ANALYSIS AND MODELLING OF PREDATION ON BIOFILM ACTIVATED SLUDGE PROCESS: INFLUENCE ON MICROBIAL DISTRIBUTION, SLUDGE PRODUCTION AND NUTRIENT DOSAGE

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ABSTRACT

The influence of predation on the biofilm activated sludge (BAS) process is studied using a unified model that incorporates hydrolysis and predation phenomena into the two stages of the BAS system: moving bed biofilm reactor pre-treatment (bacterial-predator stage) and activated sludge (predator stage). The unified model adequately describes the experimental results obtained in a cellulose and viscose full-scale wastewater plant and has been used to evaluate the role and contribution of predator microorganisms towards removal of COD, nutrient requirements, sludge production and microbial distribution. The results indicate that predation is the main factor responsible for the reduction of both nutrient requirements and sludge production. Furthermore, increasing the sludge retention time (SRT) does not influence the total biomass content in the AS reactor of a BAS process in two different industrial wastewater treatments.

Keywords: BAS unified model; moving bed biofilm reactor (MBBR); nutrient dosage; predator microorganisms; sludge production.

1. Introduction

The activated sludge (AS) process is the most common system for biological treatment of municipal and industrial wastewater (Wei et al., 2003; Kamali and Khodaparast, 2015). The main disadvantage of the AS process is the low settling of sludge, also known as “bulking” (Rankin et al., 2007), and the large amount of activated sludge produced. Wastewater pre-treatment with biofilm formation systems is an alternative that minimizes these weaknesses. Biofilm activated sludge (BAS) is composed of two aerobic stages: a moving bed biofilm reactor (MBBR) as pre-treatment, followed by an AS reactor. The MBBR is a continuously operating biofilm reactor using small carriers, to which microorganisms attach (Borkar et al., 2013). In aerobic processes, biofilm carriers are moved by blowers. Agitation generates collision between carriers, favouring detachment of biomass and resulting in better diffusion of the components in the layers of the biofilm.

The performance of biological wastewater treatment plants (WWTPs) is closely associated with the structure and functions of microbes. One of the unique characteristics of the BAS process is that microorganism populations in the two stages are different (Sointio et al., 2006). The biofilm stage generates a substantial amount of dispersed (non-floc-forming) bacteria, and the activated sludge stage, in turn, promotes the growth of microorganisms that contain a large amount of higher life forms (predator microorganisms) that live largely on dispersed bacteria.

Predation is not relevant for conventional AS process but becomes very significant in the second stage of the BAS process (Malmqvist et al., 2008). For conventional AS processes, the concentration of predator microorganisms is approximately 5%-10% of the total suspension solids (TSS) (Hauduc et al., 2013). Predator microorganisms are at the top of the food chain in the ecological system of the AS stage, and their concentration depends on the sludge retention time (SRT) (Hao et al., 2010), food sources (Sointio et al., 2006) and wastewater composition. Due to predation on fast-growing MBBR bacteria in the AS system, excess sludge production will typically be 30%-50% lower than that of a conventional AS process (Malmqvist et al., 2008).

Nutrient control in MBBR is also very relevant for the BAS process (van Haandel and van der Lubbe, 2015) because nutrients taken up by bacteria in the biofilm stage are released when the bacteria are consumed by predator microorganisms in the AS stage (Slade et al., 2004). Therefore, BAS processes can operate under nutrient limitation conditions (Rankin et al., 2007; Malmqvist et al., 2008). The BAS process is widely used in wastewater from the pulp and paper industry because this type of wastewater is typically characterized by low nutrient and high COD concentrations (Slade et al., 2004; Elsergany et al., 2015). The addition of nutrients has an important impact on the operational costs of this type of plant.

In a previous study, the authors presented a mathematical model of MBBR reactors (Revilla et al., 2016). This MBBR model confirmed the presence of predator microorganisms in the biofilm and in the bulk liquid under various inlet conditions and the dominance of heterotrophic microorganism in the outlet of MBBR reactors.

