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Enrichment and characterization of microbial consortia degrading soluble microbial products discharged from anaerobic methanogenic bioreactors

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ABSTRACT

Soluble microbial products (SMP) produced in bioprocesses have been known as a main cause to decrease treatment efficiency, lower effluent quality, and promote membrane fouling in water reclamation plants. In this study, biological degradation of SMP using selectively enriched microbial consortia in a down-flow hanging sponge (DHS) reactor was introduced to remove SMP discharged from anaerobic methanogenic reactors. On average, 68.9–87.5% SMP removal was achieved by the enriched microbial consortia in the DHS reactor for >800 days. The influent SMP fed to the DHS reactor exhibited a bimodal molecular weight (MW) distribution with 14–20 kDa and <4 kDa. Between these two types of SMP, the small MW SMP were biodegraded in the upper part of the reactor, together with most of the large MW SMP. Using 16S rRNA gene pyrosequencing technology, the microbial community composition and structure were characterized and correlated with operational factors, such as hydraulic retention time, organic loading rate, and removal of soluble chemical oxygen demand at different depths of the reactor, by performing network and redundancy analyses. The results revealed that *Saprospiraceae* was strongly correlated to the increasing SMP loading condition, indicating positive co-occurrences with neighboring bacterial populations. Different microbial diversity along with the depth of the reactor implies that stratified microbial communities could participate in the process of SMP degradation. Taken together, these observations indicate that the spatial and temporal variability of the enriched microbial community in the DHS reactor could effectively treat SMP with respect to changes in the operational factors.

1. Introduction

Biological treatment processes have been extensively used to treat wastewater containing dissolved organic materials. In these treatment processes, microbial cells are enriched to high concentrations (>1–2 g/L) to effectively degrade and mineralize organic matters to carbon dioxide. Concurrently, energy is derived for the growth of microbial cells, and soluble microbial products (SMP) are secreted into the bulk solution. SMP generally contain a wide range of soluble, complex, and heterogeneous compounds with a molecular weight (MW) ranging from 0.5 to 1000 kDa (Barker and Stuckey, 1999; Jarusutthirak and Amy, 2006; Ni et al., 2010b). They are present in the effluent discharged from the treatment processes and are primary substances contributing to the increase in effluent chemical oxygen demand (COD) (Barker et al., 1999; Jarusutthirak and Amy, 2006). They can be a cause of increasing toxicity of the effluent by themselves (Magbanua and Bowers, 2006) and an environmental hazard by acting as a precursor of disinfection by-products (Magbanua and Bowers, 2006; Tang et al., 2012; Wei et al., 2011). Accumulation of SMP in activated sludge (AS) processes not only decreases respiration rates but also reduces efficiencies in flocculation, settling ability, and dewaterbility of AS by affecting physical properties in the processes, such as sludge structure, turbidity, and viscosity (Chudoba, 1985; Reid et al., 2006). Increase of SMP in tertiary treatments can also have inhibitory effects on nitrification (Ichihashi et al., 2006).

The presence of SMP in discharged water can potentially have negative impacts to water reclamation processes. In membrane bioreactors and in membrane separation processes for water purification, SMP are reported to be responsible for membrane fouling by accumulating on the surface of membranes, blocking pores, and subsequently reducing the water flux through the membranes

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(Choo and Lee, 1996; Kang et al., 2002; Liao et al., 2006). To remove foulants deposited on the membrane surface and restore the water flux passing through the membrane, membrane backwashing or chemical cleaning is often used. In extreme cases, these foulants can no longer be removed from the membrane surface. As a result, replacement of new membrane modules is required, which can increase operation costs.

It is important to develop strategies to effectively control and remove SMP in membrane-based water treatment and reclamation processes (Laspidou and Rittmann, 2002; Liao et al., 2004). In these processes, adsorption and coagulation as pretreatments are often used to reduce SMP, and this can prevent or minimize the extent of fouling taking place on the membrane surface (Akram and Stuckey, 2008; Hu and Stuckey, 2007; Koseoglu et al., 2008; Park et al., 1999; Wu et al., 2009). The most commonly used adsorbent is activated carbon in a form of granules or powders (Akram and Stuckey, 2008; Hu and Stuckey, 2007; Trzcinski et al., 2011; Wu et al., 2009), and its use prior to microfiltration and ultrafiltration is reported as the most effective pretreatment to control SMP in secondary effluent (Kim et al., 2002; Pandey et al., 2012). However, the long-term application of activated carbon can be limited by its adsorption capacity (Akram and Stuckey, 2008; Pandey et al., 2012; Trzcinski et al., 2011; Wu et al., 2009).

Alternatively, biologically degrading SMP has been suggested to control the amount of SMP in water treatment systems (Cai et al., 2013). Biodegradation of SMP is feasible but at a slow rate due to the large MW and complex chemical structures (Jiang et al., 2008; Rittmann and McCarty, 2012). However, when appropriate conditions are provided, effective degradation of SMP discharged from an anaerobic reactor can be achieved with efficiency up to 96% on high MW SMP (>100 kDa); the degradation efficiency of SMP is observed to be more effective under aerobic conditions than anaerobic conditions (Barker et al., 2000). Microbial community compositions related to anaerobic SMP degradation have not been characterized, but a few previous studies are limited to identifying phylogenetic groups of heterotrophic bacteria utilizing SMP produced by nitrifying bacteria at the phylum and class levels (Kindaichi et al., 2004; Matsumoto et al., 2010; Okabe et al., 2005). The decrease of SMP in the system was speculated to have a correlation with the abundance of *Klebsiella* in a biological activated carbon reactor (Dong et al., 2013) and *Chloroflexi* in a membrane bioreactor (MBR) (Miura and Okabe, 2008).

