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Impact of cationic substances on biofilm formation from sieved fine particles of anaerobic granular sludge at high salinity

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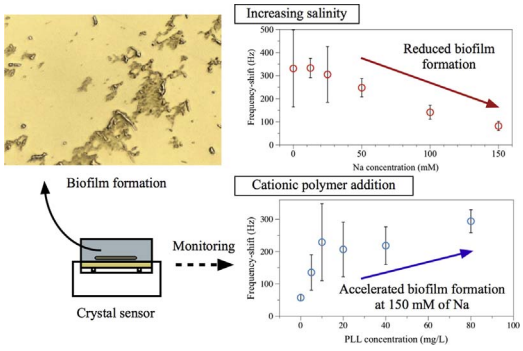
GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated early stages of biofilm formation from sieved fine particles of anaerobic granules in the presence of various cationic substances using a quartz crystal sensor to improve biofilm formation in the anaerobic treatment of saline wastewater. The biomass attached on the sensor was greatly increased with Ca within the low range (8–16 mM), which was not affected by 50 mM of Na. However, the positive effect of 16 mM of Ca was strongly reduced in the co-presence of Ca and Na when Na concentrations were in the range from 25 to 150 mM because Ca may compete with Na for the limited binding sites in biofilm. The addition of cationic polymer at 150 mM of Na increased biomass adhesion by several folds at only 10–80 mg/L compared to the addition of 16 mM of Ca. Moreover, no methanogenic inhibition was presented below the polymer content of 20 mg/L.

1. Introduction

Anaerobic biogasification as an established way for renewable energy generation is garnering much attention. Anaerobic treatment processes have been successfully applied to industrial wastewater treatment because of their simplicity, small space requirement, and low excess sludge production compared to the conventional activated sludge process (van Lier et al., 2015). The anaerobic treatment of industrial wastewater is a major stream in the field of full-scale applications of biogasification technology in Japan because the number of these plants has already been over 300 (Li and Kobayashi, 2010). Among a number of variations in reactor design, biofilm-based processes such as up-flow anaerobic sludge blanket (UASB), expanded granular sludge bed (EGSB), and fluidized bed have been the most

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widely accepted ways for industrial wastewater treatment (van Lier, 2008). The key for these biofilm-based technologies is successful immobilization of microorganisms to ensure a long sludge retention time at a high-rate treatment of wastewater. The immobilization is strongly influenced by environmental parameters, i.e., chemical/biological compositions, hydrodynamic conditions, pH, and redox potential etc. For the treatment of industrial wastewater, the chemical state of water greatly varies.

Recently, it has been reported that immobilization of anaerobic microorganisms is negatively affected by ionic substances such as Na^+ (Ismail et al., 2010) and HS^- (Kobayashi et al., 2015). A lot of researches focused on salinity in the anaerobic wastewater treatment because the problem related to high salinity still remains unsolved (Pevere et al., 2007; Jeison et al., 2008; Ismail et al., 2010; Kimata-Kino et al., 2011; Gagliano et al., 2017). High-salinity wastewater is produced from various industries, such as food-processing, textile-dyeing, tanning, and petroleum industries (Lefebvre and Moletta, 2006; Xiao and Roberts, 2010). In the field of anaerobic treatment, feedings of wastewater with 1%–15% of salinity have been reported (Xiao and Roberts, 2010). Basically, the high salt content was considered as inhibitory for microorganisms in an anaerobic reactor because the increase in osmotic pressure resulting from high salinity plasmolyzes and kills cells of non-salt-tolerant microorganisms. It has been well known that over 10 g/L of Na strongly inhibits anaerobic digestion (Kugelman and Mccarty, 1965; Feijoo et al., 1995). Currently, some researches have confirmed that the negative effects of high salt content on UASB processes appeared above the Na^+ level of 7–13 g/L (Vallero et al., 2002; Jeison et al., 2008; Ismail et al., 2010; Kimata-Kino et al., 2011). Moreover, a reduction in size and density of granular sludge has been observed in the UASB processes (Jeison et al., 2008; Ismail et al., 2010), which potentially resulted in wash-out of microorganisms. In these studies, reduced biofilm strength was also reported. Ismail et al. (2010) found a leaching of Ca^{2+} from the biofilm. Multivalent cations such as Ca^{2+}, Fe^{2+}, and Al^{3+} have been considered as the key substances (Yu et al., 2000, 2001a,b). These cations formed a complex matrix structure with extracellular polymeric substances (EPS) by ion bridges between cations and the negative functional groups of EPS, which played an important role on biofilm formation (Shi et al., 2017). Therefore, the elution of Ca^{2+} can weaken the bindings among molecules inside the biofilm, possibly reducing biofilm strength.

