Journal of Membrane Science 491 (2015) 99–109

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Application of dispersed and immobilized hydrolases for membrane fouling mitigation in anaerobic membrane bioreactors

Philip Chuen Yung Wong [a,b,*], Jia Yi Lee [a], Chee Wee Teo [a,b]

[a] School of Civil and Environmental Engineering, Nanyang Technological University, Singapore 639798, Singapore
[b] Singapore Membrane Technology Center, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, Singapore 637141, Singapore

ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form
14 May 2015
Accepted 16 May 2015
Available online 23 May 2015

Keywords:
Submerged anaerobic membrane bioreactor
Extracellular polymeric substances
Enzyme augmentation
Hydrolysis
Enzyme-immobilized membrane

ABSTRACT

Enhancing the hydrolysis of microbial macromolecules by supplementing exogenous hydrolases may improve membrane performance via structural disruptions of fouling layers and alterations to sludge characteristics. This was investigated by short batch filtration (< 1 h) and 30-day extended filtration experiments using laboratory-scale anaerobic membrane bioreactors. Crude hydrolases were either dispersed directly into the reactor, or immobilized onto microfiltration membranes. Under constant flux operation, dispersed enzymes consistently moderated increases in transmembrane pressures (TMPs) compared to the control setup. Immobilized hydrolases appeared effective in the short filtration test, but in the extended experiment, the pseudo-stable TMP was not significantly lower compared to the control TMP. With dispersed enzymes, the average TMP was almost 30% lower than the control value. This was associated with a 33% reduction in the protein content of the bulk extracellular polymeric substances, and a 45% reduction in the membrane cake density. Immobilized enzymes limited cake formation to a similar extent through hydrolysis at the base of the cake, but this was negated by the increase in gel resistance attributed to the hydrophobic attraction between the immobilization layer and proteinaceous hydrolysis products. Even as the dispersed hydrolases exhibited greater effectiveness under the conditions studied, there is scope for further enhancement in both approaches.

1. Introduction

The recent focus on energy conservation in sewage treatment [1] has directed a trend toward anaerobic processes, including the employment of anaerobic membrane bioreactors (AnMBRs). Although AnMBRs possess inherent advantages such as the elimination of outlays associated with oxygen provision and low sludge production, membrane fouling control and membrane cleaning still incur sizable expenses. For instance, approximately 47% of the operating cost of AnMBR is associated with gas scouring for fouling mitigation [2]. Several complementary or adjunctive fouling control strategies include supplementing with additives such as powdered and granular activated carbon [3,4], coagulants [5], and quorum quenchers [6]. These additives differ in their mechanistic functionalities. Powdered activated carbon adsorbs macromolecular and colloidal foulants [4], while granular activated carbon has additional membrane scouring ability [3]. Coagulants aggregate colloidal foulants and promote bioflocculation, hence reducing internal fouling and specific cake resistance [5,7]. Quorum quenchers degrade microbially produced autoinducers to inhibit intercellular communication during biofilm formation, thereby mitigating biofouling. One common characteristic amongst these additives is their passive participation in the removal of microbial macromolecules, which are one of the most significant contributors toward membrane fouling in membrane bioreactors [8,9].

Extracellular polymeric substances (EPS) and soluble microbial products (SMP) are largely constituted by microbial macromolecules. The former are heterogeneous polymeric matrices which envelope and confer adhesive properties to microbial cells [10,11], while the latter are by-products excreted during endogenous decay and substrate utilization [12]. Both comprise principally of proteins, carbohydrates, glycoproteins, and small quantities of lipids, humic substances, nucleic acids and uronic acids [13]. A different fouling control strategy involves utilizing enzymes with affinities toward macromolecules, thereby promoting their degradation. This is the basis behind membrane cleaning agents containing protease, amylase, cellulase and/or peroxidase [14,15], and underpins the supplementation of crude hydrolases to bioreactors to enhance solids hydrolysis [16,17]. The latter may be expedient for AnMBRs treating wastewaters containing particulates such as

* Corresponding author at: School of Civil and Environmental Engineering, Nanyang Technological University, Singapore 639798, Singapore.
Tel.: +65 6790 5269; fax: +65 6791 0676.
E-mail address: PCYWONG@ntu.edu.sg (P.C.Y. Wong).

http://dx.doi.org/10.1016/j.memsci.2015.05.022
0376-7388/

raw sewage, owing to net solids accumulation when the influent particulates are not promptly hydrolyzed [17,18]. Therefore, the dual benefits of mitigating membrane fouling and limiting solids buildup provide the rationale for an enzymatic approach.

Hydrolases can potentially alleviate membrane fouling by reducing the quantities, or through structural simplifications of macromolecular EPS and SMP. This is partially supported by demonstrations of the affinity of these enzymes towards EPS in sludge digestion [19,20] and biofilm degradation [21]. The benefits of supplementing dispersed or membrane-immobilized hydrolases on membrane filtration have also been reported. Hodgson et al. [22] ascribed the 2–3 fold reduction in filtration resistance following the addition of 100 mg/mL protease to a carbon-limited bacterial suspension to the enzymatic modification of EPS matrix. Howell and Velicangil [23] filtered 0.5% albumin and 0.5% hemoglobin separately using ultrafiltration membranes with 0.2–0.5% attached protease. Membrane flux were 25–78% higher compared to that of the unmodified membranes.

