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Abstract:	An important part of biological treatment system design is quantifying the sludge production and the nutrient removal capacity. The objectives of this project were to determine the model parameters associated with sludge production and the nutrient content of sludge components. A complete sludge retention experiment was conducted in a pilot-scale membrane bioreactor operated with alternating growth and famine periods. The General ASDM parameter set was found to better fit the experimental data than the Metcalf and Eddy parameter set, mainly to characterize endogenous respiration and the heterotrophic biomass concentration. An influent unbiodegradable organic particulate fraction value of 0.16 g COD/g COD was determined by calibration of the accumulated sludge total COD, suspended solids and heterotrophic biomass concentrations. The nutrient content of the accumulated endogenous residue and influent unbiodegradable organic particulate components were calibrated to 0.030 and 0.100 g N/g COD and 0.035 and 0.008 g P/g COD, respectively.

Activated sludge production parameters and nutrient content of organic sludge components

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Abstract

An important part of biological treatment system design is quantifying the sludge production and the nutrient removal capacity. Influent wastewater COD fractionation, biomass growth and endogenous respiration directly impacts the composition of the mixed liquor solids in activated sludge systems. The objectives of this project were to determine the model stoichiometric and kinetic parameters associated with activated sludge production and the nutrient content (N and P) of unbiodegradable organic sludge components. A complete sludge retention experiment was conducted over 70 days in a pilot-scale membrane bioreactor fed with a real municipal wastewater, and operated with alternating growth and famine periods. Experimental results were simulated and compared using the default values from two well-accepted model parameter sets. The *General ASDM* parameter set was found to better fit the experimental data than the Metcalf and Eddy parameter set, mainly to characterize endogenous respiration and the heterotrophic biomass concentration. An influent unbiodegradable organic particulate fraction ($f_{XU,Inf}$) value of 0.16 g COD/g COD was determined by calibration of the accumulated sludge total COD, suspended solids and heterotrophic biomass concentrations. The nutrient content of the accumulated endogenous residue (X_E) and influent unbiodegradable organic particulate ($X_{U,Inf}$) components were calibrated to 0.030 and 0.100 g N/g COD and 0.035 and 0.008 g P/g COD, respectively. These values are in the range of those reported in the literature except for the high P content found in the endogenous residue, possibly due to the presence of coagulants added for P removal in the accumulated sludge. These results were consistent under the wide range of dynamic conditions tested and could improve model prediction of sludge production and composition.

Keywords : complete sludge retention; dynamic modelling; membrane bioreactor; mixed liquor composition; wastewater fractionation.

List of abbreviations and symbols

ASM: Activated sludge model

b_H: Endogenous respiration rate of the heterotrophic biomass

BOD: 5 day carbonaceous biochemical oxygen demand

COD: Chemical oxygen demand

COD_{Inf}: Influent total COD

COD_{XE}: X_E expressed as COD

COD_{XH}: X_H expressed as COD

COD_{XU}: $X_{U,Inf}$ expressed as COD *f*: Endogenous residue fraction resulting from X_H decay

$f_{CV,XB}$: COD/VSS ratio of X_B

$f_{CV,XH}$: COD/VSS ratio of X_H

$f_{CV,XU}$: COD/VSS ratio of X_U

$f_{X,Inf}$: Influent total particulate (X) COD fraction

$f_{XB,Inf}$: Influent biodegradable particulate (X_B) COD fraction

$f_{XH,Inf}$: Influent heterotrophic biomass (X_H) COD fraction

$f_{XU,Inf}$: Influent unbiodegradable organic particulate ($X_{U,Inf}$) COD fraction

GM: General activated sludge digestion model *(General ASDM)* model

ISS: Inorganic suspended solids

i_N: Nitrogen content of a component

$i_{N,XH}$: Nitrogen content of X_H

$i_{N,XE}$: Nitrogen content of X_E

$i_{N,XU}$: Nitrogen content of $X_{U,Inf}$

i_P: Phosphorus content of a component

$i_{P,XH}$: Phosphorus content of X_H

$i_{P,XE}$: Phosphorus content of X_E

$i_{P,XU}$: Phosphorus content of $X_{U,Inf}$

MBR: Membrane bioreactor

ME: Metcalf and Eddy activated sludge model

NH_4: Ammonia

NO_3: Nitrate plus nitrite

OUR: Oxygen uptake rate

$o\text{-}PO_4$: Orthophosphate

RMSE: Root mean square error

SRT: Sludge retention time

$S_{U,Inf}$: Influent unbiodegradable soluble COD

TKN: Total Kjeldahl nitrogen

TP: Total phosphorus

TSS: Total suspended solids

VSS: Volatile suspended solids

VSS_{XB}: Volatile suspended solids associated to X_B

VSS_{XH}: Volatile suspended solids associated to X_H

VSS_{XU}: Volatile suspended solids associated to X_U

WRRF: Water resource recovery facility

X_{COD}: Particulate COD

X_B: Biodegradable particulate COD

X_E: Endogenous residue from biomass decay

X_H: Heterotrophic biomass

$X_{U,Inf}$: Influent unbiodegradable particulate organics

Y_H: Heterotrophic biomass true yield

1 INTRODUCTION

Estimating activated sludge production and composition is important for the design of water resource recovery facilities (WRRFs) as it determines the sizing of the bioreactors, the settlers, the anaerobic digesters and the sludge treatment and handling equipment. Sludge production predicted by activated sludge models (ASMs) depends mainly on the influent fractionation and on the heterotrophic biomass true yield (Y_H), endogenous respiration rate (b_H) and fraction of endogenous residue (f). These complex non-linear models are overparametrized with respect to available data and the parameters are poorly identifiable (Dochain and Vanrolleghem, 2001). Various combinations of this model parameters subset (Y_H, b_H and f) will predict, for a given influent and sludge retention time (SRT), almost identical sludge production and oxygen consumption, but different heterotrophic biomass fraction and mixed liquor composition (Dold, 2007).

The three main organic components of mixed liquor are heterotrophic biomass (X_H), endogenous residue from biomass decay (X_E) and unbiodegradable particulates from the influent ($X_{U,Inf}$). The latter two are unbiodegradable components that accumulate in the sludge and cannot be isolated nor measured independently. Their concentration and composition must be estimated from model calibration, from engineering checks (e.g. influent COD/BOD) and from lumped analyses by difference with heterotrophic biomass if the latter can be estimated (Hulsbeek et al., 2002; Melcer et al., 2003).

The operation of membrane bioreactors (MBRs) under complete retention allows to reach long SRTs and to accurately determine the sludge production without relying on good sludge settling properties. Recent studies point out that sludge accumulation reaches a plateau at long SRTs, which contradicts the accepted concept that the unbiodegradable organic particulate fractions and the inorganic particulate fractions accumulate in the sludge (Liu et al., 2005; Pollice et al., 2007). Further work is thus required to determine the fate of these sludge fractions at long SRTs.

The nutrient content of $X_{U,Inf}$ and X_E is poorly documented and will affect the effluent quality for WRRFs facing stringent effluent limits and the C:N:P ratio used to determine the valorization potential of the sludge and digester supernatant for i.e. landspreading and sidestream nutrient removal. The nitrogen and phosphorus content of sludge component was identified as an issue for further research (Melcer et al. 2003; Ekama et al., 2007; Ramdani et al., 2012; Choubert et al., 2013).

