eliminatie van gesuspendeerd CZV materiaal 1-orde kinetiek te zien gaf. Voor normale praktijkomstandigheden werden 0-orde snelheden waargenomen voor de verwijdering van opgeloste CZV en biofilm respiratie van respectievelijk 30-40 en 10-16 g $m^{-2}d^{-1}$. Bij afwezigheid van organisch substraat in de bulkvloeistof werd een bepaalde achtergrondsrespiratie waargenomen (3,5-9,0 g $m^{-2}d^{-1}$ O_2). Als gevolg van de 1-orde kinetiek, waargenomen voor een groot concentratiebereik, werden extreem hoge verwijderingssnelheden gemeten voor gesuspendeerd CZV materiaal, zelfs nog hoger dan 300 g $m^{-2}d^{-1}$. Het verschil tussen de maximale respiratie- en CZV verwijderingssnelheid wordt meer toegeschreven aan fysische biofilm adsorptieprocessen dan aan biodegradatieprocessen. De biofilm CZV eliminatiekinetiek wordt bediscussieerd voor afvalwater waarin een mengsel van gesuspendeerde en opgeloste CZV voorkomt. Hierbij wordt geïllustreerd dat de waar te nemen kinetiek-orde van het gecombineerde proces en ook de reactieconstante sterk afhankelijk is van de verhouding tussen gesuspendeerde en opgeloste CZV. In relatie tot het ontwerpen van fixed-film reactoren voor de verwijdering van organisch materiaal is aangetoond dat de concentratie van opgelost organisch substraat, waarbij overgang plaatsvindt tussen ½-orde en 0-orde kinetiek, eveneens bepalend is voor kinetiek-orde van het samengestelde CZV-totaal verwijderingsproces. De samenvattende conclusie van dit deel van het onderzoek is dat een biorotor zeer geschikt is voor de verwijdering van een mengsel van gesuspendeerde en opgeloste organische stoffen, en kan worden beschreven als een reactor waarin biochemische oxidatie van opgeloste organische verbindingen gecombineerd is met adsorptie/flocculatie processen van gesuspendeerde deeltjes organisch materiaal tot een extreem aandeel in het gehele proces.

De geschiktheid van fixed-film reactoren voor toepassing in recirculatiesystemen voor de aquacultuur is aangetoond door het ontwikkelen van een pilot-scale recirculatiesysteem voor de teelt van de Afrikaanse meerval. Het hart van de waterbehandelingsinstallatie was een oxidatiebed, ontworpen voor nitrificatie. Aangezien de nitrificatie eveneens het $\frac{1}{2}$-orde/0-orde kinetiek model bleek te volgen, kon gebruik gemaakt worden van de nitrificatiekinetiek om het zuiveringsgedrag van de reactor af te stemmen op de fluctuatie gedurende een etmaal van de ammoniumproduktie door de vis. Hierbij werd een gemiddelde 0-orde ammoniumverwijderingssnelheid van circa 0,55 g $m^{-2}d^{-1}$ NH_4-N succesvol toegepast als grondslag voor de ontwerpformules voor reactoren ter behandeling van voorbezonken visteeltwater bij een temperatuur van 25°C, pH 7 en zuurstofconcentraties van circa 7 g m^{-3}. Gebaseerd op de waargenomen biofilm kinetiek en de dynamiek in de produktie van afvalstoffen is een complete ontwerptheorie gepresenteerd voor een waterbehandelingssysteem, bestaande uit een voorbezinker en een aerobe fixed-film reactor. In deze theorie zijn de mogelijke vormen van het gedrag van de reactor beschreven in relatie tot de voorkomende concentraties van ammonium, opgeloste zuurstof en de 24-uurs variatie van de ammoniumbelasting van de reactor. Hierbij wordt aandacht besteed aan a) hydraulische belasting van de voorbezinker, b) de afmetingen van de fixed-film reactor, c) de water-recirculatiesnelheid en d) de water-verversingssnelheid.

