

# Biological phosphorus and nitrogen removal from wastewater using moving bed biofilm process

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## Abstract

In this research, an experimental study to evaluate nutrient removal from synthetic wastewater by a lab-scale moving bed biofilm process was investigated. Also, kinetic analysis of the process with regard to phosphorus and nitrogen removal was studied with different mathematical models. For nutrient removal, the moving bed biofilm process was applied in series with anaerobic, anoxic and aerobic units in four separate reactors that were operated continuously at different loading rates of phosphorus and nitrogen and different hydraulic retention times. Under optimum conditions, almost complete nitrification with an average ammonium removal efficiency of 99.72% occurred in the aerobic reactor. In the aerobic reactor, the average specific nitrification rate was 1.92 g NO<sub>x</sub>-N (NO<sub>x</sub>-N=NO<sub>2</sub>-N+NO<sub>3</sub>-N) produced/kg volatile suspended solids. hour (VSS.h). Denitrification rate increased with increasing NO<sub>x</sub>-N loading in the second anoxic reactor. The aerobic phosphate removal rate showed good correlation with the anaerobic phosphate release rate. Under optimum conditions, the average total nitrogen and phosphorus removal efficiencies were 80.9% and 95.8%, respectively. As a result of the moving bed biofilm process (MBBR) kinetic analysis, the Stover-Kincannon model was chosen for modeling studies and experimental data analysis. The Stover-Kincannon model gave high correlation coefficients for phosphorus and nitrogen removal, which were 0.9862 and 0.986, respectively. Therefore, this model could be used in predicting the behavior or design of the moving bed biofilm process.

**Keywords:** MBBR; Biofilm carriers; Biological nutrient removal (BNR); Sewage treatment; Stover-Kincannon model

## INTRODUCTION

Wastewater containing high levels of phosphorus and nitrogen cause several problems, such as eutrophication, oxygen consumption, and toxicity, when discharged into the environment (Luostarinen *et al.*, 2006). It is, therefore, necessary to remove such substances from wastewaters in order to reduce their harm to the environment (Wang *et al.*, 2006). Biological processes based upon suspended biomass are effective for organic carbon and nutrient removal from municipal wastewater plants. But there are some problems of sludge settleability and the need for large reactors, settling tanks and biomass recycling (Pastorelli *et al.*, 1999, 1997a, 1997b).

Biofilm processes have proved to be reliable for organic carbon and nutrient removal and are without some of the problems of activated sludge processes (Ødegaard *et al.*, 1994). Biofilm reactors are especially useful when slow growing organisms like nitrifiers have to be kept in a wastewater treatment process. Both nitrification and denitrification processes have been individually successful in the biofilm reactor (Wang *et al.*, 2006). There are already many different biofilm systems in use, such as trickling filters, rotating biological contactors (RBCs), fixed media submerged biofilters, granular media biofilters and fluidized bed reactors-all of which have advantages and disadvantages. For these reasons, the moving bed

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biofilm reactor (MBBR) process was developed in Norway during the late 1980s and early 1990s (Ødegaard, 2006; Ødegaard *et al.*, 1999). The moving bed biofilm process is a promising process for the enhancement of nitrification, denitrification and phosphorus removal in conventional activated sludge systems that can be used for upgrading biological nutrient removal, particularly when they have space limitations or need modifications that will require large monetary expenses (Hooshyari *et al.*, 2009). The moving bed biofilm reactor is a highly effective biological treatment process that has been developed on the basis of conventional activated sludge and biofilter processes. It is a completely mixed and continuously operated biofilm reactor, where the biomass is grown on small carrier elements that have a little lighter density than water and are kept in movement along with a water stream inside the reactor. The movement inside a reactor can be caused by aeration in an aerobic reactor and by a mechanical stirrer in an anaerobic or anoxic reactor.

Researchers have proven that MBBR possesses many excellent traits such as high biomass, high chemical oxygen demand (COD) loading, strong tolerance to loading impact, relatively smaller reactor and no sludge bulking problem (Chen *et al.*, 2008). There are presently more than 400 large-scale wastewater treatment plants based on this process in operation in 22 different countries all over the world (Rusten *et al.*, 2006). During the past decade it has been successfully used for the treatment of many industrial effluents including pulp and paper industry waste (Jahren *et al.*, 2002), poultry processing wastewater (Rusten *et al.*, 1998), cheese factory wastes (Rusten *et al.*, 1996), refinery and slaughter house wastes (Johnson *et al.*, 2000), phenolic wastewater (Hosseini and Borghei, 2005), dairy wastewater (Andreottola *et al.*, 2002; Rusten *et al.*, 1992) and municipal wastewater (Andreottola *et al.*, 2003, 2000a, 2000b; Rusten *et al.*, 1997, 1995a, 1995b, 1994). Moreover, sequencing batch operation of MBBR has been attempted for biological phosphorus removal (Helness, 2007; Pastorelli *et al.*, 1999), however, documents and practical experiences with simultaneous nitrogen and phosphorus removal in the MBBR process with continuous operation are not available in Iran and other countries. The objective of this study was to evaluate phosphorus and nitrogen removal by applying a lab-scale MBBR system with continuous operation filled with low cost biofilm carriers of FLOCOR-RMP® (The Nottingham Koi

Company, UK). For nutrient removal, the lab-scale MBBR system has been applied in series with anaerobic, anoxic (denitrifying) and aerobic (nitrifying) units represented by separate reactors. Furthermore, another aim of this research was to determine the moving bed biofilm process kinetics with regard to phosphorus and nitrogen removal by using the Stover-Kincannon, second-order (Grau) and the first-order substrate removal models.