The objectives of this project were to estimate a set of model parameters for determining sludge production associated to the activated sludge organic components ($X_{U,Inf}$, X_H, X_E) from a pilot MBR treating a real municipal wastewater subjected to growth and famine periods and with complete sludge retention, and to determine the nitrogen (i_N) and phosphorus (i_P) content of these components.

2 MATERIAL & METHODS

2.1 Complete sludge retention experiment

A complete sludge retention experiment was conducted over 70 days in a pilot-scale MBR. Influent to the pilot unit was a real municipal primary effluent. Feeding was turned on and off to create alternating growth and famine conditions. Details on the influent fractionation and the experimental setup along with further details on the analytical methods and sampling are given below.

2.1.1 Influent fractionation

Experiments were conducted with a primary effluent from the Saint-Hyacinthe (Canada) WRRF on which a detailed fractionation was conducted according to Melcer et al. (2003). About 50% of the influent organic load to the plant was attributed to agro-industries.

Influent heterotrophic biomass fraction ($f_{XH,Inf}$) was measured with a batch exponential growth test (Wentzel et al., 1995). Influent unbiodegradable soluble COD ($S_{U,Inf}$) was evaluated from the pilot-scale MBR effluent COD concentration. Particulate COD (X_{COD}) was obtained by difference between the total COD and the filterable COD measured on the filtrate of 1.5 μm pore size glass microfiber filters (Whatman, 934-AH).

The influent biodegradable particulate COD/VSS ratio ($f_{CV,XB}$) was obtained from Equations 1 and 2, assuming a ratio of 1.42 g COD/g VSS for the heterotrophic biomass ($f_{CV,XH}$; Melcer et al., 2003; Dold, 2007). The influent unbiodegradable organic particulate COD/VSS ratio ($f_{CV,XU}$) was obtained from model calibration (see section 3.2.1).

$$f_{CV} = \frac{X_{COD}}{VSS} = \frac{X_B + X_H + X_{U,Inf}}{VSS_{XB} + VSS_{XH} + VSS_{XU}} \qquad \text{(Eq. 1)}$$

With $VSS_{XH}=X_H/f_{CV,XH}$ and $VSS_{XU} = X_U/f_{CV,XU}$ and rearranging, $f_{CV,XB}$ is obtained.

$$f_{CV,XB} = \frac{X_B}{VSS_{XB}} = \frac{X_{COD} - X_H - X_{U,Inf}}{VSS - X_H/f_{CV,XH} - X_U/f_{CV,XU}} \qquad \text{(Eq. 2)}$$

Based on this fractionation, the influent biodegradable particulate COD fraction ($f_{XB,Inf}$) is obtained by the difference of the total particulate COD fraction ($f_{X,Inf}$) with the influent unbiodegradable organic particulate COD fraction ($f_{XU,Inf}$) and $f_{XH,Inf}$ values. The simulated mixed liquor heterotrophic biomass concentration (X_H), partly depending on $f_{XB,Inf}$, is thus affected by the calibration of sludge production using $f_{XU,Inf}$.

2.1.2 Experimental setup

The primary effluent feed to the pilot-scale MBR was sequentially turned on and off to create growth (periods A, C and E) and famine (periods B, D and F) conditions (Table 1). The duration of the periods were chosen to obtain a final maximal MLSS concentration corresponding to the limit for the aeration system to maintain the desired dissolved oxygen concentration and to account for a Christmas holiday period. No sludge was wasted during the 70-day experiment. An MBR was used to maintain all solids in the system and to prevent solids loss to the effluent even in the event of poor sludge settling properties. Sequencing growth and famine periods were created to obtain periods with sludge accumulation (growth) and periods of aerobic digestion (famine).

The pilot-scale MBR consisted of an aeration tank equipped with fine bubble diffusers and an external submerged hollow fiber MBR (Puron, Koch Membrane Systems, USA). The MBR characteristics and operating conditions are presented in Table 2. The MBR filtration cycle consisted of 600 seconds of permeation phase, 30 seconds of relaxation phase and 30 seconds of backflush phase at a flowrate of 2 m^3/h. Scouring airflow rate was continuously maintained at a high level of 6 Nm^3/min to prevent membrane fouling. Further details on the Puron MBR system is presented by Hirani et al. (2010).

The pilot-scale MBR was initially filled with activated sludge from the full scale WRRF aeration tanks, operated at an SRT of about 4 days (partially nitrifying). The sludge was kept under aerobic conditions throughout the experiment by controlling the dissolved oxygen at 3.2 ± 1.0 mg O_2/L in the aeration tank (LDO dissolved oxygen probe with SC1000 controller, Hach, USA) during growth periods while it reached up to saturation levels during famine periods. Mixed liquor pH and total suspended solids concentration (TSS) were monitored hourly in the aeration tank (Solitax TSS probe, Hach, USA). The TSS probe error was 1 ± 9% (n=23) over the full range of measurements (2.0 to 11.3 g TSS/L), after correction with a linear regression (r^2=0.98) based on laboratory TSS measurements.

2.1.3 Mixed liquor heterotrophic biomass

The mixed liquor heterotrophic biomass concentration (X_H) was estimated based on biomass activity by the oxygen uptake rate (OUR) test for sludge (APHA et al., 2005). The test was conducted on site in a BOD bottle using a mixed liquor sample previously oxygenated by vigorous mixing in a one-liter half-filled sampling bottle. The linear depletion of oxygen concentration with time ($r^2 \geq 0.985$; n=20) was measured every 30 seconds over periods of 5 to 60 minutes depending on activity with a portable oxygen probe connected to a controller for data acquisition (LBOD101 probe with HQ40d controller, Hach, USA). The mixed liquor heterotrophic biomass concentration was estimated from equation 3, considering that 4.33 g of oxygen is required per g of ammonia nitrogen oxidized to nitrate nitrogen (Wezernak and Gannon, 1967; Metcalf & Eddy, 2003). It was assumed that nitrate was the final product and that a negligible amount of nitrite accumulated during the batch OUR test, as the measured nitrite concentration in the MBR effluent was low (average of 0.28 mg NO_2-N/L [n=15]). The OUR and b_H values were corrected for temperature using the van't Hoff-Arrhenius relationship with a temperature coefficient of 1.029.

$$OUR_t = \left(4.33 \cdot i_{N,XH} + 1\right) \cdot \left(1 - f\right) \cdot b_H \cdot X_H \cdot \frac{e^{(-b_H)\,t/24}}{24} \qquad \text{(Eq. 3)}$$

where: OUR_t = oxygen uptake rate after t hours of sampling ($mg \cdot L^{-1} \cdot h^{-1}$)

$i_{N,XH}$ = heterotrophic biomass nitrogen content (g N/g COD)

f = fraction of heterotrophic biomass converted to endogenous residue (g COD/g COD)

b_H = endogenous respiration rate (d^{-1})

X_H = heterotrophic biomass concentration at the beginning of test (mg COD/L)

t = duration of test (h)

2.1.4 Analytical methods and sampling

Composite 24-hour samples were taken from the pilot-scale MBR influent (refrigerated at 4°C) and effluent. Grab samples were taken for mixed liquor. Daily measurements were made at the influent for chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS) and 5 days biochemical oxygen demand (BOD) according to APHA et al. (2005). Other parameters were measured two to three times per week. Nitrogen and phosphorus analyses were made with a flow injection analysis (FIA) system (Lachat, Quickchem 8500, USA). Total Kjeldahl nitrogen (TKN) and total phosphorus (TP) were measured after digestion using the Kjeldahl digestion method (APHA et al., 2005).