The success of current activated sludge models does not require the inclusion of predation, since this process is not relevant in a conventional activated sludge reactor (Henze et al., 2000). Moussa et al. (2005) and, later, Hao et al. (2011) present a model to describe a mixed culture in which nitrifiers, heterotrophs and predators (protozoa and metazoa) coexist. This predation process simplifies the complex reality of the predator-prey relationship, pooling all types of predators and assuming that the predation process is a function of bacterial concentration. However, in the BAS process, the existence of heterotrophs and predators in the inlet of the AS reactor must also be considered in any model.

Many current papers use mathematical models to simulate a conventional AS process, but no literature report uses a mathematical model for a BAS process that integrates the MBBR and AS stages. Lindblom developed a mathematical model for the AS reactor of a BAS process without modelling the MBBR stage (Lindblom, 2003); in this model, heterotrophic microorganisms generated in the biofilm stage and entering the AS stage are slowly biodegradable compounds, and therefore, heterotrophic microorganisms are not the main food source in the AS. This is a major difference from the present study.

This paper proposes and validates a novel unified model for the two steps of the BAS process: an MBBR bacterial-predator stage and an AS predator stage where the food source is mainly bacteria from the MBBR and a low concentrations of readily biodegradable COD. The novelty of the model is that it considers a BAS process in which nitrifiers, heterotrophs and predators coexist, with a different microorganism distribution in the biological reactors of each stage. The removal of COD, nutrient requirements, sludge production and microbial distribution is analysed using the proposed model as applied to a full-scale wastewater treatment plant.

2. Unified mathematical model for BAS process

The mathematical model considers the fate of both soluble (S_i) and particulate (X_i) compounds as described in the nomenclature section. The model is structured with 13 model components or state variables (Ni et al., 2011) and is segregated as follows because three types of microorganisms are considered (Gernaey et al., 2010): i) seven soluble compounds, namely, dissolved oxygen (S_{O2}), readily biodegradable compounds

(S_F), fermentation products (S_A), phosphorous (S_{PO4}), ammonium (S_{NH4}), nitrate (S_{NO3}) and organic nitrogen (S_{ND}); ii) three microorganism groups, namely, heterotrophic bacteria (X_H), autotrophic bacteria (X_A) and predators ($X_{predators}$); and iii) two types of slowly biodegradable compounds: X_S from inactivation of the microorganism groups and $X_{cellulose}$ since the model will be used for wastewater from the pulp and viscose industry and iv) inert matter (X_I) from inactivation of the microorganism groups.

Microorganisms grow under aerobic conditions in the BAS process for this study, but anoxic and anaerobic conditions for the MBBR reactor biofilm have also been considered (Table 1 and Table 2; Revilla et al., 2016).

The conversion of COD and total suspension solids (TSS) has been evaluated assuming stoichiometric conversion parameters of 0.75 and 0.90 gTSS/g COD as in previous studies (Revilla et al., 2016; Henze et al., 2000; Boltz et al., 2011; Tamis et al., 2011). The TSS, filtered COD (COD_f) and total nitrogen (TN) parameters are not introduced as variables but are computed from state variables using equations 1, 2 and 3 (Revilla et al., 2016):

$$\mathrm{TSS} = \left(0.75\,\mathrm{X_I} + 0.75\,\mathrm{X_S} + 0.90\,\mathrm{X_H} + 0.90\,\mathrm{X_{Aut}} + 0.90\,\mathrm{X_{predators}}\right) + \mathrm{X_{cellulose}} \qquad (1)$$

$$\mathrm{COD_f} = \mathrm{S_F} + \mathrm{S_A} + \mathrm{S_I} \qquad (2)$$

$$\mathrm{TN} = \mathrm{S_{NO3}} + \mathrm{S_{NH4}} + \mathrm{S_{ND}} \qquad (3)$$