## MATERIALS AND METHODS

**Experimental set-up:** The experiments were conducted using four laboratory scale moving bed biofilm reactors in series followed by a final settler. Sludge recycling was not implemented in this process. The anaerobic reactor ( $R_1$ ) was constructed for study of enhanced biological phosphorus removal (EBPR). The first anoxic reactor ( $R_2$ ) was built to minimize the effect of nitrate in wastewater entering the anaerobic reactor. One port at the top of  $R_2$  allowed the pumping of the anoxic mixed liquor out into the anaerobic reactor. The mixed liquor from the first anoxic reactor ( $R_2$ ) contains substantial soluble COD but little nitrate. Anoxic recirculation (AR) was provided to increase organic utilization in the anaerobic reactor and provide optimal conditions for fermentation uptake in the anaerobic reactor. The anoxic recirculation (AR) rate was typically 2 times the influent flow rate. The second anoxic reactor ( $R_3$ ) followed the first anoxic reactor ( $R_2$ ) and received nitrate recirculation (NR) flow from the aerobic reactor ( $R_4$ ) to provide the major portion of nitrate removal for the process. The aerobic reactor ( $R_4$ ) was built for the purpose of nitrification. One port at the end of the reactor was provided for pumping out the aerobic mixed liquor containing nitrate. Moving bed biofilm reactors placed into a water bath were equipped with aquarium heaters in order to operate at the constant temperature of  $28 \pm 1^\circ\text{C}$ . A sketch of the lab-scale moving bed biofilm reactors is shown in Figure 1 and some key parameters are listed in Table 1. Reactors were operated in an up-flow mode. Sampling ports were provided in each reactor for sample collection. All anaerobic and anoxic reactors had variable speed propellers that pushed the biofilm media downwards towards the center of the reactors. The normal propeller speed was 32 rpm. The aerobic reactor was equipped with a tube diffuser and air to the aerobic reactor was supplied by an air compres-

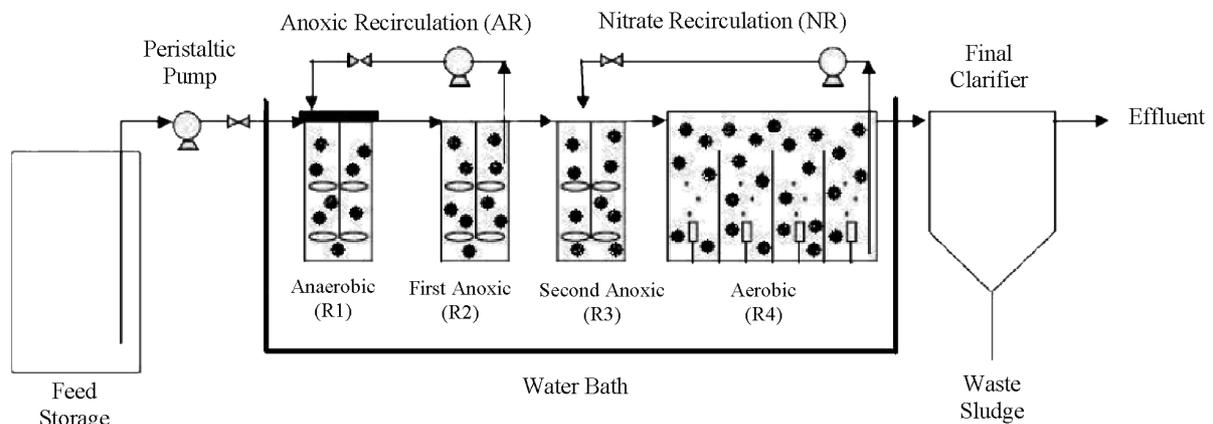


Figure 1. Schematic diagram of the lab-scale MBBR system.

Table 1. Technical data and some key parameters for the moving bed biofilm reactors.

| Parameter   | Anaerobic reactor (R <sub>1</sub> ) | Anoxic reactors (R <sub>2</sub> and R <sub>3</sub> ) | Aerobic reactor (R <sub>4</sub> ) |
|---|-------------------------------------|--|-----------------------------------|
| Volume (l)  | 3.33                                | 3.33   | 10                                |
| Filling ratio with biofilm carriers (%)                         | 50                                  | 50   | 70                                |
| Specific biofilm surface area (m <sup>2</sup> /m <sup>3</sup> ) | 130                                 | 130  | 182                               |
| Total biofilm surface area (m <sup>2</sup> )                    | 0.4329                              | 0.4329   | 1.82                              |
| Flow rate (l/d)   | 10-60                               | 10-60  | 10-60                             |
| Flow direction  | Up-flow                             | Up-flow  | Up-flow                           |
| HRT in reactors   | 80 min-8 hr                         | 80 min-8 hr  | 4-24 hr                           |
| HRT in the lab-scale MBBR system                                |                                     | 8-48 hr  |                                   |

R1 (Anaerobic reactor); R2 (First anoxic reactor); R3 (Second anoxic reactor); R4 (Aerobic reactor) HRT (Hydraulic retention time).

sor. The airflow to the reactor was measured by a rotameter and regulated with a manual valve. Synthetic wastewater was continuously fed into the bioreactors using a variable speed peristaltic pump (Masterflex L/S pump, Cole-Parmer Instrument Company, USA). Characteristics of the FLOCOR-RMP<sup>®</sup> plastic media are presented in Table 2.