Several reports have also described the use of a down-flow hanging sponge (DHS) reactor as a post-treatment to treat the effluent discharged from up-flow anaerobic sludge blanket (UASB) processes treating domestic wastewater (Abdou Saad El-Tabl et al., 2013; Kubota et al., 2014; Onodera et al., 2013; Tanaka et al., 2012; Tandukar et al., 2006). Additional 70–80% reduction in COD by the DHS reactor was reported. Although no measurement was performed to confirm the molecular size of the COD present in the UASB effluent, it is possible that the majority of the COD was primarily made of SMP, and a large fraction of the SMP was biodegraded by the microbial populations selectively enriched in the DHS reactors.

In this study, to understand biological degradation of SMP in a DHS reactor as a post-treatment process to the effluent of anaerobic methanogenic reactors, the spatial and temporal variability of the community composition and structure of the enriched microbial consortia was characterized using 16S rRNA-based pyrosequencing. In addition, the key microbial populations involved in SMP degradation and their relationships with the operational factors were identified and evaluated by applying network and redundancy analyses.

2. Material and methods

2.1. Experimental set up

Fig. 1A illustrates the use of a DHS reactor to enrich microbial consortia that could degrade SMP present in the effluent of anaerobic bioreactors. To produce the required SMP-containing effluent, two anaerobic reactors, named an anaerobic packed-bed reactor (AP) and a hybrid packed-bed reactor (HP), were operated to treat synthetic wastewater that mimicked the wastewater composition discharged from soft drink production plants (Table S1 in the Supplementary Information (SI)). The detailed information of the system performance of the AP and the HP reactors is described elsewhere (Narihiro et al., 2015). Briefly, the OLR increased from

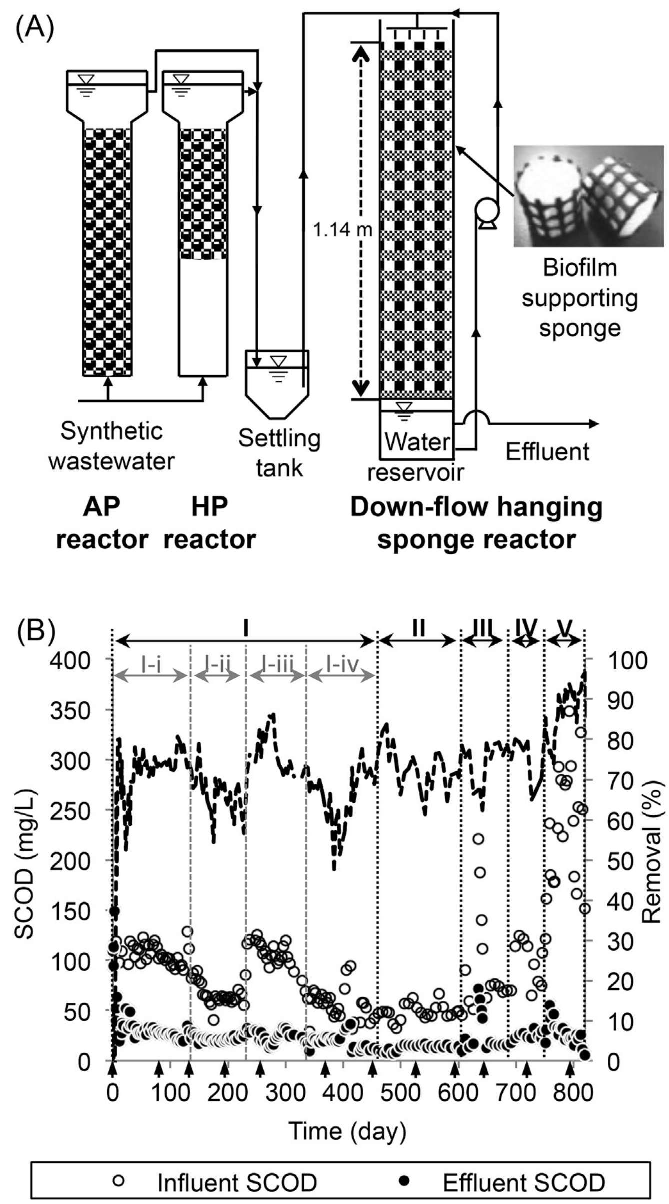

Fig. 1. (A) Schematic diagram of the anaerobic packed-bed reactors (AP and HP) and the down-flow hanging sponge (DHS) reactor. (B) Soluble chemical oxygen demand (SCOD) removal of the DHS reactor in five different phases. Phase I was divided into four different subphases based on organic loading rates (OLRs). The arrows along with the time axis indicate the operational days when biomass in the supporting sponge media was collected for microbial community analysis.