2.2 Modelling

The following subsections present the model parameters compared in this study and the approach used to determine the nutrient content of the mixed liquor components.

2.2.1 Model parameters

The default model parameter values (Y_H, b_H and f) from two well-accepted models were compared to determine which set provided a better fit to the experimental data (Table 3). Parameter sets ME and GM are from Metcalf and Eddy (2003) and from the *General ASDM model* of Biowin 3.1, respectively, and are expressed in terms of the endogenous respiration approach. These two models were selected because of their broad application by practitioners in WRRF design and very similar sludge production predictions despite dissimilar default parameter values (Dold, 2007). These models were used to calibrate sludge production data from several WRRFs operated under fully aerobic, anoxic-oxic and anaerobic-anoxic-oxic conditions (Dold, 2007). These parameters are sensitive for the prediction of sludge production and are representative of typical ranges of parameters values found in the literature (Cox, 2004; Hauduc et al., 2011).

Sludge production was calibrated by adjusting the $f_{XU,Inf}$ to fit the accumulated mixed liquor total COD, TSS, VSS and X_H. All simulations were carried out using BioWin 3.1 (EnviroSim, Canada). The model sludge mineral fraction was composed of only the accumulated influent inorganic suspended solids (ISS), neglecting the intracellular salts of other sludge components. Initial model values were obtained from steady state simulations at an SRT of 4 d using an average influent fractionation. Daily temperature variation in the aeration and MBR tanks was considered using the simulator default Arrhenius temperature coefficients.

The root mean square error (RMSE) was used as a statistical criterion of the goodness of fit of the model and to evaluate the relative model parameter sensitivity (Sin et al., 2008). A low number indicates a good fit.

2.2.3 Nutrient content

The nutrient content in X_E and $X_{U,Inf}$ was calibrated by manual trial and error using a two-step procedure. The nitrogen and phosphorus content in X_E ($i_{N,XE}$ and $i_{P,XE}$) was first adjusted to fit the nitrate build-up, resulting from the nitrification of released ammonia, and the orthophosphosphate build-up, respectively, both from biomass decay during famine periods. The nitrogen and phosphorus content in $X_{U,Inf}$ ($i_{N,XU}$ and $i_{P,XU}$) was then adjusted to fit the accumulated sludge TKN and TP using the whole dataset.

The empirical composition of heterotrophic biomass was considered as $C_{58.8}H_{73.5}O_{22.1}N_{11.6}P$, corresponding to a nitrogen and phosphorus content of $i_{N,XH}$ = 0.087 g N/g COD and $i_{P,XH}$ = 0.016 g P/g COD, and to a particulate COD to VSS ratio ($f_{CV,XH}$) of 1.42 g COD/g VSS. This composition was previously determined by elemental analysis during pilot-scale and batch experiments with a fully biodegradable soluble synthetic substrate (Ramdani et al., 2012).

3 RESULTS & DISCUSSION

In the following sections, the influent characterization is presented first. The sludge accumulation data is then presented and calibrated through adjusting the influent unbiodegradable organic particulate fraction ($f_{XU,Inf}$). The goodness of fit obtained with the two studied model parameter sets is then compared. The nutrient content of the sludge components is finally estimated from the behavior of accumulation and release of the various forms of nitrogen and phosphorus during growth and famine periods.

3.1 Influent

The average influent concentrations and fractions are presented in Table 4. The measured COD, TKN and total P concentrations are representative of a low to medium strength wastewater. The measured ratio of 2.4 ± 0.5 g COD/g BOD (n=69) is higher than the range of 1.9 to 2.0 g COD/g BOD typically observed for municipal primary effluent (Melcer et al., 2003). The ammonia fraction of the TKN and the orthophosphate fraction of total phosphorus are also low compared to the typical ranges of 0.60 to 0.85 g N/g N and 0.5 to 0.8 g P/g P. The industrial load from agro-industries at the studied plant could explain these differences when compared to typical domestic wastewaters.

The average volumetric loading during growth periods was of 1.1 ± 0.3 kg COD m^{-3} d^{-1}, corresponding to a low loading for an MBR system. The F/M ratio ranged from 0.0 during famine periods up to 0.4 g COD g VSS^{-1} d^{-1}, with an average of 0.2 g COD g VSS^{-1} d^{-1}, during growth periods. The wide range of F/M ratios resulting from the operating conditions is better put into perspective when expressed in terms of COD load per heterotrophic biomass in the system (F/M_{XH}). The average and maximum F/M_{XH} ratios during growth periods using the GM and the ME calibrated simulation results, were of 0.68 and 2.52 and of 0.40 and 1.01 g COD g X_H^{-1} d^{-1}, respectively. Highest values were obtained at the beginning of growth periods C and E, when the X_H concentration was minimal (Figure 1).

3.2 Sludge accumulation

Mixed liquor COD and TSS concentrations accumulated to as high as 13 g COD/L and 11 g TSS/L during the 70 days of experimentation (Figure 1). Accumulation of particulate matter occurred during growth periods (periods A, C and E) due to biomass growth and accumulation of unbiodegradable volatile (VSS) and inorganic (ISS) suspended solids. A decrease in COD and VSS concentration was observed during famine periods (periods B, D and F) due to biomass decay while the ISS concentration remained essentially constant. The average mixed liquor f_{CV} during the whole experiment was of 1.47 ± 0.07 g COD/g VSS (n=19; Table 5). The measured mixed liquor VSS to TSS ratio gradually decreased from 0.84 to 0.76 g VSS/g TSS over the course of the experiment, with slight increases during growth periods C and E during which the proportion of heterotrophic biomass in mixed liquor increased.

For systems under complete sludge retention (without wastage), the duration of the experiment can be used to illustrate sludge age instead of the SRT as the latter cannot be calculated from standard equations (Spérandio et al., 2013). In the current study the dynamic SRT was estimated to range from 4.0 to 51.5 days based on calculations suggested by Takács et al. (2008).

The few sudden changes observed in the probe TSS concentration measurements (Figure 1) were due to the start-up and shut down of the MBR permeate pump. The mixed liquor concentration was slightly higher in the external MBR tank during membrane filtration, so small shifts in solids masses between the external MBR tank and the aeration tank occurred at start-ups and shut-downs of the MBR pump. This can be observed at the beginning of each growth and famine period and on days 2, 10 and 29 due to pump failures. No solids were lost during these events. These operating conditions were accounted for in data processing and simulations, as can be observed in the simulated response.

3.2.1 Calibration and parameter validation

Sludge production expressed in units of COD, TSS, VSS and heterotrophic biomass was calibrated by adjusting $f_{XU,Inf}$ to a value of 0.16 g $X_{U,Inf}$/g COD_{Inf} for both the ME and the GM parameter sets. The resulting dynamic simulation results are presented in Figure 1. Model predictions were in best agreement with experimental data using the GM parameter set without any further adjustment to the model parameters. The fit with the heterotrophic biomass concentration and the VSS decrease during famine periods were significantly better, as the ME parameter set poorly estimated the heterotrophic biomass component and overestimated the VSS decrease.