**Operating procedure:** Synthetic wastewater was prepared with ordinary tap water and glucose as the main sources of carbon and energy, plus balanced macro and micro nutrients. Synthetic wastewater with the following composition was used in this study: 516.07 mg of glucose (500 mg/l as COD), 21.95-109.75 mg of KH<sub>2</sub>PO<sub>4</sub> (5-25 mg/l as phosphorus (PO<sub>4</sub>-P) basis), 141.18-705.89 mg of NH<sub>4</sub>HCO<sub>3</sub> (25-125 mg/l as nitrogen (NH<sub>4</sub>-N) basis), 90 mg of MgSO<sub>4</sub>·7H<sub>2</sub>O, 14 mg of CaCl<sub>2</sub>·2H<sub>2</sub>O and 0.3 ml of trace element solution per liter. The trace solution consisted of the following

compounds per liter: 1.5 g of FeCl<sub>3</sub>·6H<sub>2</sub>O, 0.15 g of H<sub>3</sub>BO<sub>3</sub>, 0.03 g of CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.18 g of KI, 0.12 g of MnCl<sub>2</sub>·H<sub>2</sub>O, 0.06 g of Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.12 g of ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.15 g of CoCl<sub>2</sub>·6H<sub>2</sub>O and 10 g of EDTA (Kishida *et al.*, 2006). NaOH and NaHCO<sub>3</sub> were used for alkaline pH adjustments.

Seeding sludge obtained from the Isfahan Municipal Wastewater Treatment Plant was acclimatized to the synthetic wastewater prior to the start of the experiments for a few days. The composition of

Table 2. Characteristics of the FLOCOR-RMP<sup>®</sup> plastic media.

|                  |                                    |
|------------------|------------------------------------|
| Material         | Polypropylene                      |
| Shape            | Corrugated cylinder                |
| Diameter         | 15 mm                              |
| Length           | 10 mm                              |
| Specific surface | 260 m <sup>2</sup> /m <sup>3</sup> |
| Density          | 0.94 g/cm <sup>3</sup>             |

ingredients in prepared wastewater was chosen in a way that the COD concentration of 500-2000 mg/l and different concentrations of  $\text{NH}_4\text{-N}$  ranging from 25-125 mg/l and  $\text{PO}_4\text{-P}$  ranging from 5-25 mg/l were prepared and used as feed to the system. The dissolved oxygen concentration in the aerobic reactor ranged from 2.5 to 5.5 mg/l depending on the influent organic and ammonium load. Prepared wastewater was continuously pumped into the lab-scale MBBRs at a flow rate of 10-60 l/day. Consequently the theoretical hydraulic retention time (HRT) in the lab-scale MBBR system was 8-48h.

**Sampling and analysis:** Samples were collected from influents and sampling ports of each reactor. Temperature, dissolved oxygen and pH were measured in each reactor every workday, immediately before sampling. All dissolved oxygen (DO) and pH measurements were carried out with a YSI 55 DO meter (YSI Company Inc., USA) and SCHOTT pH meter model CG-824 (SCHOTT UK Ltd., UK), respectively. The samples were analyzed immediately after filtration through 0.45  $\mu\text{m}$  filter papers. Soluble COD, ammonium ( $\text{NH}_4\text{-N}$ ), nitrate ( $\text{NO}_3\text{-N}$ ), nitrite ( $\text{NO}_2\text{-N}$ ) and soluble phosphorus ( $\text{PO}_4\text{-P}$ ) were measured in accordance with standard methods (American public health association (APHA), 1998).

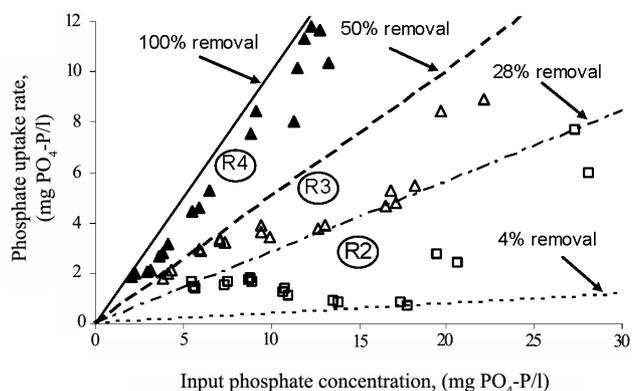
The assessment of the total suspended solids (TSS) on the fixed biomass elements was performed as follows: the biofilm was removed from ten plastic elements and diluted in a known amount of demineralized water; after filtration (0.45  $\mu\text{m}$ ) the sample was dewatered at 105°C and weighed; because of the variability of plastic element's dimension, the obtained value was referred as the total measured surface of the ten elements; total suspended solids concentration was assessed through the total surface in a cubic meter of reactor (Andreottola *et al.*, 2000b). Many models for the biomass growth processes have appeared in the wastewater treatment literature (Hooshyari *et al.*, 2009; Borghei *et al.*, 2008; Hosseiny and Borghei, 2002). Parameters such as  $\text{PO}_4\text{-P}$  and  $\text{NH}_4\text{-N}$  were used as substrates for evaluation under the assumption that the removal was exclusively due to aerobic biodegradation. The first-order substrate removal model, The Stover-Kincannon model and the second-order model often known as the Grau model are some of the models that are used to test the kinetics of substrate removal in bioreactors and are considered in this research.

## RESULTS

Enhanced biological phosphorus removal (EBPR) was carried out in this study. In the biological phosphorus removal, the phosphorus in the influent wastewater is incorporated into cell biomass, which subsequently is removed from the process as a result of sludge wasting. Phosphorus accumulating organisms ( $\text{PAO}_s$ ) are encouraged to grow and consume phosphorus in systems that use a reactor configuration that provides  $\text{PAO}_s$  with a competitive advantage over other bacteria (Tchobanoglous *et al.*, 2003). Phosphorus removal in biological systems is based on the following observations (Sedlak, 1991):

- 1- Numerous bacteria are capable of storing excess amounts of phosphorus as polyphosphates in their cells.
- 2- Under the anaerobic conditions,  $\text{PAO}_s$  will assimilate fermentation products (e.g., volatile fatty acids) into storage products within the cells with the concomitant release of phosphorus from stored polyphosphates.
- 3- Under the anoxic or aerobic conditions, energy is produced by the oxidation of storage products and polyphosphates storage increases within the cell.

