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Effect of high loading on substrate utilization kinetics and microbial community structure in super fast submerged membrane bioreactor

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HIGHLIGHTS

- Full substrate removal with super fast membrane bioreactor at high loading.
- Permeate COD lower than 50 mg/L attributed to soluble microbial products.
- Entrapment of soluble products due to membrane effective filtration size (8–14 nm).
- Major changes in the composition of the microbial culture at high substrate loading.

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ABSTRACT

The study investigated the effect of high substrate loading on substrate utilization kinetics, and changes inflicted on the composition of the microbial community in a superfast submerged membrane bioreactor. Submerged MBR was sequentially fed with a substrate mixture and acetate; its performance was monitored at steady-state, at extremely low sludge age values of 2.0, 1.0 and 0.5 d, all adjusted to a single hydraulic retention time of 8.0 h. Each MBR run was repeated when substrate feeding was increased from 200 mg COD/L to 1000 mg COD/L. Substrate utilization kinetics was altered to significantly lower levels when the MBR was adjusted to higher substrate loadings. Molecular analysis of the biomass revealed that variable process kinetics could be correlated with parallel changes in the composition of the microbial community, mainly by a replacement mechanism, where newer species, better adapted to the new growth conditions, substituted others that are washed out from the system.

1. Introduction

Development of membrane bioreactor (MBR) has been a monumental step towards reshaping the classical activated sludge process; it eliminated major design constraints on sludge age (SRT) and biomass concentration, traditionally implemented for providing good settling properties for the biomass. While the initial practice was mainly focused on higher sludge ages, attempting to visualize the response of substantially higher biomass in the reactor volume (Masse et al., 2006; Laera et al., 2009), a number of studies also investigated MBR operation at low SRT levels in the range of 2.0–5.0 d (Harper et al., 2006; Duan et al., 2009). The reported results, while limited to the assessment of system performance indicating excellent chemical oxygen demand (COD) removal, helped to define a novel role for the system as *superfast membrane bioreactor* that would be operated below a sludge age of 2.0 d, with the ability for complete removal of soluble substrate, and conservation of particulate substrate for energy recovery. Recent studies conducted with readily biodegradable substrates indicated that *superfast MBR* exhibited stable operation at extremely low SRT range of 0.5–2.0 d, provided complete removal of available organic substrate and generated significantly low levels of soluble microbial products (SMPs) within the span of 15–27 mg COD/L (Teksoy Başaran et al., 2013).

The next question, not so far covered by current studies, is how the superfast MBR operation would withstand excessively higher substrate loadings. This question constitutes the major issue within the scope of this study and it is quite intriguing, not so much perhaps whether effective substrate removal will persist or

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not, but for the way in which the microbial community will respond to it. It is also quite relevant as it would reflect the potential of treating strong industrial wastewaters in practical application (Kutluay et al., 2007).

The first approach in clarifying the impact of high substrate loading is to explore the metabolic machinery that adjusts process kinetics for substrate utilization. This approach is likely to show the direction in which the process kinetics changes and this change, if applicable, is currently ascertained by modeling. Respirometric analysis now generates oxygen uptake rate (OUR) profiles that serve as biodegradation fingerprints associated with selected operating conditions. As applied in many similar studies, evaluation of the experimental data by an appropriate model yields numerical information on applicable process kinetics under different substrate levels (Karahan et al., 2008; Orhon and Sözen, 2012).