2.1. Biological conversion processes

The structure of the biological process uses a matrix format that constitutes the model backbone (Revilla et al., 2016). The stoichiometric coefficients are incorporated into appropriate cells of the matrix and the rate of conversion for a given compound I (r_i) is obtained by multiplication of the related process stoichiometry (ν_{ij}) and kinetics (P_j) (Ni et al., 2011) as shown in equation 4:

$$r_i = \sum_{j=1}^{n} P_j \nu_{i,j} \quad (4)$$

The predation mechanism can appear in the MBBR reactors when the soluble COD loading rate (SCLR) is moderate (10-15 g COD/$m^2_{\text{carrier area}}$ day), a biofilm with predators is promoted and consequently a bacterial-predator stage is considered. However, when SCLR is high (>30 g COD/$m^2_{\text{carrier area}}$ day) a bacterial-stage is considered since predator are absent (Ødegaard, 1999; van Haandel and van Lubbe, 2015). In the AS reactor of a BAS process, predators are the dominant microorganisms acting as a predator-stage (Sointio et al., 2006).

The predation mechanism used in this work assumes a single type of predator ($X_{\text{predators}}$). This assumption can be justified by the lack of information on predation rates by biomass type (Ni et al., 2009). As proposed by Moussa (Moussa et al., 2005), the model considers that predators grow aerobically (consume S_{O2}) on the degradable fraction of the two types of available bacteria, heterotrophic microorganisms (X_H) and autotrophic microorganisms ($X_{\text{Aut,}}$) and that the predation rate is a function of bacterial concentration. When X_H and X_{Aut} are consumed by predators, large amounts of nutrients (S_{PO4} and S_{NH4}) (Lindblom, 2003) are regenerated and available to other microorganisms (Revilla et al., 2016). Moreover, when predators graze on X_H and X_A, they convert the non-biodegradable fraction of X_H into inert biomass (X_I) (Table 1). Figure 1 shows a general scheme of the reactions for the predation mechanism, where the transformation of compounds as consumed by predators is described.

A complete description of the stoichiometric matrix and process rate equations used to model the MBBR and AS reactors of the BAS is described in Table 1 and 2.

2.2. MBBR model

The MBBR model is constituted by the biofilm model and bulk liquid model. The biofilm model is based on the general one-dimensional mathematical mixed-culture biofilm (MCB) model described in Wanner and Gujer (1986), which assumes that changes in particulate and soluble compounds occur in the direction perpendicular to the wall of the carrier.

The mass balance for particulate compounds by volume fraction ($f_i(t, z)$) and for soluble components (S_i^f) in the biofilm are given by equations 5 and 6. The mass balance in the bulk liquid is given by equations 7 and 8.

$$\frac{df_i(t,z)}{dt} = \left[Uo_i(t,z) - \overline{U}o(t,z)\right] f_i(t,z) - U(t,z)\frac{df_i(t,z)}{dz}; \qquad \text{i=S, H, Aut, I, predators} \quad (5)$$

$$\frac{dS_i^f(t,z)}{dt} = D_i^f \frac{d^2 S_i^f(t,z)}{dz^2} + r_i(t,z); \qquad \text{i=F, A, NH4, PO4, NO3, O2, ND} \quad (6)$$

$$V_{MBBR}\frac{dS_i^b(t)}{dt} = Q^{in}\left(S_i^{in} - S_i^b\right) - J_i(t,z)\,AF + r_i(t)\,V_{MBBR}; \quad \text{i=F, A, NH4, PO4, NO3, ND} \quad (7)$$

$$V_{MBBR}\frac{dX_i^b(t)}{dt} = Q^{in}\left(X_i^{in} - X_i\right) + \lambda\, L(t)^2 AF\,\rho + r_i(t)\,V_{MBBR}; \quad \text{i= S, H, Aut, I, predators} \quad (8)$$

A precise description of the equations appears in previous studies (Wanner and Gujer, 1986; Revilla et al., 2016).