Under the optimum conditions (500 mg of COD/l and 12.5 mg of  $\text{PO}_4\text{-P/l}$ ), acceptable phosphorus removal efficiency up to 95.8% (89.73% on average) occurred in the lab-scale MBBR system. The results of the average phosphorus removal efficiency in anoxic ( $\text{R}_2$  and  $\text{R}_3$ ) and aerobic ( $\text{R}_4$ ) reactors are shown in Figure 2.



**Figure 2.** The average phosphate removal efficiency in anoxic ( $\text{R}_2$  and  $\text{R}_3$ ) and aerobic ( $\text{R}_4$ ) reactors.

In the anoxic reactors, most  $PAO_S$  can use nitrite in place of oxygen to oxidize their stored carbon source. According to the Figure 3, aerobic phosphate removal rate showed a good correlation with the anaerobic phosphate release rate. Anaerobic phosphate release was calculated based on the difference in phosphate concentration at the beginning and end of the anaerobic reactor ( $R_1$ ) and the biofilm surface area in this reactor.

In Figure 4, a plot of the phosphate removal rate versus the phosphate loading rate in the aerobic reactor is shown. According to the results, the phosphate removal rate showed a strong correlation with the phosphate loading rate in the aerobic reactor. The results of the MBBR kinetic analysis with respect to phosphorus removal showed that the Stover-Kincannon model was more appropriate than the first-order substrate removal and the second-order substrate removal models (Grau

model). So, in relation to the Stover-Kincannon model, Figure 5 shows a graph of the inverse of the loading removal rate,  $[V/(Q(S_i - S_e))]$ , Where V: reactor volume (l), Q: inflow rate (l/d),  $S_i$  and  $S_e$ : substrate concentration in the feed and effluent (mg/l) plotted against the inverse of the total loading rate,  $V/(QS_i)$ . Since the plot of  $[V/(Q(S_i - S_e))]$  versus  $V/(QS_i)$  was linear, linear regressions (least squares method) were used to determine the intercept,  $1/U_{max}$  and a slope of  $K_B/U_{max}$  are present on the graph. The saturation value constant ( $K_B$ ) and the maximum specific substrate utilization rate ( $U_{max}$ ) were calculated from the plotted line in Figure 5 as 8.50 g/l.day and 7.71 g/l.day, respectively. The regression line had an  $R^2$  of 0.9862, where R is the degree of regression.

Nitrification rates versus ammonium loads are shown in Figure 6 for the predenitrification MBBR

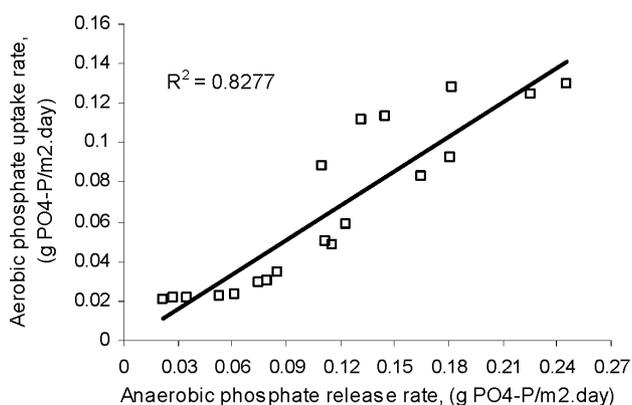


Figure 3. Aerobic phosphate uptake/release rate versus anaerobic phosphate release rate in the MBBR system.

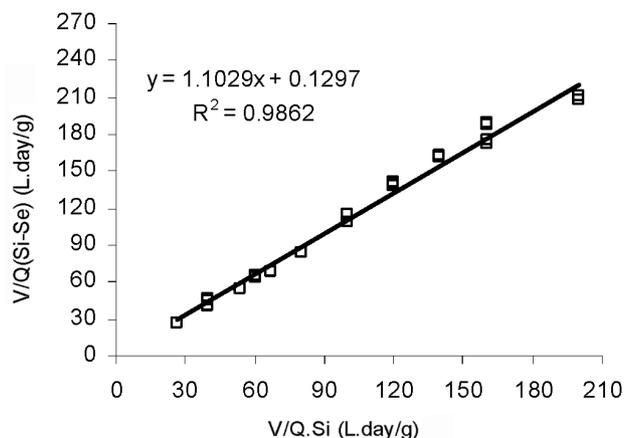


Figure 5. Stover-Kincannon model plot for phosphorus removal in the MBBR system.

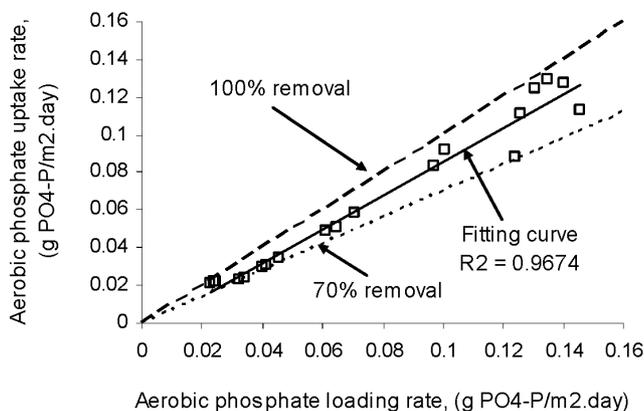


Figure 4. Phosphate uptake/release rate versus phosphate loading rate in the aerobic reactor.

