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Performance and microbial behavior of submerged membrane bioreactor at extremely low sludge ages

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ABSTRACT

The study investigated the effect of sludge age on substrate utilization kinetics, soluble microbial product generation, and composition of the microbial community sustained in a *superfast submerged membrane bioreactor* (*SS*MBR). For this purpose, a laboratory-scale membrane bioreactors (MBR) unit was operated at steady state, with three different sludge ages in extremely low range of 0.5–2.0 d, and a hydraulic retention time of 8.0 h. Substrate feeding was adjusted to 220–250 mg COD/L and involved a synthetic mixture representing the readily biodegradable COD fraction in domestic sewage. The MBR operation at sludge age of 1.0 d was duplicated with acetate feeding as the sole organic carbon source. Under different operating conditions, *SS*MBR was able to secure complete removal of available soluble/readily biodegradable substrate, with a residual microbial product level as low as 20–30 mg COD/L, partly retained and accumulated in reactor volume. Phylogenic analysis based on polymerase chain reactions-denaturing gradient gel electrophoresis analysis indicated that selected sludge ages affected the composition of microbial community. Lower sludge ages selected a community characterized by faster rates for microbial growth. Results confirmed the existence of a functional relationship between variable process kinetics and changes in the microbial community structure, even for slight variations that can be inflicted on the culture history while operating superfast MBR systems.

Keywords: Respirometry; Process kinetics; Microbial composition; Soluble microbial products; Superfast membrane bioreactor

1. Introduction

Coupling the biological reactor with membrane filtration has been one of the most ingenious innovations ever attempted for biological treatment of wastewaters. It has the potential of reshaping the activated sludge process, which still depends on an almost a century-old technology of gravity settling which basically controls system design. In fact, gravity settling dictates the level of biomass concentration that can be maintained in the reactor. Traditionally, the operating sludge age

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is selected implicitly for conditioning the biomass for upgrading its settling properties so that it may be separated from the liquid stream and re-circulated back to the reactor. In this context, *membrane bioreactors* (MBRs), which rely on *membrane filtration* of biomass instead of secondary clarifiers, offer a major step forward for the activated process [1,2]; they can be operated with lower reactor volume and smaller footprint based on their ability to separate and filter treated effluent from biomass independently from its settling properties.

So far, this innovative feature has been mostly interpreted as a means for sustaining a higher biomass level and for operating the MBR at high sludge ages as compared to conventional activated sludge process [3–6]. However, the current practice with the MBR should be challenged with the following basic concern: do we indeed require a higher sludge age and a similarly high biomass concentration? Information reported in the literature indicates otherwise: in fact, a number of studies also investigated the performance of the MBR system operated at sludge ages in the low range of 2.0–5.0 d [7–10]. The results obtained indicated effective COD removal, opened the way for a new dimension of the system as *superfast MBR* that would be sustained at sludge ages below 2.0 d, capable of completely removing of soluble COD and conservation of the particulate fraction of available substrate for energy recovery. This novel system was successfully tested with readily biodegradable substrate [11].

Accumulated information on the biodegradation characteristics of organic substrate in wastewaters provide a clear indication that the removal of biodegradable COD fractions in domestic sewage and most industrial wastewaters only requires a small fraction of the hydraulic retention time (HRT) and the sludge age adopted in the design of activated sludge systems [12,13]. In fact, exploring the possibility of maintaining a substantially higher biomass concentration with high sludge ages is closely related to empirical experiences and habits underlining the traditional practice. It is evident that once the constraint of settling is no longer a problem, the operating flexibility of the MBR should be exploited to lower the sludge age and the reactor volume to a level prescribed by biodegradation requirements alone. Furthermore, the MBR system should be operated with as low a biomass concentration as possible to facilitate and enhance membrane filtration.

Studies conducted on the treatment efficiency of MBR systems generally report effective COD removal rates above 95% [14–17]. These results should be evaluated considering the fact that wastewater characterization now differentiates soluble and particulate fractions of the biodegradable COD [18,19]. Therefore, MBR operation should essentially be concerned with the soluble biodegradable COD compounds of the influent, which need to be utilized and fully removed from the system before the liquid stream leaves the reactor. Particulate COD components become immediately entrapped and enmeshed with the biomass so that they can be totally removed from the system with excess sludge. In conventional activated sludge systems, the sludge is allowed to stay in the reactor. The potential energy of the particulate biodegradable matter is burned within the reactor with supply of excessive amount of oxygen requiring additional energy input. This energy is practically wasted for endogenous respiration in a much larger reactor volume operated at much higher sludge ages. Especially in systems such as the MBR where biomass settling is not a problem, there is possibility to recover this energy from sludge under anaerobic conditions and the opportunity of using a much smaller reactor volume devoted solely to the treatment of the liquid stream. Therefore, assessment of achievable limits for soluble COD removal is a prerequisite of practical importance for MBR systems operated at low sludge ages.

Membrane fouling is quite an important issue in most MBR applications. Studies generally focus on a number of factors including membrane configuration, wastewater characteristics, and metabolic activities of the biomass, especially related to the generation of extracellular polymeric substances (EPS) and soluble microbial products (SMP) [2,20]. The relationship between the sludge age and the EPS/SMP levels has been investigated in MBR systems operated at sludge ages higher than 10 d and yielded conflicting results [21–24]. The effect of extremely low sludge ages in superfast MBR operation on the extent of SMP formation with a potential impact on membrane fouling is yet to be clarified.