The endogenous respiration rate in the ME parameter set is half the value of that in the GM set, hence one would expect the VSS decrease to be less significant in the former during famine periods, but the higher heterotrophic biomass fraction simulated with the ME parameters (Dold, 2007) resulted in a more rapid VSS decrease. The heterotrophic biomass concentration estimated from the OUR test with the ME parameter set shows some overestimated values, sometimes reaching over 80% of the sludge total COD concentration (Figure 1).

The f_{CV} values considered for each organic particulate component (Equation 1 and Table 4) allow the COD-based model variables to be converted into volatile suspended solids concentrations. The assumed ($f_{CV,XH}$ and $f_{CV,XE}$) and calibrated ($f_{CV,XU}$) values resulted in reasonable predictions of the accumulation of volatile suspended solids in the mixed liquor without any further model adjustment using the GM parameter set (Figure 1). The f_{CV} ratio in conventional activated sludge processes typically is about 1.50 g COD/g VSS, which is somewhat higher than that typically considered for heterotrophic biomass ($f_{CV,XH}$ of 1.42). For that reason the f_{CV} of the two other main particulate COD components found in activated sludge ($f_{CV,XU}$ and $f_{CV,XE}$) are usually considered to have a higher value (e.g. 1.60) in ASM models. The work of Ramdani et al. (2012) with a synthetic feed containing no $X_{U,Inf}$, suggests that a value for $f_{CV,XE}$ of 1.48 leads to reasonable predictions of the volatile suspended solids of a mixed liquor where the relative amounts of biomass and endogenous residue change over time in terms of COD fractions. The higher f_{CV} value for X_E compared to that of X_H indicates that the former is constituted of organics that are more reduced than those in X_H, as pointed out by Ramdani et al. (2012). The value of $f_{CV,XU}$=1.60 g COD/g VSS calibrated in this study, together with values of 1.42 for heterotrophic biomass and 1.48 for endogenous residue, appears to result in adequate predictions of sludge production over the wide range of operating conditions tested (F/M, SRT).

The $f_{XU,Inf}$ value of 0.16 g $X_{U,Inf}$/g COD_{Inf} obtained from calibration may appear high when compared to typical values of 0.11 - 0.13 for raw wastewaters and to even lower values expected for settled wastewaters (Melcer et al., 2003). Much higher values of $f_{XU,Inf}$ are sometimes obtained by difference between total COD and other COD fractions measured by combination of physical-chemical and respirometric methods, instead of model calibration as in the current study (Spérandio et al., 2001; Roeleveld and van Loosdrecht, 2002; Lu et al., 2010). The value for settled wastewater estimated in this study corresponds to a raw influent $f_{XU,Inf}$ of approximatly 0.20 g $X_{U,Inf}$/g COD_{Inf}. This is based on using a plant wide model developed for this WRRF with a primary settler TSS removal efficiency of 40% (average efficiency for 8 years of historical data). This corresponds to the typical value for raw wastewaters from the plant modelling experience at Cemagref, France (Stricker et al., 2010). The high proportion of agroindustry load (50% of BOD) and the co-thickening of the waste activated sludge in the primary settler at this WRRF may have contributed to increase this fraction. However, a higher $f_{XU,inf}$ value is supported by the high measured influent COD:BOD ratio of 2.4 ± 0.5 g COD/g BOD (n=69), compared to the typical range of 1.9 to 2.0 for settled wastewater, as this is an indication of a higher than typical $f_{XU,Inf}$ (Melcer et al., 2003). The good fit between simulated and measured nitrate concentrations (see the *Nutrient content* section, Figure 4) also supports the high $f_{XU,Inf}$ value obtained, as the latter affects the influent nitrogen available to be nitrified.

These calibration results indicate that the influent characterization and the default GM parameter set were adequate and sufficient to model organic and inorganic sludge production as well as heterotrophic biomass, whereas the ME parameters led to larger errors, mainly regarding the heterotrophic biomass and during famine periods.

3.2.2 Goodness of fit and sensitivity analysis

The relationship between the calibrated model results and the experimental values for each studied parameter sets are showed in Figure 2. The linear relationships were very good ($r^2 > 0.97$) for mixed liquor COD and solids but were poor for the heterotrophic biomass, as the experimental values often were higher than the simulated values, mainly during growth periods (Figure 1). This experimental overestimation was attributed to the presence of residual substrate in the sludge during OUR measurements, resulting in some respiration attributable to substrate oxidation in addition to that of endogenous respiration and nitrification as considered in Equation 3. Relationships were increased from $r^2 = 0.12$ to 0.39 and from $r^2 = 0.39$ to 0.62 for the ME and the GM results, respectively, by considering only the measurements made during famine periods (Figure 2). This indicates that the OUR test for sludge is quite sensitive to the presence of residual substrate, even for low loaded systems operating at long SRTs. A longer sludge pre-aeration period prior to the determination of the heterotrophic biomass should minimize the oxidation of substrate during the respirometric measurements and reduce this experimental error.

The sensitivity of the initial model values on the adjustment of $f_{XU,Inf}$ for the calibration of sludge production was assessed by initializing the model at different SRTs. The initial SRT affected the intercept of the linear relationship between model results and experimental values for mixed liquor COD and solids, whereas the adjustments of $f_{XU,Inf}$ affected the slope of the linear relationship (Figure 2). The very good linear relationships between model results and experimental values using an intercept value of zero suggested that the initial model values were adequate.

The root mean squared errors (RMSE) between simulated and measured sludge TSS (n=1620; TSS probe), TSS (n=25), COD (n=22) and X_H (n=20) concentrations was calculated to evaluate the goodness of fit of the model and the sensitivity of the fitting parameter ($f_{XU,Inf}$) over these measurements. The RMSE in the calibrated model ($f_{XU,Inf} = 0.16$ $X_{U,Inf}$/g COD_{Inf}) were of 440 vs 370 mg TSS/L (TSS probe), 380 vs 310 TSS/L, 480 vs 460 mg COD/L and 1280 vs 520 mg COD_{XH}/L with the ME vs the GM parameter sets (Figure 3). The RMSE were systematically lower with the GM than with the ME parameters, especially for the heterotrophic biomass. This comparison of the goodness of fit between the two models gives a better insight of the simulation accuracy when compared to the linear relationship presented above.

The parameter value sensitivity of $f_{XU,Inf}$ with regards to the RMSE is shown in Figure 3 by the rate of change of the slope of each of the curves. Sensitivity was highest to the sludge COD, followed by TSS, while sensitivity to X_H was low. The only RMSE minima obtained with the ME parameters was with the probe TSS measurements, at a value of 0.166 g $X_{U,Inf}$/g COD_{Inf}. Values greater than 0.170 g $X_{U,Inf}$/g COD_{Inf} resulted in an error with the influent fractionation due to an overestimation of the P associated with particulate matter. The minimum RMSE for sludge TSS and COD measurements was obtained with $f_{XU,Inf}$ values between 0.153 and 0.163 g $X_{U,Inf}$/g COD_{Inf} with the GM parameters. No minima was reached for the X_H measurements with both parameter sets, as the RMSE was still decreasing with $f_{XU,Inf}$ values as low as 0.10 g $X_{U,Inf}$/g COD_{Inf}, leading to large errors in the calibration of total sludge production (TSS and COD).