Als gevolg van de vaststelling dat de 0-orde nitrificatiesnelheid beschouwd dient te worden als het kernpunt in het ontwerpen en inpassen van fixed-film reactoren in recirculatiesystemen voor de aquacultuur, zijn diverse vervolgstudies gemaakt van de gevoeligheden van het fixed-biofilm nitrificatieproces met betrekking tot de bedrijfsvoering van dergelijke recirculatiesystemen. De betrokken factoren daarbij zijn de gelijktijdige CZV belasting, de zuurstofconcentratie, pH en het zoutgehalte. Monitoring experimenten op het gebied van de ontwikkeling van biofilm nitrificatie toonden aan dat na een aanvankelijke opstartperiode, met als kenmerk een ophoping van nitriet, de nitriet-oxidatiecapaciteit zich sneller ontwikkelt dan de ammonium-oxidatiecapaciteit. Uiteindelijk resulteerde deze snellere ontwikkeling in een hogere nitriet-verwijderingscapaciteit van een volgroeide biofilm dan de bijbehorende ammonium-verwijderingscapaciteit. De effecten van de eerder genoemde factoren zijn bestudeerd voor dergelijke volgroeide biofilmmonsters. In deze studies is gebruik gemaakt van een speciaal biofilm adaptie systeem, waarin "kunstmatig" biofilmmateriaal werd gekweekt waarvan het gedrag is bestudeerd onder goed gecontroleerde laboratorium omstandigheden in een biofilm monitoring systeem.

Met betrekking tot het effect van de organische stof-belasting op de biofilm nitrificatie is onderscheid gemaakt tussen lange-termijn en kortdurende piek-belastingen met organische stof. Bij kortdurende belastingen met organisch materiaal werden verlaagde 0-orde nitrificatiesnelheden gevonden, omgekeerd evenredig met de bijbehorende CZV verwijderingssnelheid. De reductiefactor is gekwantificeerd als -0,015 g $m^{-2}d^{-1}$ NH_4-N genitrificeerd per g $m^{-2}d^{-1}$ CZV verwijderd. Deze reductiefactor geeft aan dat het nitrificatieproces, gedurende de kortdurende piek-belastingen met organische stof, gelimiteerd is door zuurstofoverdracht in de biofilmlaag. De reductie van de nitrificatie is in omvang beperkt als gevolg van de slechts gedeeltelijke oxidatie van de organische stof (circa 10%), onmiddellijk na verwijdering uit de waterfase. Lange-termijn achtergrondsbelastingen met organische stof gaven een ernstiger verlaging te zien van de 0-orde nitrificatiesnelheid: bij een gelijktijdige CZV-belasting van 5 g $m^{-2}d^{-1}$ werd ongeveer 0,6 g $m^{-2}d^{-1}$ NH_4-N genitrificeerd tegen ongeveer 0,4 g $m^{-2}d^{-1}$ NH_4-N bij een CZV-belasting van 10 g $m^{-2}d^{-1}$ (pH 7, 7 g m^{-3} O_2, 25°C). Deze afname van de nitrificatiesnelheid is toe te schrijven aan de verhoogde aanwas van niet-nitrificerend biofilmmateriaal, hetgeen resulteert in kortere verblijftijden van de nitrificerende bacteriën in de aerobe zone van de biofilm.

De zuurstofdiffusie-gelimiteerde 0-orde nitrificatiesnelheid, zoals waargenomen voor normale bedrijfscondities, is bevestigd door het effect van verhoogde en verlaagde opgeloste zuurstofconcentraties in de bulkvloeistof. Een sterk verhoogde concentratie (30 g m^{-3} O_2) resulteerde in metabolisme-gelimiteerde nitrificatiesnelheden, verschillend voor de betrokken achtergronds CZV-belasting: circa 1,4 g $m^{-2}d^{-1}$ NH_4-N en 0,6 g $m^{-2}d^{-1}$ NH_4-N voor respectievelijk 5 en 10 g $m^{-2}d^{-1}$ CZV (pH 7, 25°C, geen CZV aanwezig in de monitoring experimenten). De reductiefactor, zoals eerder waargenomen bij de gelijktijdige korte-termijn CZV verwijdering bij 7 g m^{-3} O_2, was nul of zeer laag voor dergelijke hoge zuurstofconcentraties. Een verlaagde zuurstofconcentratie (3 g m^{-3} O_2) resulteerde in een verlaagde 0-orde nitrificatiesnelheid van ongeveer 0,3 g $m^{-2}d^{-1}$ NH_4-N voor een biofilm die was gekweekt bij een achtergrond CZV-belasting van 5 g $m^{-2}d^{-1}$, waarbij de reductiefactor voor de nitrificatiesnelheid als functie van de simultane CZV-verwijdering gelijkwaardig was aan de waarde zoals waargenomen voor een zuurstofconcentratie van 7 g m^{-3}. Onafhankelijk van de zuurstofconcentratie verwijderden de biofilms organische stof, uitgedrukt als CZV, met snelheden rechtevenredig met de CZV-belasting en een efficiëntie van ongeveer 85%. De onafhankelijkheid van de zuurstofconcentratie wordt verklaard als gevolg van het zeer geringe deel van de CZV die onmiddelijk geoxideerd wordt na verwijdering uit de waterfase.