Only a few studies were conducted using MBR operation at low sludge ages. They generally reported high effluent quality and effective COD removal [7–9] also provided significant information on the composition of the microbial community sustained under fast growing conditions. The available results, although interesting, were quite general in nature and did not fully address fundamental issues such as system stability, extent of SMP generation, kinetics of substrate utilization, etc. associated with MBR operation at extremely low sludge ages.

In this context, the study was a continued effort in testing the performance of submerged MBR operated at extremely low sludge ages, as part of a comprehensive

research program devoted to explore the microbial mechanisms related to *superfast MBRs* [11,25]. Specifically, it investigated the effect of sludge age in the range of 0.5–2.0 d on (i) the process kinetics of substrate removal, (ii) the extent of SMP generation, and (iii) the composition of the microbial community.

2. Materials and methods

2.1. Experimental design rationale

In accordance with the objectives of the study, the experiments were essentially designed to evaluate the performance and the microbial mechanisms of a submerged MBR at extremely low sludge ages. For this purpose, a laboratory-scale submerged MBR unit was operated at steady state, at three different sludge ages of 0.5, 1.0, and 2.0 d, compatible with the concept of superfast MBR. The HRT was adjusted to 8.0 h in all experiments. A synthetic substrate mixture, suggested to characterize the readily biodegradable COD content of domestic sewage [18,26,27] was used as the organic carbon source. The MBR experiment conducted at sludge age of 1.0 d was duplicated using acetate as the sole organic carbon source, for comparative evaluation of system performance. The substrate concentration in the influent stream of the MBR unit was adjusted to 220–250 mg COD/L. Each MBR operation was started with an initial biomass seeding taken from a parallel fill and draw reactor continuously operated at steady state at a sludge age of 2.0 d and with similar substrate feeding. Major characteristics of MBR operation under different conditions are outlined in Table 1.

MBR performance for each specific operating condition was assessed by means of COD, EPS, and SMP measurements both in the permeate—system effluent—and in the reactor volume. Characteristics of each MBR operation at steady state were further investigated by means of respirometric analysis, assessing oxygen uptake rate (OUR) profiles in batch reactors started with acclimated biomass from the corresponding MBR operation. Parallel batch experiments were also conducted to monitor substrate/COD utilization as well as substrate storage and generation of intracellular biopolymers. Experimental data collected in batch experiments were evaluated by modeling; mainly for the assessment of process kinetics related to substrate utilization mechanisms. Biomass samples were also tested and evaluated for microbial community diversity under different operating conditions by means of molecular techniques.

2.2. Reactor setup

The laboratory-scale submerged MBR consisted of a cylindrical plexiglass reactor with an operating volume of 3 L, coupled with a hollow fiber Zee Weed*1 (GE) (Fig. 1). The surface area of PVDF membrane fiber was 0.1 m^2 with a nominal pore size of 0.04 μm. Membranes were operated with a transmembrane pressure (TMP) range of 0.1–0.5 bar at a flux of 3.75 L/m^2 h.

The submerged MBR was fed with synthetic substrate (synthetic substrate mixture and acetate) stored in a 60-L PES feed tank. The reactor was aerated constantly and stirred with a magnetic stirrer to provide aeration and mixing. MBR system was operated under constant flux conditions at which permeate was withdrawn at a constant flow by operating a permeate pump (P2). Permeate was collected in a 60-L PES permeate tank, which also served as backwash tank during the backwash operation. The membrane module was backwashed once every day by employing 15 min contact with a pH 12 NaOH solution followed by one cycle of normal operation and 15 min contact with a pH 2.5 H_2SO_4 solution. Liquid level (set-point 1⁄4 18.4 cm) in the MBR was controlled by the operation (on/off) of the wastewater feed pump (P1).

Data acquisition and control of the system was maintained via processing signals from a pH/temperature probe and a dissolved oxygen probe connected to a multimeter (Hach-LANGE sc1000, Germany). TMP was calculated from the pressure readings.

Table 1
Major characteristics of MBR operation under different conditions

Substrate Type	Sludge Age (days)	Influent COD (mg/L)	S_0/X_0 (mg COD/mg VSS)
Substrate mixture			
Run S1	0.5	220	0.72
Run S2	1.0	235	0.57
Run S3	2.0	255	0.45
Acetate			
Run A4	1.0	250	0.50

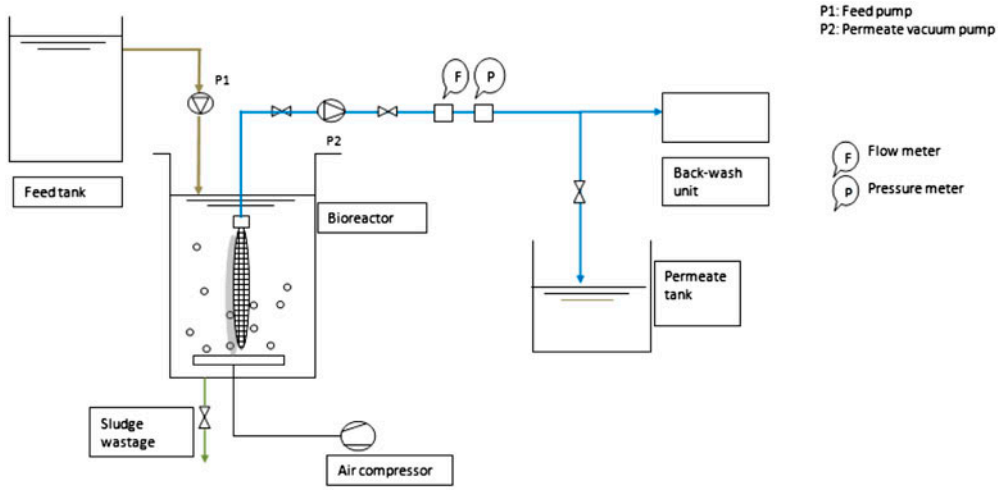

Fig. 1. Schematic flow diagram of the laboratory-scale submerged MBR system.