Recent studies show that sludge production is better predicted by models considering a slow degradation of $X_{U,Inf}$ and X_E for systems operating at higher than typical sludge retention times (Spérandio et al., 2013). This degradation was included in the current study by considering a first order degradation rate for these sludge components ($b_{XU} = b_{XE} = 0.007\ d^{-1}$). The goodness of fit of the simulation results could not be improved by considering the slow degradation of the two unbiodegradable components. It resulted, as expected, in a higher $f_{XU,Inf}$ calibration value, but also in higher RMSE values. Moreover, the $f_{XU,Inf}$ value required to reach the minimal RMSE could not be reached, as increasing $f_{XU,Inf}$ resulted in a particulate phosphorus mass in the influent inconsistent with the influent fractionation.

The goodness of fit between the measurements and the model predictions obtained by linear regression and RMSE showed that the GM parameter set better fitted the experimental data than the ME parameter set. The slow degradation of $X_{U,Inf}$ and X_E did not improve the simulation results in any case, even after a 70 d of solids retention.

The model parameters from both the Metcalf and Eddy and the General ASDM models were adequate to predict sludge production as a whole for SRTs ranging from 4 to beyond 30 days, which covers most of the activated sludge WRRFs in operation. When a more accurate prediction of the heterotrophic fraction of the mixed liquor and the solids reduction associated with its endogenous respiration is needed, care should be taken when using the Metcalf and Eddy parameters since it led to a much higher heterotrophic fraction and a greater solids decrease. The prediction of the volatile sludge destruction and biogas production during anaerobic digestion of low SRT excess activated sludge could also be overestimated when sludge composition is estimated with the ME parameters, while the GM should lead to more accurate values.

3.3 Nutrient content

The measured nitrogen and phosphorus concentrations in the mixed liquor are shown in Figure 4. The TKN and TP accumulated in the mixed liquor during growth periods. During famine periods, TKN decreased while TP remained constant and biomass decay resulted in the buildup of soluble nitrogen (NH_4 and NO_3), as released ammonia from decay was nitrified to nitrates, and of orthophosphate. The average mixed liquor N and P content during the whole experiment were 0.101±0.011 g N/g VSS (n=22) and 0.028±0.002 g P/g VSS (n=19), respectively (expressed in terms of COD in Table 5). The following sections present the determination of the nutrient content in mixed liquor organic particulate components using the GM as a basis, as the latter resulted in a better fit of the experimental data in previous sections.

3.3.1 X_H and X_E nutrient content

The N and P content in X_E was calibrated to 0.030 g N/g COD_{XE} and 0.035 g P/g COD_{XE} by fitting the soluble nutrients build-up during famine periods (Figure 4, dotted lines). Since the nutrient release during these periods was solely attributed to biomass decay, and thus only a function of the nutrient content of X_H and X_E, the nutrient content in X_E was calibrated prior to the nutrient content in $X_{U,Inf}$.

The N and P content in X_H and the N content in X_E calibrated in this study were in accordance with results obtained from previous work from a lab-scale experiment on synthetic sludge without X_U content (Table 5; Ramdani et al., 2012). However, the P content of X_E, appeared significantly higher in this study with a value of 0.035 instead of 0.003 g P/g COD. Simulations with the latter value resulted in almost twice as much P release during famine periods compared to observed results.

The precipitation of some released o-PO_4 may have led to the high P content in X_E obtained. Some possible causes of precipitation would be the accumulation of coagulants in the MBR mixed liquor because of the co-thickening of the waste activated sludge containing some ferric sulfate coagulant in the primary settler at the studied WRRF and to the discharge in the sewer of alum sludge from the drinking water treatment plant. Precipitation as calcium phosphates was unlikely under the experimental conditions tested, as the mixed liquor pH was dropping from about 7.0 to 4.9 because of nitrification during the three famine periods. If, however, precipitation occurred, the calibrated $i_{P,XE}$ should be lowered concurrently with an increase in $i_{P,XU}$. The P content in X_E and X_U presented here is thus to be interpreted with care.

The N content of the endogenous residue appears to be significantly lower than that of the heterotrophic biomass (Table 5). This has some impact on the proportion of ammonia released from decay and the associated oxygen demand for its nitrification. As the N content in X_H and X_E appear to differ, Equation 4 was used in place of Equation 3. That accounts for the difference in the nitrogen content of these components by considering their respective i_N values. The estimated OUR due to nitrification obtained from the 4.33 term was increased by 16% through considering the separate i_N values found in this study. For convenience, the nutrient content of X_E and other COD components in the influent is often assumed to be equal to or less than that of X_H (Table 5) as this detailed fractionation is unsensitive for many model applications but it becomes important for some modeling tasks such as quantifying nutrient assimilation and modelling for very low effluent nutrient limits (Roeleveld and van Loosdrecht, 2002; Stricker et al., 2010).

$$\mathrm{OUR}_{t} = \left(4.33 \cdot \left(\mathrm{i}_{\mathrm{N,XH}} - f \cdot \mathrm{i}_{\mathrm{N,XE}}\right) + \left(1 - f\right)\right) \cdot b_{\mathrm{H}} \cdot X_{\mathrm{H}} \cdot \frac{e^{(-b_{\mathrm{H}})\,t/24}}{24} \qquad \text{(Eq. 4)}$$

The composition of the endogenous residue is unknown as this component cannot be isolated from others but its nutrient content and degree of oxidation provides some insight. Microbial cell walls contain glycan strands cross-linked by peptides chains, causing resistance to biodegradation but the endogenous residue is unlikely to be composed of these nitrogen-rich peptidoglycans as all of the amino acids appear to be recycled at relatively high rates during cell turnover (Appels et al., 2008; Park and Uehara, 2008). A significant portion of the dissolved organic nitrogen in oceans may be remaining peptidoglycan, which contains amide nitrogen (McCarthy et al., 1998). The cell membrane P content is high due to its content in phospholipids. It is likely that the cell membrane will be degraded last during decay since it strengthens the envelop that protects the living cell from its surroundings. Cellular material with high N content such as RNA, is known to be degraded during endogenous metabolism (Dawes and Ribbons, 1964). These observations could explain the lower N and higher P content in the cell residue compared to that in heterotrophic biomass. It is interesting to note that DNA and RNA, which account for about 24% of cell mass, exert a quite low theoretical oxygen demand (ThOD; 0.76 and 0.63 g COD/g for cytosine and guanine, respectively) when compared to that of heterotrophic biomass ($f_{CV,XH}$ = 1.42 g COD/g VSS). Their biodegradation during endogenous respiration could thus explain in part the higher f_{CV} found for endogenous residue (1.48 g COD/g VSS). Fats, such as phospholipids, exert a much higher ThOD (i.e. 2.03 g COD/g for $C_8H_6O_2$) and their resistance to biodegradation would contribute to increase both the f_{CV} and the P content of endogenous residue (Henze et al., 2008).