2.3. AS process model

The aeration tank of the AS process is modelled as a continuous stirred-tank reactor (CSTR) and the generic equations 9 and 10 describe the mass balance.

$$V_{AS}\frac{dS_i(t)}{dt} = Q\left(S_i^{in} - S_i^b\right) + r_i(t)V_{AS}; \qquad \text{i=F, A, NH4, PO4, NO3, O2, ND.} \quad (9)$$

$$V_{AS}\frac{dX_i(t)}{dt} = Q\left(X_i^{in} - X_i^b\right) + r_i\,(t)V_{AS}; \qquad \text{i=S, H, Aut, I, cellulose, predators.} \quad (10)$$

The conversion rates r_i of the MBBR and AS models are obtained by summing the product of the stoichiometric coefficients and the process rate expression, as obtained in a previous study (Revilla et al., 2016).

2.4. Secondary clarifier model

The most widely used model for secondary clarifiers is the one-dimensional model proposed by Takács et al., 1991, known as double-exponential settling velocity, which can predict TSS concentrations in the effluent of BAS. This model assumes a non-reactive (no biological reactions) secondary clarifier, and therefore, the concentration of soluble compounds is the same in the effluent of the BAS process and the outlet stream of the AS reactor (Hreiz et al., 2015).

The general equation is as follows

$$\nu_{s,j}(\mathrm{TSS}) = \max\left\{0, \min\left\{\nu_0' , \nu_0 \left(\exp^{r_h\left(\mathrm{TSS}_j - f_{ns}\mathrm{TSS}_{AS}\right)} - \exp^{r_n\left(\mathrm{TSS}_j - f_{ns}\mathrm{TSS}_{AS}\right)}\right)\right\}\right\} \qquad (11)$$

2.5. Calibration and validation of the unified model

The proposed dynamic model was developed using Aspen Custom Modeler (ACM) software, which solves rigorous models using a specific language that customizes the models for the processes under study. The method of lines (MOL) was used to solve the system of equations, and BFD1 was the discretization method. The adjustment of parameters was done by NL2SOL algorithm for least-squares minimization of the deviation between experimental and theoretical values.

The BAS process for the treatment of wastewater from the cellulose and viscose industry is designed under nutrient-limitation conditions (Malmqvist et al., 2008). This enables the use of a simple strategy for calibration of models, where the biological degradation of organic matter under nutrient limitation dominates (Revilla et al., 2016). The nitrogen and phosphorus parameters $i_{N,BM}$ and $i_{P,BM}$ (nitrogen and phosphorous content of biomass), and $i_{N,XI}$ and $i_{P,XI}$ (nitrogen and phosphorous content of inert matter) were adjusted at steady state with average experimental values for each case.

Validation of the model was carried out using the calibrated input model parameters generated from a set of experimental values (Hao et al., 2011). The experimental data were measured every 7 days (Figure 3 and 4) during the operational time in each case study and standard deviations (SD) between the experimental and simulated concentrations were used to validate of the model.

3. Materials and methods

3.1. Set-up of the full-scale BAS plant

The full-scale BAS plant design is shown in Figure 2. The plant consists of a fine grid of 6 mm to eliminate larger solids, followed by a 1,600-m^3 equalization tank used to i) adjust the inlet flow peaks, ii) dose the nitrogen as urea (40% w/w) and phosphorous as phosphoric acid (72%), and iii) adjust pH to 7-8 with NaOH to neutralize acid effluent. After the equalization tank, there are two MBBR reactors in-series (biofilm stage), referred to as MBBR$_1$ and MBBR$_2$. The 5,331-m^3 MBBR reactors were filled with BiofilmChip P carriers from AnoxKaldnes™ to 10% of the total volume. The carriers have an effective specific surface area of 900 m^2/m^3 and are 45 mm in diameter and 3 mm in length. The carriers move freely due to agitation generated by a blower (airflow 31,600 Nm^3/h).

Later, a 47,000-m^3 AS reactor with two blowers (air flow 31,600 Nm^3/h) was included in the process. It was necessary to recycle sludge from secondary clarifiers to the AS reactor in order to maintain a high biomass concentration. Finally, two parallel secondary clarifiers with a unit volume of 4,143 m^3 were used.

3.2. Stream characterization and operational conditions

The sampling method was removal of 24-h mixed samples for the influent of BAS, outlet stream of AS and effluent of BAS. However for the outlet streams of $MBBR_1$ and $MBBR_2$, grab samples were collected *in situ* during operation.