2.3. Respirometric analyses

The respirometric procedure for the assessment of substrate and storage products consisted of running 2-L volume batch reactors. Tests were first started with the biomass alone to obtain the initial endogenous respiration level. After observing the endogenous decay level, substrate was added into the reactor, using the same S_0/X_0 ratio as in the MBR system at selected SRT and HRT.

OUR profiles were performed using a respirometer (RA-1000; Manotherm). During the respirometric tests, the DO concentration and the temperature were kept above 2 mg/L and at 20°C, respectively. Possible interference of ammonia consumption for nitrification was avoided by adding nitrification inhibitor (Formula 2533TM, Hach Co.).

2.4. Analytical measurements

For COD measurements, samples taken from the reactor were filtered using 0.45 μm PVDF syringe filters and directly analyzed for COD according to ISO 6060 methodology [26]. Mixed liquor suspended solids and volatile suspended solids concentrations were determined using the methodology described in Standard Methods [28].

The *polyhydroxyalkanoates* (PHA) content of the washed (K–P buffer solution) and freeze-dried biomass were subjected to extraction, hydrolization, and esterification in a mixture of hydrochloric acid, 1-propanol, and dichloroethane at 100°C [29]. The resulting organic phase was extracted with water to remove free acids. The propylesters were analyzed by gas chromatograph (Agilent 6890 N) according to methodology also described by Beun et al. [30]. Acetate samples were filtered through 0.22 μm cartridge filters and analyzed by gas chromatography using a flame-ionization detector with a HP-FFAP capillary column (0.53 mm, 30 m, 1 μm, Agilent).

Soluble protein and carbohydrate fractions were analyzed after employing a physicochemical (sodium hydroxide–formaldehyde) extraction method as described by Li et al. [31]. Samples in 5 mL were taken from the reactor mixed liquor, placed in Eppendorf tubes, and centrifuged for 10 min at 4°C at 4,000× g. The supernatant was transferred to a sterile tube and re-centrifuged for 20 min at 4°C at 13,200× g and the supernatant was analyzed for soluble protein and carbohydrate. Protein and carbohydrate analyses were conducted by applying the Lowry and phenol-sulfuric acid methods [32], respectively. All samples were run in duplicates.

2.5. Molecular studies

Microbial composition was investigated for the purpose of assessing changes in the microbial community structure induced by culture history—i.e. different sludge age levels using Denaturing Gradient Gel Electrophoresis (DGGE). Observed results were used for similarity analysis based on clustering of the band patterns.

Molecular analyses were carried out in order to identify different sludge age and substrates caused a change in the microbial community in terms of population dynamics. DGGE, followed by similarity analyses based on clustering of the band patterns, was performed to investigate microbial composition.

2.6. DNA extraction

Samples were taken in duplicates from each reactor operated at steady state and stored in the freezer until the DNA was extracted, which was done using FastDNA® spin kit for soil (MP Biomedicals LLC, Illkirch, France) following the manufacturer's instructions. DNA quantity was measured by Qubit fluorometer (Invitrogen, Carlsbad, CA, USA).

2.7. Polymerase chain reaction (PCR) amplification

Eubacterial 16S rRNA genes were amplified using VfGC-Vr primer pair in replicate PCRs. Each PCR mixture consisted of 10 ng of DNA template, 250 mM of each dNTPs, 2.5 mM $MgCl_2$, and 1 U of Taq polymerase. Finally, adequate amount of sterile water was added to the PCR mixtures to reach final reaction volume of 25 μL. Following thermal cycling conditions were applied for all PCR reactions; pre-incubation at 94˚C for 5 min; 33 cycles of 30 s at 94˚C for denaturation; 30 s at 55˚C for annealing; 60 s at 72˚C for extension; and incubation at 72˚C for 10 min. PCR products were checked by 1.5% agarose gel electrophoresis.

2.8. Denaturing gradient gel electrophoresis

DGGE was performed on a D-Code apparatus (Bio-Rad, Hercules, USA) using 10% (wt/vol) polyacrylamide gels (37.5:1; acrylamide: bisacrylamide) with a denaturing gradient ranging from 35 to 65% (100% denaturant contains 40% [vol/vol] formamide and 7 M urea in 1xTAE buffer). Approximately, 20–25 μL of PCR products with loading dye (2:1 vol/vol) were loaded on the gels, electrophoresis was performed at constant 60˚C at 70 V for 960 min in 1xTAE buffer. Gels were stained with SYBR Green I (1:5,000) and visualized under UV using GelDoc imaging system (Bio-Rad, CA, USA).

2.9. Analysis of DGGE patterns

BioNumerics software 5.1 [32] was used to analyze the DGGE patterns. Similarity matrices and dendograms of DGGE profiles were created using the Dice correlation coefficient (band-based). Band patterns were calculated using unweighted pair-group method arithmetic average (UPGMA). Structural diversities of microbial communities were inspected using the Shannon diversity index (*H*).

Shannon diversity index (*H*) accounts for both abundance and evenness of present species, providing more information on community composition and it was calculated on the basis of the bands on the gel tracks, using densitometric curves.