The second step is to assess whether adjustment/acclimation to a high level of substrate involves changes in the community composition. This is ascertained by molecular analysis of the community structure. Different methodologies were implemented for determination of microbial community structures of different activated sludge systems and natural systems, including fluorescence *in situ* hybridization (FISH), terminal restriction fragment length polymorphism (t-RFLP), ribosomal spacer analysis (RISA), analysis of 16S rRNA clone libraries, and polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) (Boon et al., 2002; Gilbride et al., 2006; Ye et al., 2011). The combination of PCR amplification of 16S rRNA genes with DGGE stands out among these tecniques. DGGE provides useful means to directly characterize bacterial populations within many samples, while retaining the possibility of a more proper genetic analysis by sequencing of specific bands, uncovering the presence of highly complex bacterial communities. Moreover, using the band intensity on the obtained DGGE pattern relative abundance of microbial species can also be determined (Murray et al., 1996). It has been shown that DGGE is a powerful tool for monitoring the diversity of particular microbial systems (McCaig et al., 2001). This technique has been used to distinguish microbial community composition (Muyzer et al., 1993), to observe population shifts (Ferris and Ward, 1997), and to follow the succession of bacterial populations over time (Simpson et al., 2000). Combining the two approaches – i.e. process kinetics by respirometry/modeling and community analysis by DGGE – has not been so far attempted in similar works. However it provides, as in this study, a meaningful assessment of the response of the microbial community acclimated to high substrate loading.

In this context, the main objective of the study was to evaluate the effect of high substrate loading on the behavior and performance of superfast submerged membrane bioreactor. This effect was primarily assessed on (i) COD removal efficiency, (ii) substrate utilization kinetics, and (iii) changes inflicted on the composition of the microbial community. For this purpose, the submerged MBR operation was monitored at steady-state, at extremely low SRT values of 2.0, 1.0 and 0.5 d, all adjusted to a single hydraulic retention time (HRT) of 8.0 h.

2. Methods

2.1. Basis of experimental design

Experiments were essentially designed to visualize and interpret the impact of a substantial increase in the influent substrate concentration on process kinetics and microbial diversity at different sludge ages in the range of 0.5–2.0 d, compatible with the concept of superfast MBR. For this purpose, a synthetic substrate mixture, suggested to characterize the readily biodegradable COD content of domestic sewage (Cokgor et al., 1998), was used as the organic carbon source. Acetate was also used as substrate in another set of experiments for comparison purposes.

The synthetic mixture and acetate were selected as organic substrates to test the limits of superfast MBR performance, mainly because soluble/readily biodegradable COD will be the only relevant fraction for MBR performance, since other COD fractions with larger particle size distribution (PSD) than the membrane will be physically removed by entrapment and adsorption.

The substrate concentration in the influent stream of the MBR unit was initially adjusted to 200 mg COD/L. Each MBR operation was repeated, when the substrate feeding was increased to 1000 mg COD/L. The MBR unit 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. The substrate feeding to the fill and draw reactor was also adjusted to 200 mg COD/L; it was gradually increased and sustained at 1000 mg COD/L to supply acclimated biomass to MBR experiments carried out with the same substrate concentration. MBR experiments with acetate feeding of 200 and 1000 mg COD/L were only conducted for SRT of 1.0 d. Major characteristics of MBR operation under different conditions are outlined in Table 1.

Aside from COD measurements in the permeate 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. 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 assessed for microbial community diversity under different operating conditions by means of molecular analysis, especially when the substrate loading was increased in the MBR operation for the two substrates tested in the experiments.

2.2. Experimental setup

2.2.1. MBR system

The lab-scale submerged MBR (Fig. 1) consisted of a cylindrical plexiglas reactor with an operating volume of 3 L, and was equipped with a hollow fiber Zee Weed*1 (GE) membrane module. The total membrane surface area of PVDF membrane fiber was 0.1 m^2 with a nominal pore size of 0.04 mm. 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 synthetic substrate was pumped into the bioreactor from a synthetic feed tank (60 L, PES). The bioreactor was aerated constantly and stirred with a magnetic stirrer to provide aeration

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	200	0.72
Run S2	1.0	200	0.57
Run S3	2.0	200	0.45
Run S4	0.5	1000	0.88
Run S5	1.0	1000	0.57
Run S6	2.0	1000	0.41
Acetate			
Run A7	1.0	200	0.50
Run A8	1.0	1000	0.50