The full-scale BAS process ran continuously for six months with two types of influent: a wastewater mixture from a cellulose and viscose fibre plant (case study A) for 64 days and wastewater from a cellulose plant (case study B) for 121 days following the plant schedule. Each case study had different operational conditions including nutrient dosage, hydraulic retention time (HRT) and sludge retention time (SRT). The operational conditions for both case studies are illustrated in Table 3. It is observed that HRT and SRT are much lower in MBBR reactors than in the AS reactor.

3.3. Analytical methods

Characterization of the streams was based on the measurement of COD_f, nitrogen forms (S_{NO3}, S_{NH4} and TN), S_{PO4} and TSS. The soluble and particulate compounds were differentiated by filtration through 1.20-µm filters (Henze et al., 2000) prior to analyses. Analysis of the soluble compounds (nitrogen forms, S_{PO4} and COD_f) was performed using Dr. Lange cuvette tests (LCK138, LCK305, LCK339, LCK348, LCK514 and LCK014), and TSS was determined according to standard methods (APHA, 1998).

A Leitz Wetzlar ORTHOLUX 2 POL microscope was used to observe biomass in the MBBR and AS reactor.

4. Results and discussion

4.1. Experimental values and simulation results for the full-scale BAS plant

The experimental concentrations of soluble compounds (COD_f, S_{PO4}, TN, S_{NO3} and S_{NH4}) and particulate compounds (TSS) in the influent and outlet stream of AS and effluent of the BAS process during the operational time (185 days) are shown in Figures 3 and 4. Variability in the concentrations of the influent of BAS at full scale was related to upstream processes and driven by cellulose and viscose production. Reference values were used to maintain the confidentiality of the information (c, p, n and s as observed in Figure 3 and 4).

Figure 3 shows the experimental COD_f concentrations in the influent and effluent, and Table 3 details the average quantity of COD_f removed in each biological reactor comprising the BAS process. Figure 3 shows the adequate and stable evolution of COD_f in the effluent of the BAS process over all operational time for both case studies, and Table 3 shows that the overall removal of COD_f: in case study A is 76%. Removal in case study B is higher (85%) because the inert fraction of COD_f in the influent (S_I) is lower (15%) in case study B than in case study A (25%) (Revilla et al., 2016). It is also observed in Table 3 that COD_f is mainly eliminated in the $MBBR_1$, which, followed by the AS reactor and $MBBR_2$, is the reactor with the lowest amount removed. Similar results were obtained in previous studies (Rankin et al., 2007; Sointio et al., 2006) in a BAS process for pulp mill wastewater.

Figure 3 shows the experimental phosphorus (S_{PO4}) and nitrogen (S_{NO3}, S_{NH4} and TN) concentrations in the influent and effluent of the BAS process; it is observed that the concentrations of S_{PO4} in the effluent are approximately 75% of the influent concentration in both case studies. These concentrations are higher than expected for a conventional AS process (Malmqvist et al., 2008).

The TN in the influent of the BAS process is mainly composed of organic nitrogen (S_{ND}) from urea (Figure 3) that is rapidly hydrolysed by heterotrophic microorganisms (Henze et al., 2000) in the MBBR reactors to ammonia nitrogen (S_{NH4}). Excess S_{NH4} is oxidized to nitrate nitrogen (S_{NO3}) by autotrophic microorganisms (X_{Aut}) (Mozumder et al., 2014) in the AS reactor. Consequently, TN in the effluent is mainly composed of S_{NO3}.

The experimental concentrations of TSS in the influent, the outlet stream of the AS reactor and the effluent are shown in Figure 4. TSS in the influent is composed mainly of cellulose fibres ($X_{cellulose}$) that will be hydrolysed in the AS reactor by heterotrophic microorganisms (Ruiken et al., 2013). As expected, the TSS in the outlet stream of AS reactor increased 10-fold due to the growth of microorganisms. Moreover, Figure 4 also shows the removal of TSS from the AS reactor in the secondary clarifiers: 98.5% in case study A and 98.7% in case study B.