3.3.2 $X_{U,Inf}$ nutrient content

The N and P content in X_U was calibrated to 0.100 g N/g COD_{XU} and 0.008 g P/g COD_{XU} by fitting the nutrient concentration in the accumulated sludge. The N content obtained for $X_{U,Inf}$ is higher than reported values in Table 5 but in the range of 0.02 to 0.10 g N/g COD reported by Hulsbeek et al. (2002). The P content obtained for $X_{U,Inf}$ is lower than reported values in Table 5.

These high and low N and P content for $X_{U,Inf}$ might be attributed to the high proportion of agro-industrial loading at the WRRF studied, mainly from milk and dairy transformation industries. The nutrient content in X_U is dependent on the source and type of wastewater but the values obtained in this study are similar to the few ones reported. Values of $i_{N,XU}$= 0.12 and $i_{P,XU}$=0.009 would have resulted in excess total nitrogen and phosphorus in the influent, which confirms that the calibrated values are in accordance with the wastewater fractionation.

4. CONCLUSION

This work on the determination of sludge production parameters and nutrient content of unbiodegradable organic sludge components allowed to conclude the following:

- The General ASDM model default parameter set for Y_H, b_H and f was adequate and sufficient to model organic sludge production as well as heterotrophic biomass over a wide range of operating conditions.
- The goodness of fit between the measurements and the model predictions indicates that the General ASDM parameter set better fitted the experimental data than the Metcalf and Eddy parameter set.
- The COD to VSS ratio values of 1.60, 1.42 and 1.48 g COD/g VSS for the unbiodegradable particulate organics from the influent ($X_{U,Inf}$), for the heterotrophic biomass (X_H) and for the endogenous residue (X_E) , respectively, resulted in an appropriate conversion of the COD-based model into volatile suspended solids over a wide range of operating conditions.
- A slow degradation rate over a period of 70 days for $X_{U,Inf}$ and the X_E did not improve model predictions and led to some fractionation discrepancies.

- The nutrient content of X_E and $X_{U,Inf}$ were determined by model calibration to be 0.03 and 0.10 g N/g COD and 0.035 and 0.008 g P/g COD, respectively. These results are in accordance with literature data, except for the high P content found in the endogenous residue, possibly due to the precipitation of orthophosphates with coagulants in the accumulated sludge.

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Period	Condition	Days	Duration (d)
A	Growth	0-17	18
B	Famine	18-23	6
C	Growth	24-31	8
D	Famine	32-52	21
E	Growth	53-59	7
F	Famine	60-69	10

Parameter	Unit	Value
Volume aeration tank	L	4600
Volume external MBR tank	L	2300
Feed flowrate	m^3/h	1.1
Hydraulic retention time	h	6.3
Dissolved oxygen	mg O_2/L	3
Recycle to feed ratio	m^3/m^3	5
Membrane area	m^2	90
Membrane permeation flux	$L \cdot m^{-2} \cdot h^{-1}$	15

Table3

Parameter	Symbol	Unit	Parameter set[a]	
			ME	GM[b]
Heterotrophic biomass true yield	Y_H	g COD/g COD	0.570	0.666
Endogenous respiration rate	b_H	d^{-1}	0.12	0.24
Endogenous residue fraction	f	g COD/g COD	0.15	0.20

[a]ME: Metcalf and Eddy (2003) activated sludge model; GM: General ASDM model of BioWin 3.1

[b]Values converted from the death-regeneration approach to the endogenous respiration approach (Dold, 2007)

Parameter	**Symbol**	**Unit**	**Avg**	***SD***	**n**
Total suspended solids	TSS	mg/L	106	21	70
Volatile suspended solids	VSS	mg/L	86	20	70
Chemical oxygen demand	COD	mg O_2/L	301	79	70
5 d biochemical oxygen demand	BOD	mg O_2/L	136	52	69
Total Kjeldahl nitrogen	TKN	mg N/L	25.5	6.4	13
Ammonia	NH_4	mg N/L	11.2	3.6	13
Nitrate	NO_3	mg N/L	0.9	1.4	13
Nitrite	NO_2	mg N/L	0.3	0.3	13
Total phosphorus	TP	mg P/L	3.6	1.4	14
Orthophosphate	o-PO_4	mg P/L	0.9	0.3	13
Heterotrophic fraction	$f_{XH,Inf}$	g COD/g COD	0.10	0.04	9
Unbiodegradable soluble fraction[a]	$f_{SU,Inf}$	g COD/g COD	0.066	0.038	12
Particulate COD/VSS ratio	f_{CV}	g COD/g VSS	1.61	0.26	10
f_{CV} for X_H fraction[b]	$f_{CV,XH}$	g COD/g VSS	1.42	n.a.	n.a.
f_{CV} for X_E fraction[b]	$f_{CV,XE}$	g COD/g VSS	1.48	n.a.	n.a.
f_{CV} for X_U fraction	$f_{CV,XU}$	g COD/g VSS	1.60	n.a.	n.a.
f_{CV} for particulate substrate[c]	$f_{CV,XB}$	g COD/g VSS	1.74	n.a	n.a.
Ammonia fraction of TKN	f_{NH4}	g N/g TKN	0.446	0.12	13
o-PO_4 fraction of TP	f_{SP}	g P/g P	0.283	0.16	13

[a] $S_{U,Inf}$ estimated from pilot-scale MBR effluent COD
[b] From previous work (Ramdani et al., 2012)
[c] From Equation 2

Table5
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	COD:VSS ratio		N content		P content		Reference
	f_{CV}	Source[a]	i_N	Source[a]	i_P	Source[a]	
	g COD/g VSS	-	g N/g COD	-	g P/g COD	-	
Mixed liquor	1.47±0.07	M	0.068 ± 0.008	M	0.019 ± 0.002	M	This study
X_H	1.42	A	0.070	A	0.022	A	Biowin 3.1[b]
	n/a	n/a	0.086	A	n/a	n/a	ASM1, Henze et al., 2000
	n/a	n/a	0.070	A	0.020	A	ASM2, Henze et al., 2000
	1.48	A	0.077	A	0.017	C	Stricker et al., 2010
	1.42	M	0.087	M	0.016	M	Ramdani et al., 2012[c]
	1.42	A	0.087	A	0.016	A	This study
X_E	1.60	A	0.070	A	0.022	A	Biowin 3.1
	n/a	n/a	0.060	A	n/a	n/a	ASM1, Henze et al., 2000
	n/a	n/a	0.030	A	0.010	A	ASM2, Henze et al., 2000
	1.48	A	0.058	C	0.013	C	Stricker et al., 2010
	1.48	M	0.030	M	0.003	M	Ramdani et al., 2012[c]
	1.48	A	0.030	C	0.035	C	This study
X_U	1.60	A	0.035	A	0.011	A	Biowin 3.1
	1.48	A	0.058	C	0.013	C	Stricker et al., 2010
	1.60	C	0.100	C	0.008	C	This study

[a] A: Assumed; C: Calibrated; M: Measured; n/a: not applicable
[b] Assumed biomass composition of $C_{41.3}H_{64.6}O_{18.8}N_{7.04}P$
[c] Measured biomass and endogenous residue composition of $C_{58.8}H_{73.5}O_{22.1}N_{11.6}P$ and $C_{312.5}H_{404.1}O_{153.8}N_{21.3}P$