3. Results and discussion

3.1. MBR performance

System performance was monitored for the submerged MBR unit sustained at steady state at three different SRT levels of 2.0, 1.0, and 0.5 d, all operated with a HRT of 8.0 h. Substrate feeding was adjusted to 220–250 mg COD/L, slightly increasing at higher SRTs, mainly to test the limits of substrate removal potential at extremely high rate operation. As previously mentioned, the selection of synthetic mixture and acetate as organic substrates was also closely related to testing the performance limits of superfast MBR, which will be primarily affected by soluble/readily biodegradable COD; in fact, other COD fractions with larger particle size than the effective filtration size of the membrane will be physically entrapped and/or removed by adsorption.

Assessment of system performance was based on COD measurements both from the effluent/permeate and the reactor volume during a period of at least 15 d at steady-state operation. Observed COD profiles remained quite stable as illustrated in Fig. 2: for the synthetic mixture, the influent COD was consistently reduced down to the range of 10–20 mg COD/L in the effluent/permeate stream, depending on the selected SRT (Fig. 2(a)). Similarly, acetate feeding in the MBR operation at SRT of 1.0 d, yielded an average permeate COD of 20 mg/L (Fig. 2(b)), while acetate was completely depleted.

It should be noted that one of the major functions of the superfast MBR is to provide partial substrate removal, mainly limited with soluble COD fractions so that the particulate components be entrapped with biomass and processed for energy recovery. This study is only used acetate as the sole organic carbon source, which was fully utilized; hence, the excess sludge would be slightly higher than the level associated with conventional biological reactors sustained at higher sludge ages, but it would contain a higher fraction of active biomass, mainly because operation at extremely low sludge age would significantly reduce the relative magnitude of particulate metabolic products.

3.2. Substrate utilization kinetics

Assessment of process kinetics for relevant substrate utilization mechanisms essentially consisted of calibrating the model adopted for the study with the OUR profiles representing MBR operations at steady

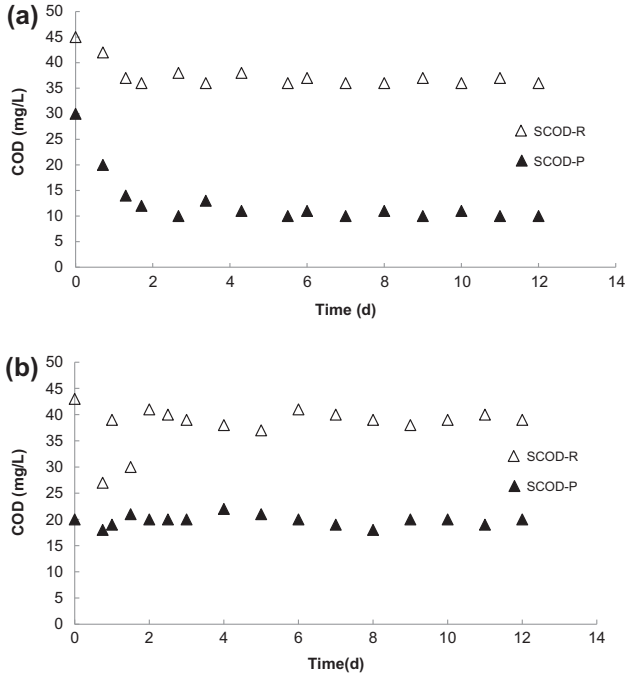

Fig. 2. Observed COD profile in the permeate (P) and soluble COD profile in the reactor (R) volume of the MBR operated at a sludge age of 1.0 d and fed with (a) substrate mixture; (b) acetate.

state under different conditions. Model calibrations were also verified with the experimental data reflecting substrate COD and intracellular storage biopolymers. This procedure, commonly adopted in similar modeling studies [6,33,34], identified numerical values of the model coefficients and state variables substrate and biomass fractions for each MBR operation. This way, the impact of significant increase in the substrate concentration could be interpreted in terms of changes in the values of respective model coefficients.

3.3. Model structure

The adopted model was structured to remain equally applicable for the two substrates utilized in the experimental studies. Metabolism of acetate, a simple/readily biodegradable substrate, involves conversion to *polyhydroxybutyrate* (PHB) together with direct utilization for microbial growth. Conversely, the basic template defining organic carbon removal in ASM1 —*Activated Sludge Model No.1*—modified for endogenous respiration [12,35,36] would be suitable for the synthetic substrate mixture, with only two different readily biodegradable COD components, S_{S1} and S_{S2}, because the mixture could not be characterized with single biodegradation mechanism, as will be explained in detail in the next section. Therefore, the selected model structure reflected the basic backbone of ASGM —*Activated Sludge Model for Growth and Storage* —successfully implemented in many similar studies [37,38].

The model components consisted of two readily biodegradable COD, S_{S1} and S_{S2} (only S_S in case of acetate); internally stored biopolymers, X_{STO}; two active heterotrophic biomass fractions, X_{H1} and X_{H2}; and finally dissolved oxygen, S_O, the basic parameter for the evaluation of the OUR profiles. In accordance with the selected components, the model provided mathematical description of five biochemical processes: direct microbial growth on S_{S1} and S_{S2}; storage of S_S (acetate) as $X_{STO;}$ secondary growth on X_{STO}; and endogenous respiration of X_H. Soluble and particulate microbial products S_P and X_P were also accounted for as part of endogenous respiration with the simplifying assumption of decay-associated processes adopted in many similar studies [33,34,39]. Matrix representation for parts of the model implemented for the substrate mixture and acetate are given in Table 2. Rate expressions and basic stoichiometry were defined in a way compatible with previous activated sludge models, ASM1 and ASM3. Switching functions were omitted in the process rate expressions because S_O and nutrients were supplied in excess in all experiments.

It should be noted that OUR profiles have been extensively used as the sole experimental tool/data for model calibration and simulation of organic carbon removal systems. They are estimated to be reliable, mainly because they are able to produce statistically identifiable results.