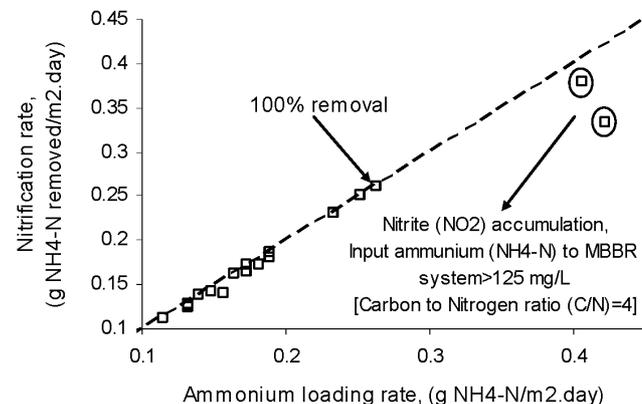
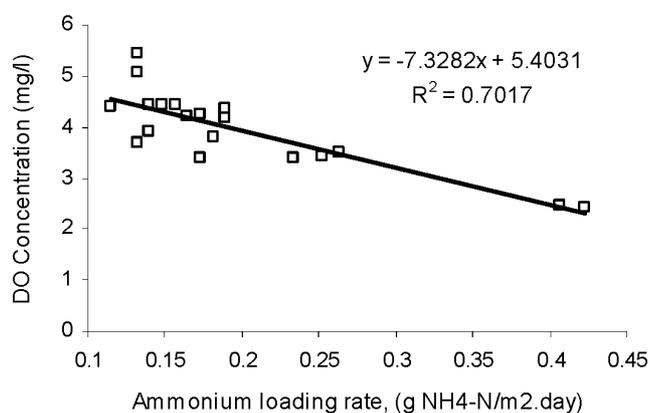
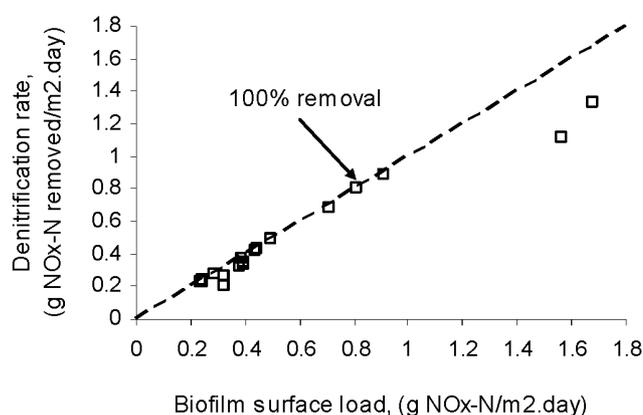


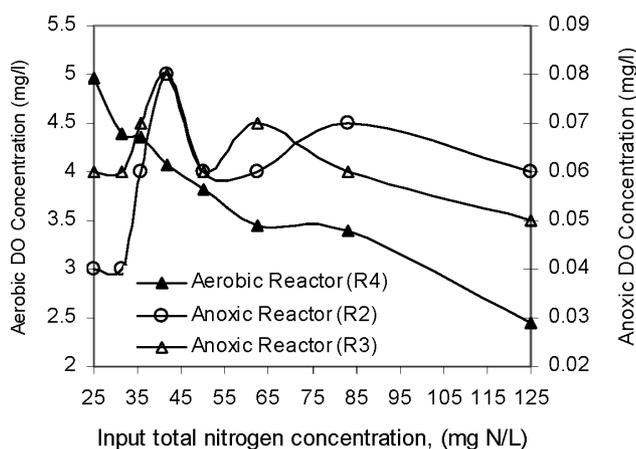
Figure 6. Nitrification rate versus ammonium loading rate in the aerobic reactor.



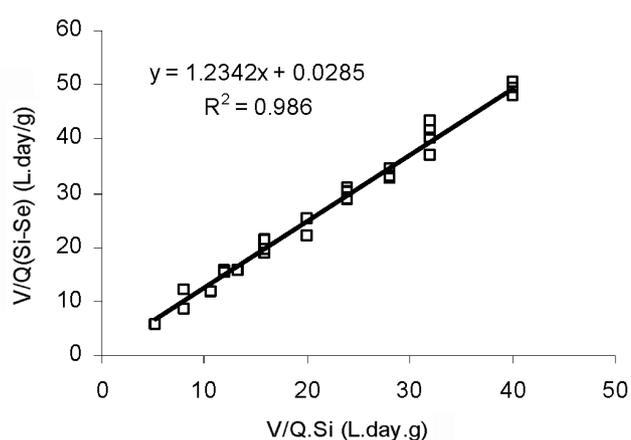
**Figure 7.** Dissolved oxygen (DO) concentration versus ammonium loading rate in the aerobic reactor.



**Figure 9.** Denitrification rate versus  $\text{NO}_x\text{-N}$  loading rate in the anoxic reactor.



**Figure 8.** Dissolved oxygen (DO) variation profile of the anoxic and aerobic reactors.



**Figure 10.** Stover-Kincannon model plot for nitrogen removal in the MBBR system.

system consisting of nitrate recirculation (NR). The data have been calculated based on lab-scale influent and effluent  $\text{NH}_4\text{-N}$  concentrations and the biofilm surface area in the aerated reactor ( $R_4$ ). These results demonstrated close to complete (99.72% ammonium removal on average) nitrification in the aerobic reactor under optimal conditions (500 mg COD/l and 62.5 mg  $\text{NH}_4\text{-N/l}$ ).

The relationship between DO concentrations and ammonium loading rates in the aerobic reactor are shown in Figure 7. Oxygen or ammonia may be the rate-limiting substrate for nitrification. The DO variation profiles of the anoxic and aerobic reactors are demonstrated in Figure 8. As indicated, DO concentration in the aerobic reactor decreased with increasing ammonium loading rates.