Fig1

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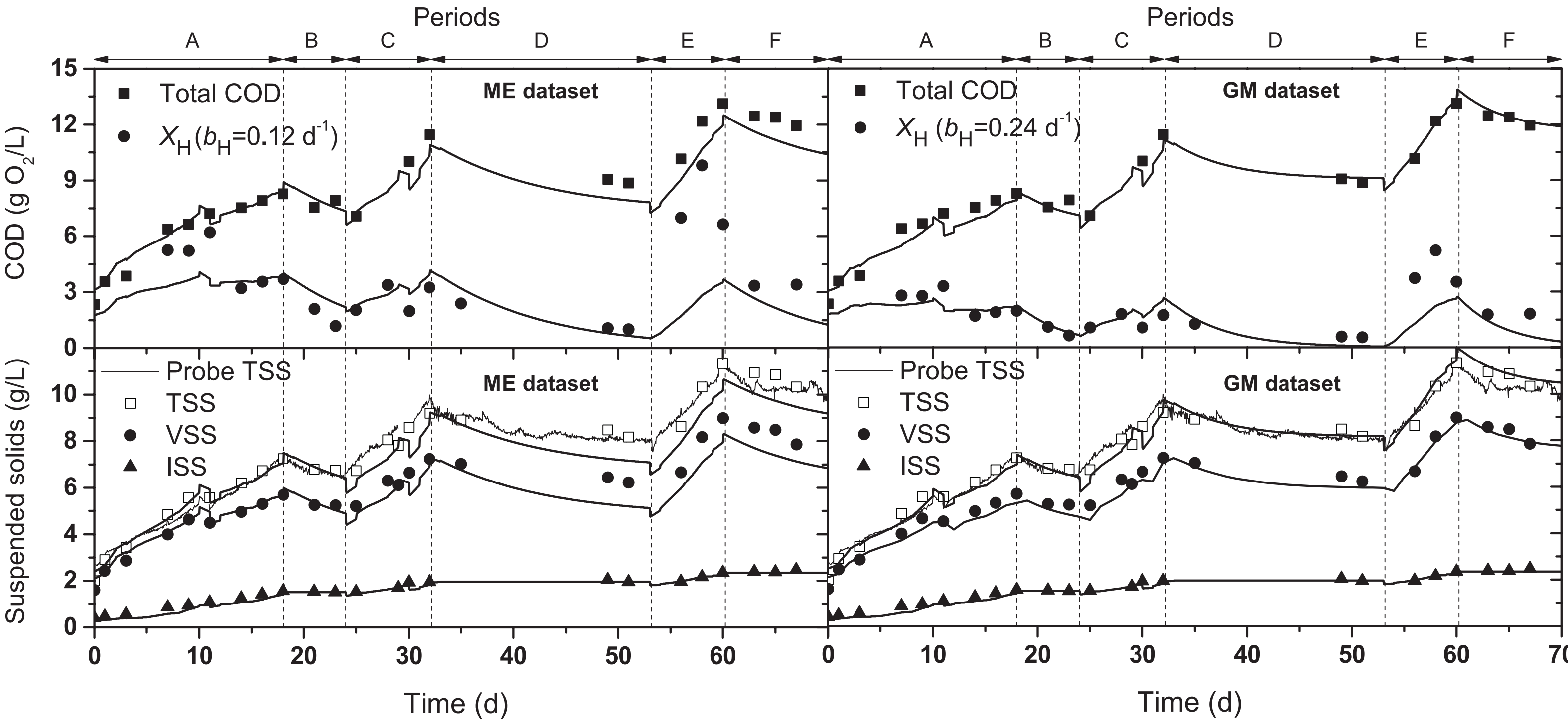

Fig2

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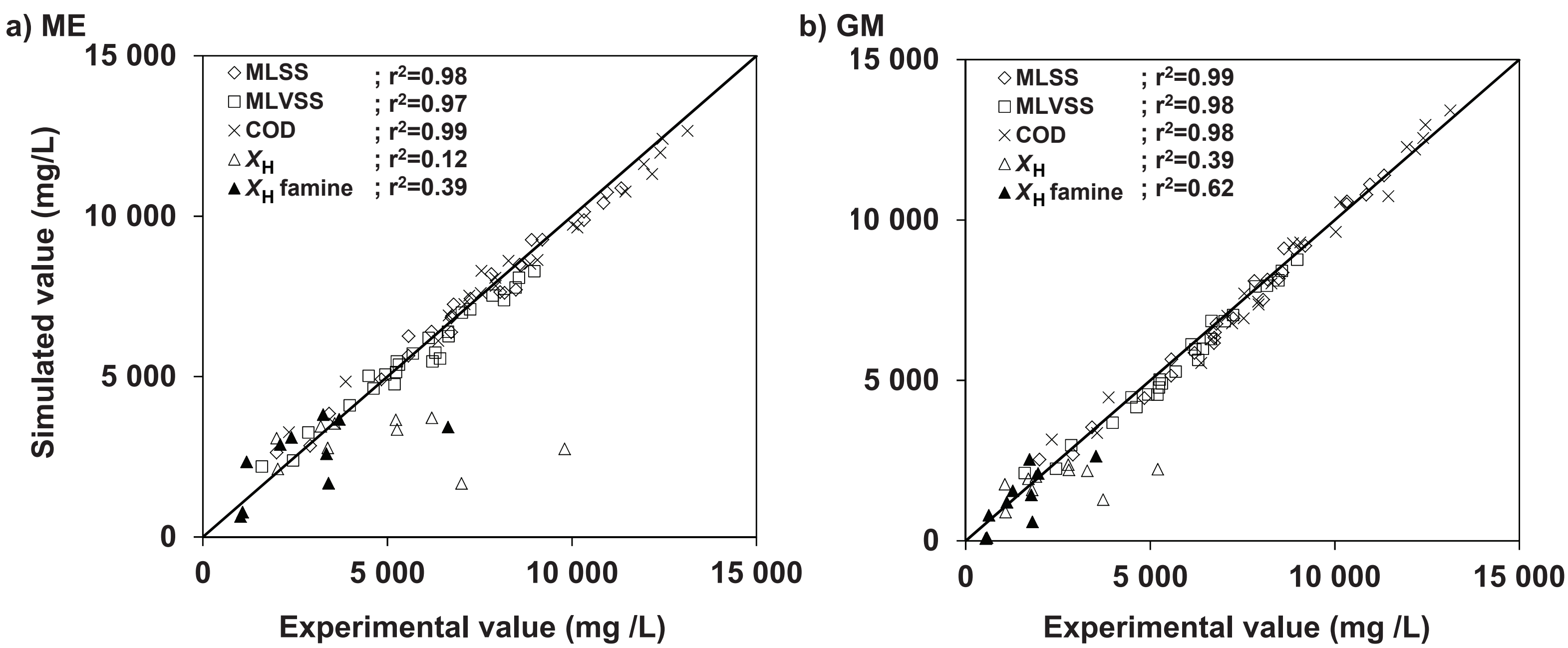