In fact, OUR profiles yield identifiable model coefficients used for the kinetics of organic carbon removal, as in this study. Conclusive evidence has been presented on this subject by Insel et al. [40] in a detailed analysis of the aerobic hydrolysis: Basically, OUR is a biodegradation fingerprint and different parts of the OUR profile exhibit variable sensitivity for different model coefficients; so each model coefficient is determined in the corresponding OUR region, where the profile is most sensitive. Detailed information on this approach is also presented in the literature [33,40].

Recent studies also give details of the parameter identifiability procedure to be implemented in similar evaluations [38,41]: The procedure basically involves the use of the UNCSIM module proposed by Brun et al. [42] used for the calculation of *collinearity indices* in order to define the best identifiable parameter subsets. It also shows that storage kinetics was also identifiable when the data was considered together with the OUR profiles.

Table 2
Matrix representation of the adopted model structure

Processes	Components								Rate equations
	$S_{S1}(S_{AC})^*$	S_{S2}	X_{H1}	X_{H2}	X_{STO}	S_P	X_P	S_{O2}	
Growth of X_{H1}	$-\frac{1}{Y_H}$		1					$-\frac{1-Y_H}{Y_H}$	$\hat{\mu}_{H1}\left(\frac{S_{S1}}{K_{S1}+S_{S1}}\right)X_{H1}$
Growth of X_{H2}		$-\frac{1}{Y_H}$		1				$-\frac{1-Y_H}{Y_H}$	$\hat{\mu}_{H2}\left(\frac{S_{S2}}{K_{S2}+S_{S2}}\right)X_{H2}$
Aerobic storage of S_{AC}	−1				Y_{STO}			$-(1-Y_{STO})$	$k_{STO}\left(\frac{S_{S1}}{K_{S1}+S_{S1}}\right)X_{H1}$
Growth on X_{STO}			1		$-\frac{1}{Y_H}$			$-\frac{1-Y_H}{Y_H}$	$\hat{\mu}_{STO}\left(\frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H}\right)X_H$
Endogenous decay			−1	−1		f_{ES}	f_{EX}	$-(1-f_{ES}-f_{EX})$	$b_H X_H$
Respiration of X_{STO}					−1			−1	$b_{STO} X_{STO}$
Parameters	COD	COD	cell COD	COD	COD	COD	COD	O_2	

*S_{S1} defines S_{AC} in the modeling of acetate utilization.

AQUASIM, a frequently used simulation program, was utilized for model calibration and evaluation based on experimental data collected in this study [43]. Model calibration was implemented by means of an iterative calibration protocol, involving manual calibration of model components in each iteration step and fitting all the model outputs on real-time data [44]. The model outputs were found to be sensitive to all selected model coefficients.

3.4. Model calibration for the substrate mixture

In the experiments conducted with the substrate mixture, no appreciable substrate storage could be observed. Accordingly, the selected model was implemented excluding components and processes related to storage. The calibration exercise simultaneously utilized OUR and COD profiles obtained in batch reactors, characterizing continuous MBR operation under different conditions. Model calibration yielded the most suited values for the model coefficients defining process stoichiometry and kinetics for each experimental set. Figs. 2 and 3 illustrate interpretation of the OUR and COD data by modeling, and show the close fit between the experimental profiles and the model simulation using calibrated model coefficients and state variable listed in Table 3.

The first significant outcome of the evaluation was that it was not possible to calibrate the model for interpreting the fate of the substrate mixture by means of a single biodegradation component and process. It required two active biomass fractions X_{H1} and X_{H2} each separately utilizing different fractions, S_{S1} and S_{S2} of the substrate mixture with kinetics. As shown in Table 3, model calibration could also distinguish substrate fractions utilized by X_{H1} and X_{H2}; apparently, S_{S1}, identified in the range of 150–180 mg/L for

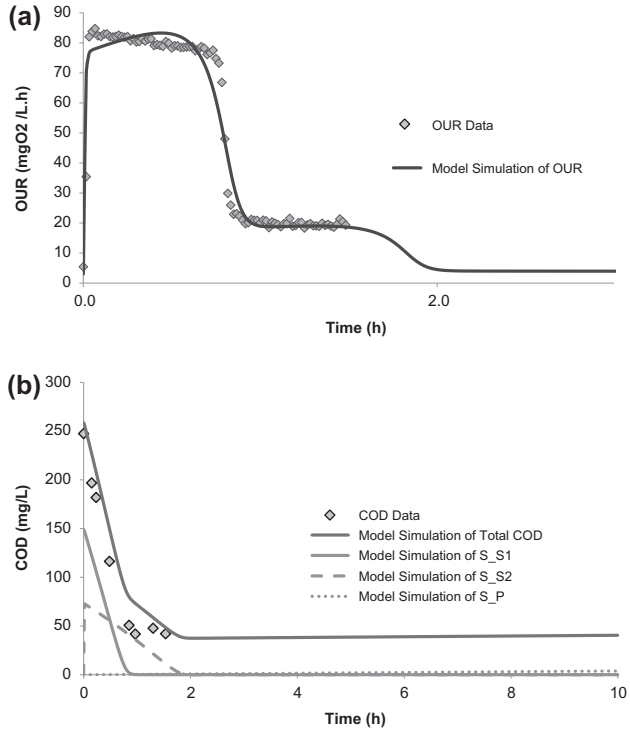

Fig. 3. Model calibration of the (a) OUR profile and (b) COD profile for the MBR system operated at a sludge age of 0.5 d and fed with 220 mgCOD/L substrate mixture.