Figure 9 shows denitrification rates versus  $\text{NO}_x\text{-N}$

loads ( $\text{NO}_x\text{-N} = \text{NO}_2\text{-N} + \text{NO}_3\text{-N}$ ) in the second anoxic reactor ( $R_3$ ). The data have been calculated based on lab-scale influent and effluent  $\text{NO}_x\text{-N}$  concentrations and the biofilm surface area in the second anoxic reactor ( $R_3$ ). As indicated in Figure 9, denitrification rate increased with increasing  $\text{NO}_x\text{-N}$  loading. The results of the MBBR kinetic analysis with regard to nitrogen removal showed that as in the case of phosphorus removal, the Stover-Kincannon model was more appropriate than the first-order substrate removal and the second-order substrate removal models (Grau model). So, with respect to the Stover-Kincannon model, Figure 10 shows a graph of the inverse of the loading removal rate,  $[V/(Q(S_i - S_e))]$  plotted against the inverse of the total loading rate,  $V/(QS_i)$ . As in Figure 5, the straight line portion of the intercept,  $1/U_{\max}$  and a slope of  $K_B/U_{\max}$  are present on the graph (Fig. 10). The saturation value constant ( $K_B$ ) and maximum specific sub-

strate utilization rate ( $U_{\max}$ ) were calculated as above, with values of 43.31 g/l.day and 35.09 g/l.day, respectively. The regression line had a  $R^2$  of 0.986 (Fig. 10).

## DISCUSSION

**Biological phosphorus removal:** Biological P-removal using enhanced biological phosphorus removal (EBPR) was carried out in this study. In systems, PAO<sub>s</sub> are thought to play a significant role in phosphorus removal. The first microbial strains isolated by EBPR were *Acinetobacter* species (Okunuki *et al.*, 2004). Biological phosphorus removal is initiated in the anaerobic reactor where acetate (and propionate) is taken up by PAO<sub>s</sub> and converted to carbon storage products that provide energy and growth in the subsequent anoxic and aerobic reactors. The phosphorus removal efficiency depends heavily on the operating conditions (Tchobanoglous *et al.*, 2003; Chuang *et al.*, 1998). According to Figure 2, maximum phosphorus removal occurs in the aerobic reactor ( $R_4$ ), because under aerobic conditions, energy is produced by the oxidation of storage products and polyphosphate storage within the cell increases.

In the anoxic and aerobic reactors stored polyhydroxybutyrate (PHB) is metabolized, providing energy from oxidation and carbon for new cell growth (Tchobanoglous *et al.*, 2003). The energy released from PHB oxidation is used to form polyphosphate bonds during cell storage so that soluble orthophosphate is removed from solution and incorporated into polyphosphates within the bacteria cell. Cell growth also occurs due to PHB utilization and the new biomass with high polyphosphate storage accounts for phosphorus removal (Okunuki *et al.*, 2004; Tchobanoglous *et al.*, 2003). If phosphorus removal efficiency is calculated as aerobic phosphate uptake vs. biomass weight, the average value is 0.827 g PO<sub>4</sub>-P removed/kg TSS.h or 1.047 g PO<sub>4</sub>-P removed/kg VSS.h. As indicated in Figure 3, aerobic phosphate removal has increased with increasing anaerobic phosphate release. It should be noted that, COD is the primary source of volatile fatty acids (VFA<sub>s</sub>) for the phosphorus accumulating organisms. The conversion of COD to VFA<sub>s</sub> occurs quickly through fermentation in the anaerobic reactor. So, the more acetate is available, the more cell growth, and, thus, more phosphorus removal (Tchobanoglous *et al.*, 2003). The results suggest that phosphate removal in aerobic reactor may be inhibited by phosphate release in the anaerobic reactor. It should be noted that, the competition between phos-

phorus accumulating organisms (PAO<sub>s</sub>) and other heterotrophs, primarily determine the biological phosphorus removal (Chuang *et al.*, 1998).

**Biological nitrogen removal:** Total nitrogen removal in wastewater treatment plants is most commonly and most economically achieved in a two stage-system, i.e. nitrification and denitrification. Nitrification transforms ammonia to a more oxidized nitrogen compound such as nitrite or nitrate, which is then converted to nitrogen gas in the subsequent denitrification process (Wang *et al.*, 2006). This latter step includes the production of nitric oxide (NO), nitrous oxide (N<sub>2</sub>O), and nitrogen gas (N<sub>2</sub>). All three products are gases and can be released into the atmosphere (Tchobanoglous *et al.*, 2003; Sedlak, 1991). Nitrification and denitrification are usually carried out in different reactors because nitrification occurs under aerobic conditions while denitrification prevails in the absence of oxygen (Wang *et al.*, 2006). In general, *Nitrosomonas* and *Nitrobacter* are assumed to be responsible for nitrification in wastewaters and denitrification is achieved by denitrifying organisms (such as *Pseudomonas*, *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter* and *Bacillus*), although an organic carbon source is required (Sedlak, 1991). As indicated in Figure 6, nitrification increases with increasing ammonium loading. The results suggest that the nitrification may be inhibited by substrate (ammonium) concentration so increasing the ammonium load leads to increasing the nitrification rates. Normally, the aerobic reactor ( $R_4$ ) was found to have very low heterotrophic activity and significantly higher nitrification rates. It may be assumed that reactor 4 has a biofilm with a thinner layer of heterotrophs and a significantly higher density of nitrifiers. So, excellent NH<sub>4</sub>-N conversion has been obtained at overall loads up to 0.4231 g NH<sub>4</sub>-N/m<sup>2</sup>.day, which is the highest load tested. If nitrification rate is calculated as g NO<sub>x</sub>-N produced/m<sup>2</sup>.day, the average value is 0.119 g NO<sub>x</sub>-N produced/m<sup>2</sup>.day. During the experimental work, the TSS biofilm concentration was found to be 0.595 kg TSS/m<sup>3</sup> on average; and the volatile suspended solids to total suspended solids ratio (VSS/TSS) resulted 79%. Thus the average specific nitrification rate in the aerobic reactor can be expressed as 1.517 g NO<sub>x</sub>-N produced/kg TSS.h or 1.92 g NO<sub>x</sub>-N produced/kg VSS.h. Andreottola *et al.* (2000b) have observed an average nitrification rate of 1.84 g NO<sub>3</sub>-N/kg VSS.h. Three factors, the load of organic matter, the ammonium concentration and the oxygen concentration, primarily determine the nitrification rate. Organic load controls nitrification and should be as low as possible. To get