Although earlier studies investigated the granular sludge at 2.5, 10, and 20 g/L of Na^+ concentrations (Jeison et al., 2008; Ismail et al., 2010; Gagliano et al., 2017), only limited studies discussed the effect of salinity on the properties of anaerobic biofilms compared to those focusing on the methanogenic activity and reactor performance. In particular, little is known about the early step of biofilm formation (i.e., adhesion of cells and attached microcolony development) at high salinity. The most critical step in biofilm formation is the adhesion as the first stage of biofilm formation. In the presence of highly concentrated salt, enrichment of multivalent cations is important for a successful biofilm formation and maintenance. Ismail et al. (2010) and Gagliano et al. (2017) tested the effect of 1 g/L Ca^{2+} in the continuous operations of UASB reactors treating saline wastewater (20 g/L). These studies clarified that Ca^{2+} enrichment enhanced the biofilm strength under high salinity. However, Gagliano et al. (2017) observed that the UASB reactor performance was negatively affected by the addition of 1 g/L of Ca^{2+}. Given these findings, there is still a need for optimization in the wide range verification of additive compounds and their concentrations.

This study focused on the early steps of anaerobic biofilm formation at a wide variety of concentrations of cationic substances including Na^+, Ca^{2+}, and cationic polymers having different molecular weights. The adhesion step has been considered to be the most critical step because it is the first stage of the biofilm formation (Zhang et al., 2016). In this study, during the short-term experiment of biofilm formation from sieved small particles of granular sludge, a slight mass change by the adhesion of microorganisms was evaluated using the quartz crystal microbalance (QCM) sensor to examine a wide range of experimental conditions. On the basis of these approaches, we investigated the impacts of cationic substances on the early step of biofilm formation. How to overcome the negative aspect of salinity in biofilm development was also examined.

2. Materials and methods

2.1. Materials and sensor preparation

Biofilm formation was evaluated by adsorption of biomass to the surface of a coated crystal sensor. The QCM experiment was performed using an AT-cut quartz crystal having a disk diameter of 25.4 mm. The fundamental resonant frequency was approximately 5 MHz. The series resonance residence and frequency were analyzed using a frequency counter (QSR-F-5, SRC, Japan). After cleaning with piranha solution (H_2O_2/H_2SO_4), a quartz crystal disk was rinsed with hexane, tetrahydrofuran, and ethanol in sequence. Then, the surface of the quartz crystal disk was coated with Humiseal® (Chase Corp., MA), which was 20 times diluted by hexane using a spin coater model (SC4001, Aiden, Japan) at 1000 rpm to reduce the interference caused by the electrolyte. The surface was coated again in the same way with 0.5 g/L of poly vinyl chloride in tetrahydrofuran as a solid support to ensure negative surface charge similar to those of microorganisms. In addition, the hydrophobicity of PVC is relatively low and close to that of microorganisms compared to those of other polymer materials used for microbial immobilization (Habouzit et al., 2011; Nguyen et al., 2016). The hydrophobicities of anaerobic microorganisms in UASB reactors have been investigated elsewhere. The contact angles of most acidogens were below 45° and those of methanogens ranged from 32° to 77° (Daffonchio et al., 1995), whereas that of PVC was 72° (Nguyen et al., 2016). Anaerobic microorganisms for the QCM experiment were granular sludge taken from a mesophilic full-scale EGSB reactor. The granular sludge was washed with PBS (7 mM of $Na_2HPO_4{\cdot}7H_2O$, 3 mM of $NaH_2PO_4{\cdot}H_2O$, 9 mM of NaCl, and 1 mM of KCl) and then transferred to a glass bottle to release EPS using a cation exchange resin (CES, Dowex Marathon C-Na 20–50 mesh, Sigma-Aldrich, MO) in the PBS. The sludge, CES, and PBS were mixed at a ratio of 5:40:55 (weight ratio) and stirred at 600 rpm for 2 h after the headspace of the sealed glass bottle was flushed with N_2 gas. Subsequently, the mixture was centrifuged at 8000 rpm for 10 min. The supernatant was filtered through a 0.45 μm pore-size syringe filter and collected as EPS. The residual sludge mixed with CES was sieved using a stainless steel test sieve (opening size: 300 μm) to remove big sludge particles and CES. The filtrate was used for the following experiments.

2.2. Biofilm formation experiment combined with QCM measurement

The experimental apparatus for resonance frequency measurements was composed of an oscillator, a flow cell equipped with a QCM sensor, two closed glass bottles (feed bottles) with a magnetic stirrer, two peristaltic pumps connected to the flow cell, and a temperature-controlled incubator (Fig. 1). The flow cell with a volume of about 2 mL consisted of two pieces. The bottom piece was used to hold the reference and counter electrodes, and the top piece was the main body for retention of the solutions. The quartz crystal disk prepared as mentioned above was located inside the flow cell and sealed by two O-rings pressed together by screws. The disk surface inside the flow cell was in the vertical direction as shown in Fig. 1 to avoid the effect of sedimentation. The two solutions (sol A and sol B) were introduced from the feed bottles into the flow cell using peristaltic pumps at a flow rate of 1 mL/min and flowed out through an outlet port. Sol A was prepared via mixing given concentrations of cationic substances (Na, Ca, and poly-L-lysine (PLL)) and 0.25 mL of 30 g/L $Na_2S{\cdot}9H_2O$, being diluted to 25 mL using reverse-osmosis-purified water. In the experiments on