MBR operation at three different sludge ages, covered *volatile fatty acids* and *ethanol* and S_{S2}, similarly assessed as 70–75 mg/L, included the remaining fractions of *glutamic acid* and *glucose*. The second major observation was the clear indication that the kinetics of substrate utilization was significantly altered when the MBR was adjusted to different sludge age levels, at approximately the same substrate loading. In fact,

Table 3
Model assessment of biodegradation kinetics and state variables

Parameters	Unit		Substrate mixture			Acetate
			Sludge Age			
			0.5 d	1.0 d	2.0 d	1.0 d
Model coefficients						
Maximum growth rate for X_{H1}	$\hat{\mu}_{H1}$	1/d	9.3	7.7	6.0	4
Half saturation coefficient for X_{H1}	K_{S1}	mg COD/L	10	13	20	2
Maximum storage rate of PHB by X_{H1}	k_{STO}	1/d	–	–	–	4.3
Half saturation coefficient for storage	K_{STO}	mg COD/L	–	–	–	0.3
Maximum growth rate on PHB for X_{H1}	$\hat{\mu}_{STO}$	1/d	–	–	–	3.9
Maximum growth rate for X_{H2}	$\hat{\mu}_{H2}$	1/d	7.3	3.7	3.0	–
Half saturation constant for X_{H2}	K_{S2}	mg COD/L	3	3	3	–
Yield coefficient	Y_H	g cell COD/g COD	0.66	0.66	0.66	0.66
Endogenous decay rate	b_H, b_{STO}	1/d	0.22	0.22	0.22	0.22
Fraction of biomass converted to S_P	f_{ES}	–	0.08	0.08	0.08	0.08
Fraction of biomass converted to X_P	f_{EX}	–	0.12	0.12	0.12	0.12
State variables						
Heterotrophic biomass component	X_{H1}	mg COD/L	330	380	420	400
Heterotrophic biomass component	X_{H2}	mg COD/L	77	90	90	–
Initial substrate	S_{ST}	mg COD/L	220	235	255	250
Substrate fraction	S_{S1}	mg COD/L	150	165	180	–
Substrate fraction	S_{S2}	mg COD/L	70	70	75	–
S_{S1}/S_{ST}		mg COD/mg COD	0.68	0.70	0.71	–
Initial storage pool	X_{STO1}	mg COD/L	–	–	–	15

the magnitude of $\hat{\mu}_{H1}$ escalated for lower sludge ages: it gradually increased from 6.0/d at sludge age 2.0 to 7.7/d and 9.3/d when the sludge age was reduced to 1.0 and 0.5 d, respectively. A similar increase was also observed at a slower pace—in the range of 3.0–7.3/d—for $\hat{\mu}_{H2}$. The increase was quite significant when the $\hat{\mu}_{H2}$ value increased from 3.7/d at sludge age of 1.0 d to 7.3/d when the sludge age was further lowered to 0.5 d. Microbial growth on carbohydrate fractions of the substrate always remained lower under different conditions of MBR operation (Fig. 4).

3.5. Model calibration for acetate

Calibration of the experimental data obtained from MBR operation at sludge age of 1.0 d with acetate feeding as the sole organic carbon sources indicated a single microbial community with substantially lower $\hat{\mu}_H$ level of 4.0/d. This is quite understandable as a fraction of acetate diverted to intracellular storage of PHB, aside from direct microbial growth. Ciggin et al. [41] similarly reported a low $\hat{\mu}_H$ value of 2.5/d for sequencing batch reactors operated at a sludge age of 2.0 d. This level is also in agreement with the 1.0–3.5/d range associated with utilization kinetics of acetate and other substrates with simultaneous storage [37,45,46].

As indicated in Table 3, PHB formation occurred at a similar maximum storage rate, k_{STO} of 4.3/d under the selected operating conditions, starting from a pool of 15 mg COD/L to around 85 mg COD/L (Fig. 5(a)). This way, the PHB accumulation was observed 70 mg COD/L in the batch experiment, corresponding to around 28% of the initial acetate concentration. It shows that the level of observed storage stays much lower in MBR operation at extremely low sludge ages, compared with the range of 60–70% reported for higher sludge ages under pulse feeding [30,41,47]. The relative magnitude of storage compared to direct utilization for microbial growth was also similarly assessed by model simulation in Fig. 5(b).

3.6. Molecular analysis of microbial community

PCR-DGGE methodology was applied to determine the possible effect of sludge age (SRT) and type of substrate on the microbial community structure sustained in the superfast MBR. Four samples were collected for microbial community analyses after the reactors reached steady state. Three of the samples (S1, S2, and S3) were collected from the MBRs fed with readily biodegradable substrate mixture, operated at different sludge ages of 0.5, 1, and 2 d, respectively. The fourth sample (A4) was taken from the