Fig3

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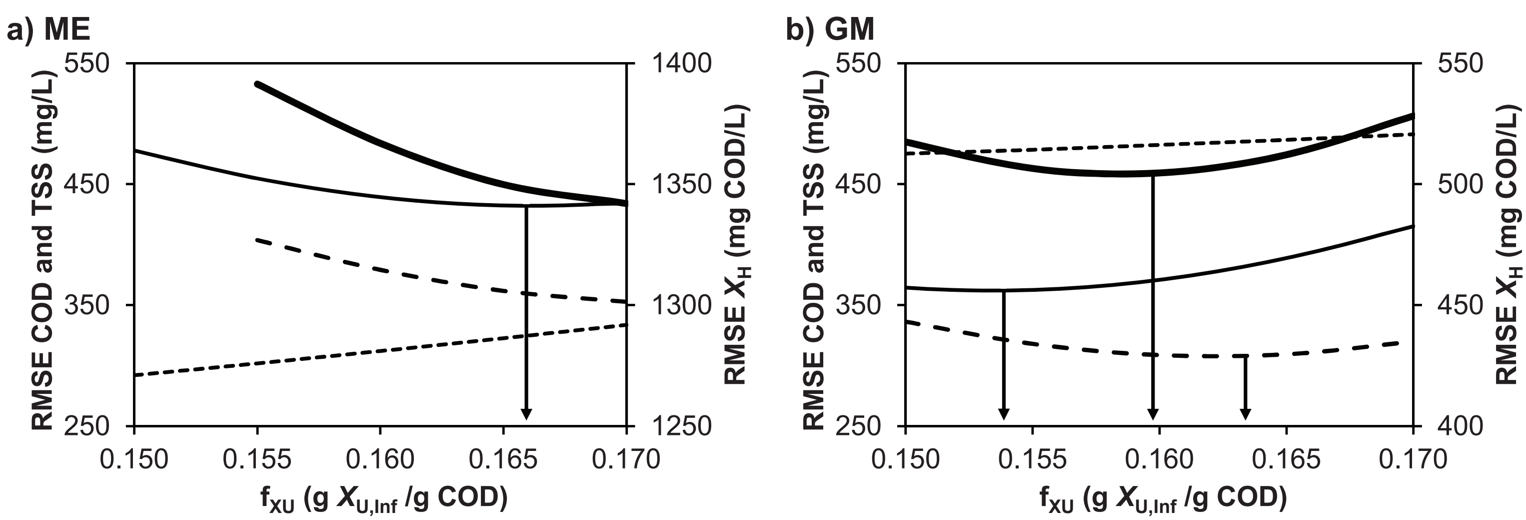

Fig4

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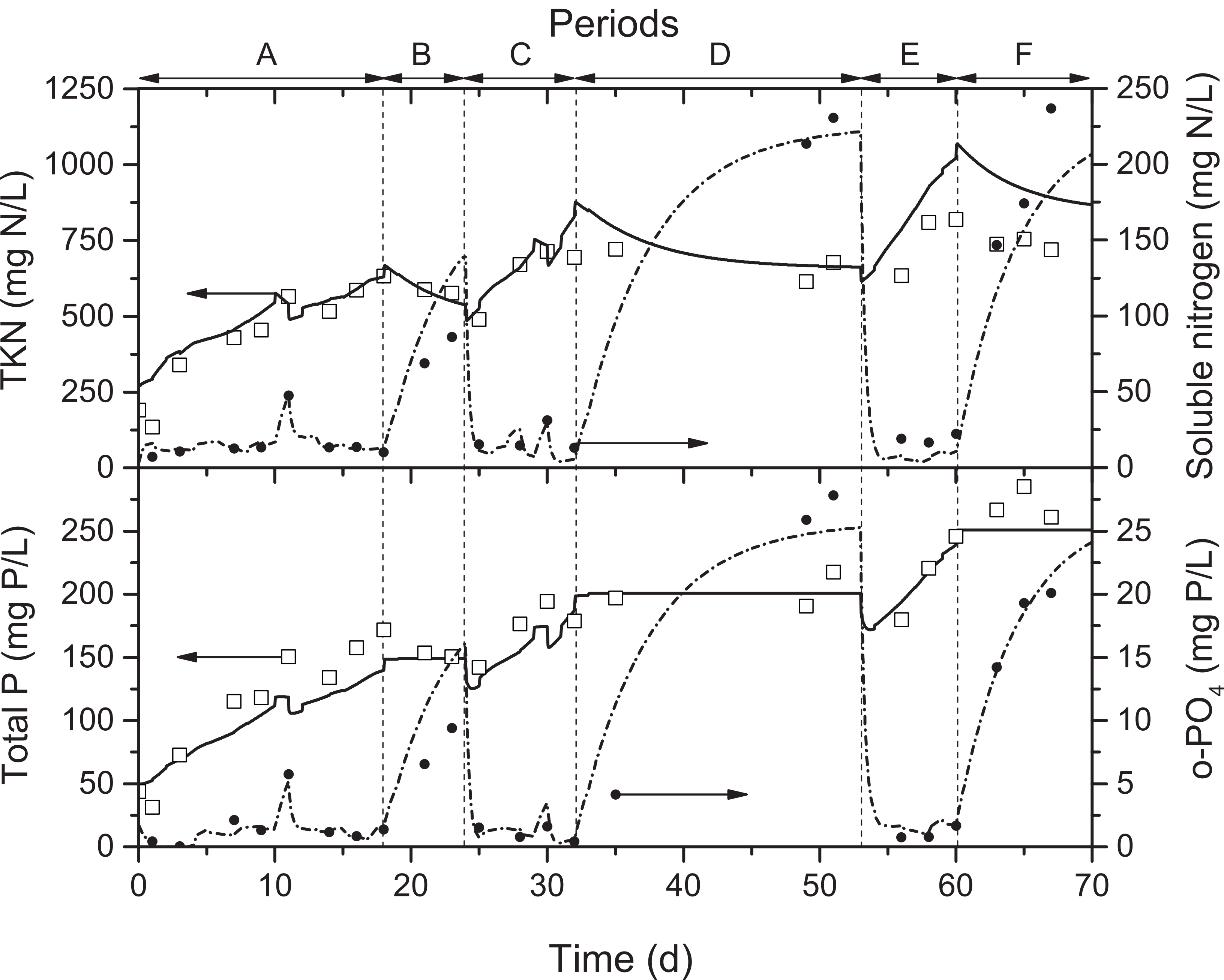

Table 1. Pilot-scale MBR experiment setup

Table 2. Pilot-scale MBR characteristics and operating conditions

Table 3. Parameter sets compared for model calibration

Table 4. Influent characterization

Table 5. COD:VSS ratio and nitrogen and phosphorus content of particulate sludge components

Figure 1. Measured (symbols and thin lines) and simulated (thick lines) COD, heterotrophic heterotrophic biomass (X_H; Eq. 3) and suspended solids (TSS and VSS) accumulation during growth (A, C and E) and famine (B, D and F) periods for the ME (left hand side) and GM (right hand side) datasets for $f_{XU,Inf}$ =0.16.

Figure 2. Measured and simulated sludge COD, solids and heterotrophic biomass concentrations with the a) ME and b) GM parameter sets.

Figure 3. Root mean squared error for the a) ME and b) GM parameter sets for COD (bold line), probe TSS (line), TSS (long dashes) and X_H (short dashes) with corresponding minima (arrows).

Figure 4. Measured (dots) and simulated (lines) nitrogen and phosphorus accumulation in particulate sludge components during growth periods (A, C and E), and release in solution during famine periods (B, D and F).