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Fractionating soluble microbial products in the activated sludge process

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ABSTRACT

Soluble microbial products (SMP) are the pool of organic compounds originating from microbial growth and decay, and are usually the major component of the soluble organic matters in effluents from biological treatment processes. In this work, SMP in activated sludge were characterized, fractionized, and quantified using integrated chemical analysis and mathematical approach. The utilization-associated products (UAP) in SMP, produced in the substrate-utilization process, were found to be carbonaceous compounds with a molecular weight (MW) lower than 290 kDa which were quantified separately from biomass-associated products (BAP). The BAP were mainly cellular macromolecules with an MW in a range of 290–5000 kDa, and for the first time were further classified into the growth-associated BAP (GBAP) with an MW of 1000 kDa, which were produced in the microbial growth phase, and the endogeny-associated BAP (EBAP) with an MW of 4500 kDa, which were generated in the endogenous phase. Experimental and modeling results reveal that the UAP could be utilized by the activated sludge and that the BAP would accumulate in the system. The GBAP and EBAP had different formation rates from the hydrolysis of extracellular polymeric substances and distinct biodegradation kinetics. This study provides better understanding of SMP formation mechanisms and becomes useful for subsequent effluent treatment.

1. Introduction

Soluble microbial products (SMP), resulting from microbial growth and decay, comprise a wide range of compounds with high or low molecular weights (MWs) (Noguera et al., 1994; Barker and Stuckey, 1999; Laspidou and Rittmann, 2002a). SMP are usually the major component of the soluble organic matter, e.g., soluble chemical oxygen demand (COD), in effluents from biological wastewater treatment plants (Barker and Stuckey, 1999; Aquino, 2004; Shon et al., 2004; Jarusutthirak et al., 2005; Rosenberger et al., 2006). Hence, their presence is a matter of great interest not only in terms of achieving current discharge standards, but also because they effectively affect the lower limit for treatment plants (Barker and Stuckey, 1999; Gao et al., 2004; Grunheid et al., 2005; Labbs et al., 2006; Holakoo et al., 2006; Ichihashi et al., 2006; Magbanua and Bowers, 2006).

SMP have been classified into two groups based on the bacterial phase from which they are derived: the utilization-associated products (UAP) derived from the original substrate in microbial growth and the biomass-associated products (BAP) generated in the endogenous phase (Namkung and Rittmann, 1986; Grady et al., 1999; Laspidou and Rittmann, 2002a; Jarusutthirak and Amy, 2006). Regarding the characterization, most of previous related studies have focused on the global characteristics of SMP (Barker and Stuckey, 1999). SMP have been found to have a very wide range of MW distribution and different structures/functions (Barker and

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Stuckey, 1999; Grady et al., 1999; Rosenberger et al., 2006; Labbs et al., 2006; Magbanua and Bowers, 2006; Jarusutthirak and Amy, 2006). The MW distribution of SMP has been extensively examined, because it is useful in assessing the efficiency and suitability of the subsequent treatment facilities (Barker and Stuckey, 1999). It is found that a majority of SMP has a MW less than 1 kDa or greater than 10 kDa, and that a minority of SMP has a MW between 1 and 10 kDa (Jarusutthirak and Amy, 2006). However, it is also reported that the SMP exhibited a bimodal distribution with 30% of MW $<$ 1 kDa and 25% of MW $>$ 100 kDa (Barker and Stuckey, 1999). These contradictory findings about the MW of SMP suggest that SMP might be further fractionated for better understand their formation mechanisms and characteristics.

In terms of the measurement methods, many analytical methods have been employed to characterize SMP. Gel-Permeating chromatography (GPC) has been widely used to determine the MW distribution of SMP from different origins (Barker and Stuckey, 1999; Labbs et al., 2006; Jarusutthirak and Amy, 2006). Fourier transform infrared spectroscopy (FTIR) and dissolved organic carbon (DOC) have been utilized to analyze the functional group characteristics and total content of SMP, respectively (Grunheid et al., 2005; Jarusutthirak and Amy, 2006). Chemical analysis has also been performed for the determination of polysaccharides and proteins present in SMP (Barker and Stuckey, 1999; Aquino, 2004; Holakoo et al., 2006). However, in these studies the characteristics of global SMP, rather than their sub-fractions, i.e., UAP and BAP, are explored. Furthermore, SMP are a complex mixture of organic compounds with various compositions and characteristics, and thus it is impossible to elucidate the comprehensive characteristics of SMP and quantification of UAP and BAP by using a single chemical approach. If UAP and BAP could be accurately and quantitatively identified, it would become possible to examine how refractory the individual compounds are and to determine which type of SMP is the most difficult to remove from biological treatment plant effluents.