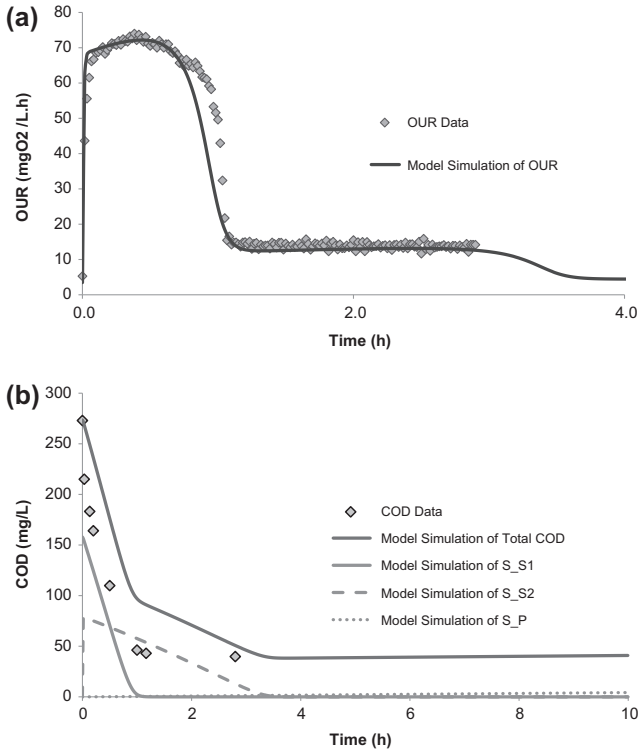

Fig. 4. Model calibration of the (a) OUR profile and (b) COD profile for the MBR system operated at a sludge age of 1 d and fed with 235 mgCOD/L substrate mixture.

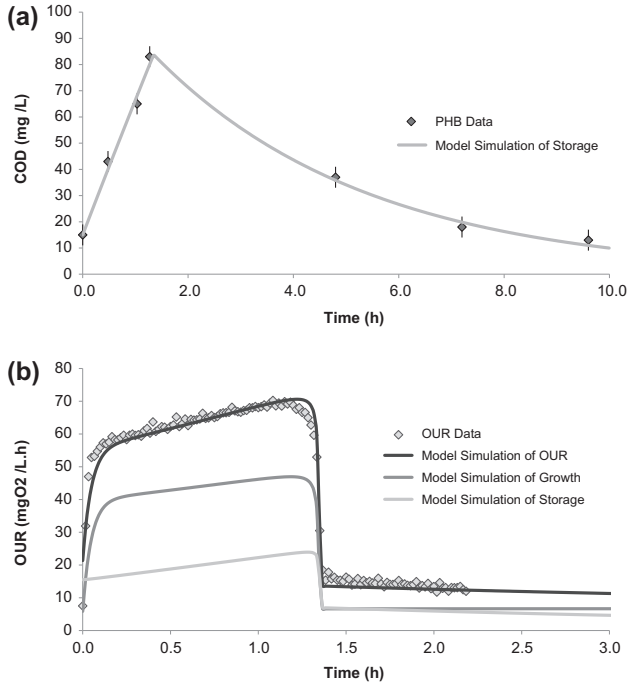

Fig. 5. Model calibration of the (a) PHB profile and (b) OUR profile operated for the MBR system at a sludge age of 1.0 d and fed with 250 mg/L acetate.

reactor fed with acetate operated at sludge age of 1.0 d. Identification of samples are given in Table 1.

Clustering of the profiles according to pairwise similarities were obtained using Dice coefficient, and band patterns were calculated using UPGMA. The similarity (Dice) coefficient was used as the main parameter in assessing changes in the microbial community induced by operation at different SRTs and substrate type. Dendograms obtained from the samples are illustrated in Fig. 6.

Analysis of the DGGE profiles in Fig. 6 showed significant differences in the structure of microbial communities at different operating conditions. As shown in Table 4, the dissimilarity between S1 and S2 was calculated as 22.22%. Similar differences were also obtained for S2 and S3 comparison (46.23%). Additionally, microbial communities of SRT 0.5 and 2.0 d activated sludge samples were differentiated by 53.82%.

Moreover, comparing structures of microbial communities sustained at SRT 2.0 d acclimated to different substrates (S2 vs A4) resulted in 61.29% dissimilarity, which shows the dramatic effect of the type of substrate on the microbial community composition.

Fig. 6. Clustering of DGGE profiles of samples with different SRT values (Scale bar represents % similarity).

Table 4
Dissimilarity of microbial culture in super fast MBR at different operating conditions

Samples	Dissimilarity (%)
Effect of sludge age	
S1 vs S2	22.22
S2 vs S3	46.23
S1 vs S3	53.82
Effect of substrate	
S2 vs A4	61.29

In addition to dissimilarity analysis, information obtained from DGGE was used to calculate the Shannon

Diversity Index (H), Species Richness (S), and Evenness (E_H), mainly to provide additional information for exploring and comparing the structure of microbial community sustained under different conditions. Results, as shown in Table 5, indicated that the structure of A4 sample, fed with acetate, exerted the lowest diversity ($H = 0.982$). Since the readily biodegradable substrate mixture consisted of different compounds, it selects a more diverse community structure. Acetate, being a uniform substrate, selects a community that is more uniform and less diverse [34].

Additionally, Table 5 also shows the effect of sludge age on the diversity of the microbial community. It can be seen that increasing sludge age selects a more diverse community, which is a consequence of the ability of slower growing organisms to survive at higher sludge ages.

3.7. Evaluation of results

Experimental results, supplemented by respirometric evaluation of biodegradation characteristics and molecular analysis of biomass, provided significant and conclusive indications on the response of the microbial community, which essentially determined the observed performance of the submerged MBR.

The operation of the submerged MBR system was monitored for more than six months with feedings of two different readily biodegradable substrates at a range of extremely low sludge ages. Under all different operating conditions, the *superfast submerged* membrane bioreactor (*SS*MBR) fed with a synthetic substrate mixture was able to secure effective COD removal, keeping the effluent/permeate COD in the low range of 10–20 mg/L. In the parallel MBR operation fed with acetate, the effluent did not contain any detectable acetate level during the observation period, leading to suggest that the permeate COD essentially consisted of residual soluble microbial products, while available biodegradable substrate was fully removed. This observation underlines the merit of superfast MBR in practical applications, since the soluble COD fraction in domestic sewage usually remains below the level 200 mg/L used in this study [18,19,48], indicating that the remaining particulate COD fractions will also be retained by simple entrapment and adsorption onto biomass in the reactor.