nitrification, the DO level in the aerobic reactor must be sufficiently high to penetrate through the outer layer of oxygen consuming heterotrophs and into the nitrifying bacteria (Rusten *et al.*, 1995a; Hem *et al.*, 1994). According to Figures 7 and 8, DO concentration in the aerobic reactor decreases with increasing ammonium loading rate from 115.4 to 423.1 mg NH<sub>4</sub>-N/m<sup>2</sup>.day. The nitrification rate is found to be almost linearly dependent upon the oxygen concentration, up to more than 10 mg O<sub>2</sub>/l.

The results also show that the liquid film diffusion is an important parameter for the moving biofilm reactors. According to the results of the average effluent soluble COD concentration from each reactor, the denitrification process in the second anoxic reactor (R<sub>3</sub>) preceding the aerobic reactor (R<sub>4</sub>) in predenitrification system was found to consume most of the biodegradable organic matter. Thus, in the aerobic reactor the average biodegradable soluble COD (BSCOD) load is considerably lower and does not interfere with nitrification. According to Rusten *et al.* (1995a), degradation of organic matter will slow down or stop the nitrification process. Heterotrophs and nitrifiers will compete for available oxygen, and the rapidly growing Heterotrophs will dilute (or wash out) the nitrifiers in the biofilm (Rusten *et al.*, 1995a). As shown in Figure 9, the maximum denitrification rate is 1.3298 g NO<sub>x</sub>-N removed/m<sup>2</sup>.day. The denitrification rate may be limited by the nitrate concentration, the biodegradable organic matter concentration or by the oxygen concentration (or rather the presence of oxygen). If oxygen is supplied to the reactor with the inlet wastewater or recirculated wastewater, biodegradable organic matter will be consumed in the process of oxygen respiration and thus reduce the available amount for denitrification. Finally, the results indicate that the lab-scale MBBR system has an acceptable total nitrogen removal efficiency of 80.9% under optimum conditions (500 mg COD/l and 62.5 mg NH<sub>4</sub>-N/l).

**Nutrients removal kinetics:** Mathematical models are used in fundamental research of biological processes to examine the hypotheses, to determine the importance of relationships between variables, to guide the experimental design, and to evaluate the experimental results. These models are also used to control and predict the treatment plant operation performance and to optimize the plant design and the results of the scale-up pilot studies (Borghei *et al.*, 2008). There are several models which have been used to describe the overall kinetics of biological reactors. Here, the first-order

substrate removal I, second-order substrate removal (Grau model) and the Stover-Kincannon models have been selected for considering phosphorus and nitrogen removal during the moving bed biofilm process. It has been assumed that steady-state conditions prevail throughout the reactors and the experimentation. The results of the MBBR kinetic analysis with regard to phosphorus and nitrogen removal show that the Stover-Kincannon model is more appropriate than the first-order substrate removal and the Grau models (Figures 5 and 10). Using this model, the saturation value constants ( $K_B$ ) and maximum utilization rates ( $U_{max}$ ) are 8.5035 g/l.day and 7.71 g/l.day for phosphorus removal and 43.305 g/l.day and 35.088 g/l.day for nitrogen removal, respectively. The Stover-Kincannon model can also be used to determine the volume required to decrease the influent nutrient concentration from  $S_i$  to  $S_e$  or to determine the effluent nutrient concentration for a given volume of a MBBR system and influent nutrient concentration. Consequently, the results of the kinetic studies obtained from the lab-scale experiments can be used for estimating treatment efficiency of a full-scale process under similar operational conditions. Therefore, the Stover-Kincannon model could be used in the design of the moving bed biofilm process.

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## References

- Andreottola G, Foladori P, Ragazzi M, Tatano F (2000a). Experimental comparison between MBBR and activated sludge system for the treatment of municipal wastewater. *Water Sci Technol.* 41: 375-382.
- Andreottola G, Foladori P, Ragazzi M (2000b). Upgrading of a small wastewater treatment plant in a cold climate region using a moving bed biofilm reactor (MBBR) system. *Water Sci Technol.* 41: 177-185.
- Andreottola G, Foladori P, Ragazzi M, Villa R (2002). Dairy wastewater treatment in a moving bed biofilm reactor. *Water Sci Technol.* 45: 321-328.
- Andreottola G, Foladori P, Gatti G, Nardelli P, Pettena M, Ragazzi M (2003). Upgrading of a Small Overloaded Activated Sludge Plant Using a MBBR System. *J Environ Sci Health. Part A.* A 38: 2317-2328.