Therefore, the main objective of this study is to elucidate SMP formation mechanisms with chemical and mathematical approaches via: (1) conducting an integrated analysis on SMP characteristics and quantitatively identifying UAP and BAP separately; and (2) further fractionating the SMP of activated sludge under aerobic conditions. In this study, we present the integrated attempt to investigate and quantify the sub-fractionation of SMP of activated sludge through selectively combining MW determination by GPC with chemical analysis by oxygen utilization rate (OUR), polysaccharide and protein determination, 3-dimensional excitation emission matrix (EEM) fluorescence spectroscopy, FTIR and DOC measurements. Another feature of this work is the development of a mathematical model to further quantitatively and qualitatively describe the production kinetics and sub-fractionation of SMP.

2. Materials and methods

2.1. Sludge, reactor and wastewater

Activated sludge cultivated in a bench-scale sequencing batch reactor (SBR) with a working volume of 2 L (see Figure A of Supplementary Materials) was employed to investigate its SMP generation. The sludge was collected from an aeration tank in the Wangxiaoying Municipal Wastewater Treatment Plant, Hefei, China, as inoculum for the SBR. The SBR was operated for 4 h per cycle with a hydraulic retention time (HRT) of 8 h. The sludge retention time (SRT) was set at 20 days by controlling the amount of sludge wasted from the reactor in each cycle. It was operated at 20 °C with 3 min of influent filling, 212 min of aeration, 20 min of settling, and 5 min of effluent withdrawal. Air was applied to the reactor at a flow rate of 0.4 $m^3\,h^{-1}$. It was fed with a synthetic wastewater at chemical oxygen demand (COD) of 800 mg L^{-1}. The wastewater composition was as follows: acetate, 750 mg L^{-1}; NH_4Cl, 190 mg L^{-1}; KH_2PO_4, 224 mg L^{-1}; $MgSO_4$, 90 mg L^{-1}; KCl, 37 mg L^{-1}; and trace element solution (in mg L^{-1}): EDTA, 50; $ZnSO_4\cdot 7H_2O$, 22; $CaCl_2\cdot 2H_2O$, 8.2; $MnCl_2\cdot 4H_2O$, 5.1; $FeSO_4\cdot 7H_2O$, 5.0; $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 1.1; $CuSO_4\cdot 5H_2O$, 1.8; $CoCl_2\cdot 6H_2O$, 1.6. The influent pH value was adjusted to 7.0 through dosing 1 M HCl and NaOH.

2.2. Experiments

Sludge was sampled from the SBR when no organic substrate was present in the medium. The samples were washed twice with distilled water to remove bulk soluble organic materials. A batch reactor (Fig. A in Supplementary Materials) was inoculated with 1 L of diluted activated sludge (65% inoculum of the sampled sludge from the SBR) and aerated continuously to keep the dissolved oxygen (DO) concentration above 4 mg L^{-1} in the entire aerobic phase. The sludge concentration in the reactor was 1600 mg MLVSS (mixed liquid volatile suspended solids) L^{-1}. A reactor without the organic substrate dose was also operated as a control. After wastewater was dosed, the OUR was monitored. The feeding wastewater composition of batch experiments was same as the synthetic wastewater of the SBR influent. The experiments were conducted at 20 °C and pH of 7.0. All the experiments were performed repeatedly and the reproducible observations were reported in this paper.

2.3. Analysis

DOC was determined using a total organic carbon (TOC) analyzer (V_{CPN}, Shimadzu Co., Japan). The acetate concentration was measured using a gas chromatograph (6890NT, Agilent Inc., USA) equipped with a flame ionization detector and a 30 cm × 0.25 mm × 0.25 mm fused-silica capillary column (DB-FFAP). The total SMP were determined from the difference as follows:

$$\text{Total SMP(as COD)} = \text{Soluble COD} - 1.07 * \text{Acetate} \quad (1)$$

$$\text{Total SMP(as TOC)} = \text{Total DOC} - 0.4 * \text{Acetate} \quad (2)$$

Fractionated SMP, i.e., UAP and BAP, were obtained from total SMP measurement based on associated phases.

The MW distribution of the organic matters in SMP was determined using a GPC (Waters 1515, Waters Co., USA) with deionized water as eluent at a flow rate of 1.0 ml min^{-1}. The detection was carried out at 35 °C with a diode array UV detector at 254 nm and simultaneously with a refractive index detector. The column was calibrated with the standard