In fact, the *SSMBR* was observed to also retain a fraction of the residual microbial products of higher size that the effective filtration size of the membrane as the soluble COD in the reactor volume, S_T remained consistently higher that the permeate COD, S_E. This observation was also reported in a number of similar MBR studies, where the effective SMP fraction kept in the reactor volume, $S_{MBR,}$ was calculated based on the fact that it would accumulate the same way as biomass [11,49]:

$$S_{MBR} = S_T\left(\frac{HRT}{SRT}\right)$$

where HRT is the hydraulic retention time and SRT, the solids retention time—the sludge age—selected for MBR operation. The accurate level of residual SMP concentration, S_R, accounting for retention and accumulation in the reactor may be calculated as outlined in Table 6.

$$S_R = S_E + S_{SMP}$$

Data displayed in Table 6 show that the residual SMP detected in submerged MBR operation at extremely low SRT and HRT levels also remained quite low, i.e. in the range of 22–33 mg/L. The calculated low SMP range was also supported by similarly low levels of proteins and carbohydrates as commonly associated with SMPs and EPSs also measured in the reactor volume. As schematically represented in Fig. 7, the total amount of carbohydrates and proteins were measured as 2.60–5.14 mg/L and exhibited an increasing trend with higher sludge age values.

The superfast MBR operation sustained a fast-growing microbial community, which could fully

Table 5
Shannon diversity index (H), species richness (S) and evenness index (E_H) for microbial cultures sustained at super fast MBR operation at SRT of 2.0, 1.0 and 0.5 d

Sample	Shannon diversity ındex (H)	Species richness (S)	Evenness (E_H)
S1	1.156	18	0.064
S2	1.158	18	0.064
S3	1.208	26	0.046
A4	0.982	13	0.076

Table 6
SMP generation based on COD measurement in the reactor volume and permeate

Parameters	Sludge Age			
	Substrate Mixture			Acetate
	0.5 d	1.0 d	2.0 d	1.0 d
Influent COD, C_{S1}	220	235	255	250
Soluble COD in reactor volume, S_T	23	36	37	39
Effluent COD, S_E	15	10	20	20
Remaining SMP in the Reactor, S_{MBR}	15	12	6	13
$S_R = S_E + S_{MBR}$	30	22	26	33
Y_{SP}	0.12	0.09	0.11	0.13

utilize and deplete the available biodegradable substrate. The observations identified and highlighted two significant factors, i.e. type of organic substrate and culture history/sludge age, exerting a significant impact on the composition and metabolic response of the selected microbial culture: (i) the results showed that the concept of a single overall heterotrophic biomass adopted and implemented in activated sludge models may, at times, be an unacceptable approximation; in fact, the model evaluation in this study could identify two biomass components, specifically utilizing the volatile fatty acids and carbohydrates components in the readily biodegradable substrate mixture. A similar substrate differentiation and sequential utilization was reported while testing acetate and glucose for their storage yields [50]. Furthermore, the growth response of the microbial community for acetate utilization was substantially slower as compared with the substrate mixture. This result, supported by similar findings in the literature, suggests that protein synthesis mechanism of active heterotrophic biomass is sustained at much lower level when the external substrate is jointly utilized for storage along with direct microbial growth [41,51]; (ii) molecular analysis of biomass provided conclusive indication that even simple/readily biodegradable substrates would not select the same microbial culture, under different growth conditions. Consequently, kinetics of substrate utilization was changed as a microbial community with a different/altered composition was sustained at each MBR operation with a different sludge age. The results confirmed the existence of a functional relationship between variable process kinetics and changes in the microbial community structure, even for slight variations that can be inflicted on the culture history while operating superfast MBR systems.

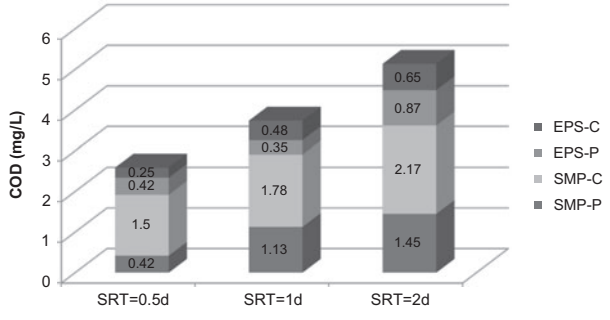

Fig. 7. Generation of carbohydrates and proteins as a function of sludge age.

4. Conclusions

This study provided conclusive experimental evidence on the validity of a novel mode of operation for submerged MBR at extremely low sludge ages. The *superfast MBR* was successfully tested to secure complete removal of 220–250 mg/L of soluble/readily biodegradable substrate, with a residual microbial product level as low as 20–30 mg COD/L, partly retained and accumulated in the reactor volume. The results suggested that, in practice, the superfast MBR would have the mechanistic potential of retaining the particulate COD fractions in domestic sewage and similar wastewaters through entrapment and adsorption onto biomass with no appreciable biodegradation while removing the soluble COD fractions in compliance with effluent limitations. This way, the major fraction of the energy potential in the wastewater would be conserved in the biomass/excess sludge, for energy generating processes (biogas production, etc.).

The results also challenged the current understanding of activated sludge systems with a heterotrophic biomass with uniform properties by means of conclusive experimental indications that both the composition and the kinetic characteristics of the microbial community exhibit significant changes as a function of

growth conditions, i.e. sludge age, even the narrow limits associated with *superfast MBR* operation.

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