Presently, the costs of crude protease and amylase produced via submerged fermentation are around US$5/kg [24] and US$10/kg [25], respectively, which do not make their use overtly favorable. However, such costs are likely to be substantially moderated when solid-state fermentation of reusable substrates such as agro-industrial wastes [26,27] and wastewater sludge [28] becomes more widespread. For example, the manufacturing cost of cellulase can be reduced from US$20/kg to US$0.20/kg by switching from submerged to solid-state fermentation [27]. A similar extent in cost reduction for protease and amylase will improve the economic feasibility of the proposed strategy. The outlook is further enhanced by continued advancements in protein engineering and molecular farming techniques [29].

The objective of this research was to investigate the effects of enzyme augmentation on filtration performances of AnMBRs treating synthetic sewage. Supplementation in the form of dispersed and membrane-immobilized hydrolases was examined and contrasted. Batch filtration tests were performed under supra-critical flux, while protracted continuous filtration was conducted under a moderate flux to assess the effectiveness of these hydrolases as fouling control agents. Apart from assessing filterability using transmembrane pressure (TMP), the compositions of membrane foulants were analyzed to deduce fouling mitigation mechanisms.

2. Materials and methods

2.1. Laboratory scale AnMBRs

Batch and extended filtration experiments were performed using two submerged AnMBRs with working volumes of 0.7 L and 5 L, respectively. The schematic of the latter is shown in Fig. 1. The setup for the smaller reactor was similar except for the absence of computer control and feeding apparatus. For both AnMBRs, headspace biogas was recirculated at 1.5 L/min to provide adequate mixing and to reduce solids deposition on the membrane surfaces. The gas flow translated to superficial velocities of 20 m/h and 4.7 m/h for the smaller and larger bioreactors, respectively. Flat-sheet polyvinylidene fluoride membranes (HVLP, Millipore, USA) with a nominal pore size of 0.45 μm were installed in two custom-made membrane modules (Ying Kwang, Singapore) with effective membrane areas of 196 cm^2 and 0.05 m^2. These membranes were pre-wetted and washed with distilled water to remove any antimicrobial preservatives before use.

Seed sludge was obtained from an anaerobic digester treating municipal sludge (Ulu Pandan Water Reclamation Plant, Singapore). The 5 L AnMBR was fed with synthetic wastewater constituted by twice diluted standard sewage (OECD 303 A), and 250 mg/L of dog food (ALPO, Purina, USA) grinded to less than 2 mm. ALPO dog food was used as the particulate organic component because of its compositional similarity with primary sludge [30]. The soluble and particulate chemical oxygen demand (COD) of the feed were 150 mg/L and 400 ± 50 mg/L, respectively, giving a total COD of 550 ± 50 mg/L. Sludge employed in the batch experiments was cultivated for one month with the same feed in a fed-batch manner in a 2 L carboy (1.12 kg COD/m^3/d volumetric loading rate, ~20 g/L reactor total suspended solids (TSS), pH 7 ± 1) without wasting.

2.1.1. Batch filtration

The final feed for the batch-cultivated sludge was provided around 4 h before the experiment. Reactor TSS was diluted to 2500 mg/L using tap water, and pH adjusted to 7.0 ± 0.2 using HCl and NaOH (5 N each). Each set of batch experiment comprised of a "test" and "control" pair with and without enzyme supplementation, respectively. Filtration in the control run was terminated when the TMP reached approximately 40 kPa. The test run was then conducted for at least the same elapsed time. Flowrate and TMP were measured by a variable area rotameter (FR2L09BVBN, Key Instruments, USA) and a digital pressure gauge (DPG1000B, Cecomp Electronics, USA), respectively. Tap water was added manually via an external port to maintain a constant liquid level.

Purified enzymes at doses of 35 and 14 mL (equivalent to 90 and 36 mL/g feed COD) were dispersed in the sludge suspension for the first two experimental sets prior to filtration. The corresponding operating flux were 40 and 20 LMH for the higher and lower doses, respectively. The third experimental set using membrane-immobilized hydrolases was performed at 40 LMH. These supra-critical flux values were selected to observe accelerated fouling behaviors over short time periods. Critical flux as determined by flux-stepping 1 LMH every 10 min [31], until the rate of TMP increase exceeded ~0.05 kPa/min, were approximately 13 and 19 LMH for the control and enzyme-supplemented runs, respectively. The dispersed enzyme experiments were conducted on consecutive days and the immobilized enzyme experiment 3 weeks thereafter.

The extent of fouling mitigation was quantified by the percentage reduction in TMP, ΔTMP, and a temporal retardation factor, t_t/t_c. The former is defined as

$$\Delta TMP = \left(\frac{TMP_c - TMP_t}{TMP_c}\right) \times 100\%, \quad (1)$$

where TMP_c and TMP_t represent the time-varying TMP values of the control and test bioreactors at the same instant, respectively. In the latter scale factor, t_c denotes the time taken for the control setup to reach a certain TMP_c, and t_t symbolizes the corresponding time taken for the test reactor to reach the same TMP. t_t/t_c and ΔTMP allow assessments on the relative effectiveness of the augmentation strategies by reducing the influences of nuisance factors and lurking variables.

2.1.2. Extended filtration

The 5 L AnMBR underwent 3 months of acclimatization until the solids concentration was steady at around 2300 mg TSS/L before the experiment commenced. Hydraulic and solids retention times were fixed at 11.7 h and 50 days, respectively. Through feedback control of the permeate suction pump, a flux of 8.5 LMH was maintained, inclusive of 2 min of relaxation for every 8 min of suction. 3 N HCl or 3 N NaOH was automatically dosed to maintain the pH at 7.0 ± 0.5. Inflow, membrane flux, and liquid level were regulated by a programmable logic controller (Renu Electronics, India) that executed 70 min of substrate feeding every 140 min. Temperature was separately controlled at 30–32 °C, using a heating tape and controller (TOHO Electronics, Japan). Biogas