The simulated concentrations of COD_f, TSS, TN, S_{PO4}, S_{NO3} and S_{NH4} in the outlet stream of the AS reactor and the effluent of BAS are show in Figures 3 and 4 as continuous and dotted lines. Good agreement is observed between experimental and simulated concentrations, as confirmed by the small standard deviations (SD) shown in Table 4. For the two case studies, the values of SD for all compounds are lower than 14%; these low SD values validate the unified proposed model under operational conditions.

4.2. Microorganism distribution in BAS reactors

A mathematical model is used to evaluate the microbial distribution profile (Moussa et al., 2005; Hao et al., 2011) in the bulk liquid of reactors involved in the BAS process.

Figure 5 shows the percentage of heterotrophic microorganisms, inert matter and suspended biodegradable compounds from inactivation, cellulose fibres, predators and autotrophic microorganisms in the bulk liquid of the $MBBR_1$, $MBBR_2$ and AS (X_H, X_I, X_S, $X_{cellulose}$, $X_{predator}$ and X_{Aut}) for both case studies at steady state. The mathematical model details the microorganism populations in the two stages (biofilm and AS); the major particulate compounds in the $MBBR_1$ and $MBBR_2$ reactors are heterotrophic microorganisms, and in the AS reactor, they are inert matter and predator microorganisms (Figure 5). This is expected because MBBR reactors (short HRT) remove the most $COD_{f,}$ such that the growth of heterotrophic microorganisms is the main biological process. However, HRT in the AS reactor is approximately 10 times higher than that in MBBR reactors (Table 3), and predation and inactivation processes are the main biological processes at this AS stage. The difference in microorganism populations at each stage is one of the main characteristics of the BAS process (Wei et al., 2003). Other differences among the fractions of particulate compounds in each reactor of the BAS process and their causes were analysed:

i) The heterotrophic microorganisms (X_H) in the MBBR reactors are 50% of TSS in case study A and 60-70% in case study B (Figure 5), removing 23.6 COD_f ton/day in the two MBBR reactors in case study A and 24.1 COD_f ton/day in case study B (Table 3). However, at the AS stage, fewer tons of COD_f are removed for both case studies (11.8 and 4.2 ton/day for case study A and B, respectively), and the percentage of heterotrophic microorganisms is low (5-10%). The main food source at the AS stage is what is left over from MBBR reactors, mainly heterotrophic microorganisms instead of COD_f.

ii) Predator microorganisms are absent in the $MBBR_1$ for both case studies since the soluble COD loading rate (SCLR) is high in the $MBBR_1$ (Ødegaard, 1999). In the $MBBR_2$, predator microorganisms are also absent in case study A, but represent 13.2% of the TSS for case study B (Figure 5) due to an SCLR value below 15 g COD/m^2 day (Revilla et al., 2016). In the AS reactor, the predator microorganisms and the inert material are the main particulate compounds in the TSS: $X_{predators}$ is 32% in case study A and 26% of total TSS in case study B, and inert matter (X_I) is 57% in case study A and 69% in case study B. This high percentage of inert matter is explained because the predator microorganisms graze on active bacteria and convert the non-biodegradable fraction of X_H into inert biomass (Moussa et al., 2005). The presence of predator microorganisms such as ciliates (Wei et al., 2003) was observed microscopically in the AS reactor.

As the quantity of COD that reaches the AS reactor is small, X_H is under starvation conditions and COD_f is removed rapidly by X_H. In general, the longer the starvation period is, the greater is the extent of inactivation and, as a consequence, the higher is the inert fraction at AS (Ni et al., 2011). In addition, predation on X_H and X_{Aut} generates high amounts of X_I (Moussa et al., 2005; Ni et al., 2009, 2011; Hao et al., 2011). As a consequence, the inert fraction (X_I) is the main particulate compound in AS reactor.

iii) Slowly biodegradable compounds ($X_{cellulose}$ and X_S) must be hydrolysed to S_F by X_H and then used by X_H as a food source. Biological hydrolysis of cellulose fibres ($X_{cellulose}$) strongly depends on SRT (Ruiken et al., 2013). Therefore, in this work, it is assumed that hydrolysis of $X_{cellulose}$ only occurs in the AS reactor (Table 3), where the SRT is high enough to break up cellulose fibres (average values of 19 and 30 days for case study A and B, respectively). Most $X_{cellulose}$ in the AS reactor is hydrolysed, but for each case study, a small fraction (0.05%) remains.