- APHA, AWWA, WEF (1998). *Standard methods for the examination of water and wastewater*. 20<sup>th</sup> Eds., Washington DC., USA.
- Borghei SM, Sharbatmaleki M, Pourrezaei P, Borghei (2008). Kinetics of organic removal in fixed-bed aerobic biological reactor. *Bioresource Technol.* 99: 1118-1124.
- Chen S, Sun D, Chung JS (2008). Simultaneous removal of COD and ammonium from landfill leachate using an anaerobic-aerobic moving-bed biofilm reactor system. *Waste Manage.* 28: 339-346.
- Chuang SH, Ouyang CF, Yuang HC, You SJ (1998). Evaluation of phosphorus removal in anaerobic-anoxic-aerobic system-via polyhydroxyalkanoates measurements. *Water Sci Technol.* 38: 107-114
- Dulkadiroglu H, Cokgor EU, Artan N, Orhon D (2005). The effect of temperature and sludge age on COD removal and nitrification in a moving bed sequencing batch biofilm reactor. *Water Sci Technol.* 51: 95-103.
- Helness H (2007). *Biological phosphorous removal in a moving bed biofilm reactor*. Doctoral Dissertation, Norwegian University of Science and Technology, Norway.
- Hem LJ, Rusten B, Ødegaard H (1994). Nitrification in a moving bed biofilm reactor. *Water Sci Technol.* 28: 1425-1433.
- Hooshiari B, Azimi A, Mehrdadi N (2009). Kinetic analysis of enhanced biological phosphorus removal in a hybrid integrated fixed film activated sludge process. *Int J Environ Sci Tech.* 6: 149-158.
- Hosseini SH, Borghei SM (2005). The treatment of phenolic wastewater using a moving bed bio-reactor. *Process Biochem.* 40: 1027-1031.
- Hosseiny SH, Borghei SM (2002). Modelling of organic removal in a moving bed biofilm reactor (MBBR). *Scientica Iranica.* 9: 53-58.
- Jahren SJ, Rintala JA, Ødegaard H (2002). Aerobic moving bed biofilm reactor treating thermomechanical pulping whitewater under thermophilic conditions. *Water Res.* 36: 1067-1075.
- Johnson CH, Page MW, Blaha L (2000). Full scale moving bed biofilm reactor results from refinery and slaughter house treatment facilities. *Water Sci Technol.* 41: 401-407.
- Kishida N, Kim J, Tsuneda S, Sudo R (2006). Anaerobic/oxic/anoxic granular sludge process as an effective nutrient removal process utilizing denitrifying polyphosphate-accumulating organisms. *Water Res.* 40: 2303-2310.
- Luostarinen S, Luste S, Valentin L, Rintala J (2006). Nitrogen removal from on-site treated anaerobic effluents using intermittently aerated moving bed biofilm reactors at low temperatures. *Water Res.* 40: 1607-1615.
- Ødegaard H, Rusten B, Westrum T (1994). A new moving bed biofilm reactor-applications and results. *Water Sci Technol.* 29: 157-165.
- Ødegaard H, Rusten B, Siljudalen J (1999). The development of the moving bed biofilm process-from idea to commercial product. *Eur Wat Manage.* 2: 36-43.
- Ødegaard H (2006). Innovations in wastewater treatment: the moving bed biofilm process. *Water Sci Technol.* 53: 17-33.
- Okunuki S, Kawaharasaki M, Tanaka H, Kanagawa T (2004). Changes in phosphorus removing performance and bacterial community structure in an enhanced biological phosphorus removal reactor. *Water Res.* 38: 2433-2439.
- Pastorelli G, Andreottola G, Canziani R, Darriulat C, de Fraja Frangipane E, Rozzi A (1997a). Organic carbon and nitrogen removal in moving-bed biofilm reactors. *Water Sci Technol.* 35: 91-99.
- Pastorelli G, Andreottola G, Canziani R, de Fraja Frangipane E, de Pascalis F, Gurrieri G, Rozzi A (1997b). Pilot-plant experiments with moving bed biofilm reactors. *Water Sci Technol.* 36: 43-50.
- Pastorelli G, Canziani R, Pedrazzi L, Rozzi A (1999). Phosphorus and nitrogen removal in moving-bed sequencing batch biofilm reactors. *Water Sci Technol.* 40: 169-176.
- Rusten B, Ødegaard H, Lundar A (1992). Treatment of dairy wastewater in a novel moving bed biofilm reactor. *Water Sci Technol.* 26: 703-711.
- Rusten B, Siljudalen JG, Nordeidet B (1994). Upgrading to nitrogen removal with the KMT moving bed biofilm process. *Water Sci Technol.* 29: 185-195.
- Rusten B, Hem L, Ødegaard H (1995a). Nitrification of municipal wastewater in moving-bed biofilm reactors. *Water Environ Res.* 67: 75-86.
- Rusten B, Hem L, Ødegaard H (1995b). Nitrogen removal from dilute wastewater in cold climate using moving bed biofilm reactors. *Water Environ Res.* 67: 65-74.
- Rusten B, Siljudalen JG, Strand H (1996). Upgrading of a biological-chemical treatment plant for cheese factory wastewater. *Water Sci Technol.* 34: 41-49.
- Rusten B, Kolkinn O, Ødegaard H (1997). Moving bed biofilm reactors and chemical precipitation for high efficiency treatment of wastewater from small communities. *Water Sci Technol.* 35: 71-79.
- Rusten B, Siljudalen JG, Wien A, Eidem D (1998). Biological pretreatment of poultry processing wastewater. *Water Sci Technol.* 38: 19-28.
- Rusten B, Eikebrokk B, Ulgenes Y, Lygren E (2006). Design and operations of the Kaldnes moving bed biofilm reactors. *Aquacult Eng.* 34: 322-331.
- Sedlak R (1991). *Phosphorus and Nitrogen Removal from Municipal Wastewater: Principles and Practice*. 2<sup>nd</sup> Eds., Lewis Publishers, New York.
- Tchobanoglous G, Burton FL, Stensel HD (2003). *Wastewater engineering: treatment and reuse*. 4<sup>th</sup> Eds., McGraw Hill, New York.
- Wang XJ, Xia SQ, Chen L, Zhao JF, Renault NJ, Chovelon JM (2006). Nutrients removal from municipal wastewater by chemical precipitation in a moving bed biofilm reactor. *Process Biochem.* 41: 824-828.