Lage pH-waarden in de bulkvloeistof resulteerden in een aanzienlijke verlaging van de biofilmactiviteit, zowel voor de verwijdering van ammonium als nitriet. Echter, er werd geen ophoping van nitriet waargenomen in experimenten waarin de biofilm werd gevoed met alleen ammonium. Indien de biofilm werd gevoed met uitsluitend nitriet werd bij pH 6 een kennelijke 0-orde nitrietverwijdering waargenomen, terwijl bij pH 7 ½-orde kinetiek optrad met hogere snelheden dan bij pH 6. De verlaging van de ammoniumoxidatiecapaciteit bij lage pH-waarden wordt verklaard in termen van overgang van de beperkende factor (opgeloste zuurstof/alkaliniteit), terwijl de verlaging van de nitriet-verwijderingscapaciteit wordt beschouwd als het gevolg van een mogelijke substraatinhibitie door niet-geïoniseerd salpeterigzuur.

Tot slot werd het gedrag van fixed-biofilm in zeewater bestudeerd in verband met de gewenste ontwikkeling van zeewater-recirculatiesystemen voor de aquacultuur. In zeewater gekweekte biofilms gaven een veel langere opstartperiode te zien, waarin zich nitriet ophoopte. Daarnaast was de uiteindelijke maximale ammoniumverwijderingssnelheid aanmerkelijk lager dan in zoetwatersystemen: bij 24°C was de 0-orde ammonium-verwijderingssnelheid respectievelijk ongeveer 0,3 g $m^{-2}d^{-1}$ NH_4-N tegen ongeveer 0,6 g $m^{-2}d^{-1}$ NH_4-N (pH 7 in zoetwater, pH 8,3 in zeewater, beide systemen 7 g m^{-3} O_2). Hoewel de nitriet-oxidatiecapaciteit van een zoetwaterbiofilm gevoeliger is voor verhoogde zoutgehaltes dan de bijbehorende ammonium-oxidatiecapaciteit, werd toch geconcludeerd dat adaptatie van een zoetwaterbiofilm aan verhoogde zoutconcentraties niet alleen mogelijk is, maar ook een effectieve methode om de opstartperiode van een zeewater-recirculatiesysteem aanmerkelijk te bekorten. De daarbij te verwachten verhoogde nitrietproductie is in een marien systeem niet zo ernstig als in een zoetwatersysteem, aangezien nitriet bij hoge saliniteiten veel minder toxisch is voor aquatische organismen.

In dit proefschrift is aangetoond dat het ½-orde/0-order kinetiek model voor de verwijdering van opgelost substraat, gecombineerd met 1-orde kinetiek voor de verwijdering van gesuspendeerde deeltjes organisch materiaal, een geschikt instrument is bij het verklaren van het gedrag van fixed-biofilms. Het is ondervonden dat het gedrag van fixed-biofilms bijzonder gecompliceerd is, hoewel de specifieke informatie, verkregen in dit onderzoek, beschouwd kan worden als een waardevolle bijdrage aan een meer rationele benadering in het ontwerpen en bedrijven van aerobe fixed-film reactoren in de biologische waterbehandeling –rationeler dan de meeste oorspronkelijke, veelal op "folklore" gebaseerde ontwerpgrondslagen.

CURRICULUM VITAE

De auteur, geboren op 6 februari 1954 te Wormerveer, behaalde in 1972 het diploma Atheneum-B aan de Christelijke Scholengemeenschap de Populier te 's-Gravenhage. Aansluitend studeerde hij Milieuhygiene aan de (toen nog) Landbouwhogeschool te Wageningen en behaalde in 1979 het ingenieursdiploma met als hoofdvakken Kennis van de Bodemverontreiniging, en Microbiologie, en als bijvak Vegetatiekunde & Plantenoecologie. Direct na het afronden van deze studie werd hij aangesteld als wetenschappelijk medewerker/universitair docent bij de vakgroep Waterzuivering van de (huidige) Landbouwuniversiteit Wageningen. In deze functie werd een reeds lopend onderzoek afgerond met betrekking tot het mobiliseren van zware metalen uit een gesuspendeerde model-vaste fase, waarna de onderzoeks-aktiviteiten vooral werden geconcentreerd op het aandachtsveld beschreven in dit proefschrift. Gedurende de laatste jaren in het dienstverband met de Landbouwuniversiteit was de auteur tevens studiecoördinator van de studierichting Milieuhygiene. Per 1 maart 1988 werd de funktie wetenschappelijk research-coördinator aanvaard bij Serasea b.v. te 's-Hertogenbosch, waar de werkzaamheden vooral zijn gericht op het ontwikkelen van verwerkingstechnologie voor verontreinigde baggerspecie.