In contrast to $X_{cellulose}$, X_S can be hydrolysed by suspended bacteria in the MBBR reactors depending on SCLR (Helness and Ødegaard, 2005). In case study B, X_S decreases slightly at $MBBR_2$ because SCLR is lower than 20 g COD/m^{2}day, and hydrolysis is not neglected; however, in case study A, X_S increases at $MBBR_2$ due to an SCLR higher than 20 g COD/m^{2}day (Revilla et al., 2016). As a consequence, the fraction of X_S and $X_{cellulose}$ is higher in the MBBR that in the AS reactor (Figure 5).

iv) The presence of X_{Aut} is fixed by the inlet COD_f/S_{NH4} ratio of the biological reactor (Mozumder et al., 2014). Figure 5 shows that the MBBR reactors do not contain autotrophic microorganisms in case studies A and B. However, in the AS reactor, a small fraction of X_{Aut} is observed—0.5% in case study A and 0.2% in case study B—because of the low COD_f/S_{NH4} inlet ratio of the AS reactor. For high COD_f/S_{NH4} ratios, the growth rate of X_H is high enough (Lee and Park, 2007), and X_{Aut} does not coexist with X_H; conversely, for low COD_f/S_{NH4} ratios, X_{Aut} coexists with X_H (Bassin et al., 2015).

As a summary of the microorganisms distribution of the BAS process, it is observed that the first reactor of the MBBR is the bacterial stage, the second reactor of the MBBR is the bacterial-predator stage and the AS reactor is the predator stage.

4.3. Nutrient dosage in the BAS process

A ratio of 100:5:1 (COD_f:N:P) has traditionally been used as a “rule of thumb” for setting nutrient levels in biological processes (Ammary, 2004). However, studies of BAS processes treating wastewater from the pulp and paper industry indicate that nitrogen and phosphorus requirements in relation to COD_f are not always as high as the above ratio (Rankin et al., 2007). In this work, the COD_f:N:P ratios used are much lower than the “rule of thumb” (Slade et al., 2004), as shown in Table 3. The large percentage of COD_f removed in the BAS process confirms that the ratio can be much lower than the ratio indicated by the “rule of thumb”, with a positive economic effect on the overall process due to the high cost of nutrients (Revilla et al., 2014).

To illustrate why this low level of COD_f:N:P is sufficient in the BAS process, the simulation results under a steady state of nutrients were obtained for MBBR and AS reactors in Table 5. It is observed that the simulation results for S_{PO4} and S_{NO3} in the AS reactor are much higher than in MBBR reactors, but the simulation result for S_{NH4} in the AS reactor is much lower. The unexpected increase in S_{PO4} after running the simulation in the activated sludge reactor is due to two biological processes: predation and inactivation (Hao et al, 2011). During these processes, phosphorous compounds inside heterotrophic microorganisms are released into the water. However, the simulation result for S_{NH4} in the AS reactor is very low because S_{NH4} recovered due to predation results in a low COD_f/S_{NH4} ratio, and S_{NH4} is oxidized to S_{NO3} by autotrophic microorganisms (Lee and Park, 2007). As a result, the simulation result for S_{NO3} is high, and the S_{NH4} concentration is low in the AS reactor of the BAS process.

To confirm the influence of predation on the concentrations of phosphorus and nitrogen forms in the AS reactor of a BAS process, the proposed mathematical model was used to switch predation on and off (Moussa et al., 2005). The simulation of S_{NH4}, S_{NO3} and S_{PO4} at steady state when predation is switched on and off are shown in Table 6. These values are all lower in absence of predators than in the presence of predators, reinforcing the importance of predator microorganisms.

These results demonstrate the importance of predation in the AS reactors of the BAS process for nutrient dosage. The increase in phosphorus and nitrogen concentrations in the AS reactor due to predation enables the use of low doses of nutrients in the inlet stream of the BAS process without decreasing COD_f removal efficiency. This is a great advantage for the overall process (Rankin et al., 2007).

4.4. Sludge production in the BAS process.

The treatment and disposal of sludge from a wastewater treatment plant is expensive and can account for up to 60% of the total operating costs of wastewater treatment (Ramdani et al., 2010). Reducing sludge production thus presents an obvious economic interest. A main characteristic of the BAS process is that the production of sludge is much lower than in conventional AS processes (Rankin et al., 2007; Malmqvist et al., 2008).

In this section, the influence of predation is analysed by comparison of the fraction of particulate compounds and concentration of TSS in the AS reactor using the proposed model. The comparison is performed at steady state under the same operational conditions, but switching predation processes on and off. Table 6 shows the simulated results with and without predators. It is observed decreases in TSS concentration of 42% and 44% in case study A and B, respectively, when predation was on (Wei et al., 2003; Malmqvist et al., 2008). These results are explained by the large decrease in the fraction of X_H when predators are activated, since the main food source in the AS reactor of BAS for predator microorganisms are the heterotrophic microorganisms that leave the second MBBR reactor (Sointio et al., 2006). As shown in Table 6, the presence of high fractions of predator leads to an increase in the inert fraction.

4.5. Influence of the SRT on biomass content in the BAS process

Another option for decreasing sludge production is to extend the SRT (Liu and Wang, 2015). However, an increase in SRT results in an increase in the inactivation processes, which may lead to a higher concentration of inert matter. As a consequence, biological wastewater treatment could lose efficiency (Hreiz et al., 2015). The level of inert matter in the AS reactor of the full-scale BAS plant under study is high (Figure 5) due to inactivation and predation mechanisms (observed previously by Hao et al., 2011).

Therefore, it is especially important to control SRT to avoid efficiency losses in the treatment and accumulation of inert matter.

Case studies A and B operate under different SRT conditions suited to different industrial wastewaters (Table 3). An analysis of both case studies allows observation of the effect of SRT on the fraction of particulate compounds and biomass content in the AS reactor. Figure 6 shows the dynamic behaviour of the simulated fraction of particulate compounds in both case studies until they reach a steady state after 150 days. This allows comparison of the behaviour of all biomass content in the AS reactor for two different SRTs and industrial wastewaters at the steady state. Case study B operates with a higher SRT (30 days) than case study A (19 days), resulting in similar concentrations of TSS in both case studies at the steady state (8.5s g/m^3 in case study A and 8.6s g/m^3 in case study B), namely, the inert material (X_I) that is the main fraction of TSS.

For wastewater from the cellulose industry (case study B), it is possible to operate using high SRT values because the increase in X_I is compensated by a reduction in the amount of predators ($X_{Predators}$), heterotrophic microorganisms (X_H) and autotrophic microorganisms (X_{Aut}) (Moussa et al., 2005) resulting in similar concentrations of TSS in both case studies. Therefore, the mathematical model can be used to determine the fraction of particulate compounds at various operating conditions of SRT and thus avoid the accumulation of high amounts of inert material (Moussa et al., 2005; Ni et al., 2009, 2011) in the AS reactor during a BAS process.

5. Conclusions

A novel unified model for the BAS process is proposed to study microbial behaviour in the biofilm (MBBR) and AS stages and to evaluate the influence of predation mechanisms on nutrient dosage, sludge production and microbial distribution. The first MBBR reactor is the bacterial stage, the second MBBR reactor is the bacterial-predator stage and the AS reactor is the predator stage. The results demonstrate that predation is the main cause of reductions in nutrient requirements (up to 44%) and sludge production (up to 46%) compared to the conventional AS process.

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