Bioresource Technology 255 (2018) 29–38

Contents lists available at ScienceDirect

Bioresource Technology

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

Changes in spectroscopic signatures in soluble microbial products of activated sludge under different osmotic stress conditions

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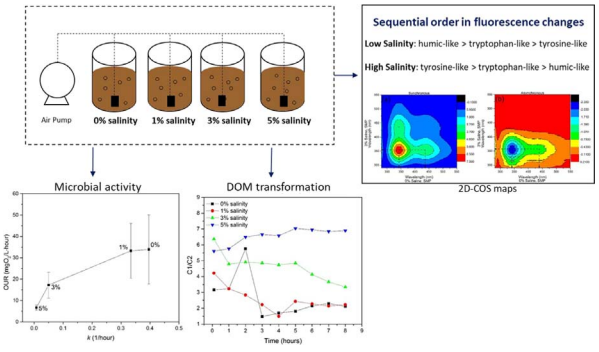
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
EEM-PARAFAC
Dissolved organic matter
Oxygen uptake rate
Soluble microbial products
Saline wastewater
Two-dimensional correlation spectroscopy

ABSTRACT

Spectroscopic techniques were used to examine the subtle changes in soluble microbial products (SMP) of batch activated sludge bioreactors working at different salinities (i.e., 0%, 1%, 3%, and 5% NaCl). The changes in different fluorescent constituent were tracked by excitation-emission matrix combined with parallel factor analysis (EEM-PARAFAC), and the sequential production was further identified by two-dimensional correlation spectroscopy (2D-COS). Greater enrichment of tryptophan-like component and large-sized biopolymer were found in SMP for higher saline bioreactors, suggesting the SMP sources from bound extracellular polymeric substances and excreted intercellular constituents. 2D-COS revealed the opposite sequences of the fluorescence changes in SMP between the low and the high saline bioreactors, following the order of "tyrosine-like > tryptophan-like > humic-like fluorescence" for the latter. This study clarified the dominant mechanisms involved in SMP formation during elevating salinity, which were well supported by the changes in SMP spectroscopic features, microbial activity, and organic degradation rates.

1. Introduction

Activated sludge (AS) systems, depicted as a complex ecosystem, are the most frequently adopted for biological wastewater treatment processes (Ramdani et al., 2010; Saunders et al., 2015), in which a mixed consortia of microorganisms perform multiple functions in treating wastewater via organic matter degradation and nutrient removal (Wang et al., 2017; Zhang et al., 2016). AS serves as the main degradation agent even in advanced treatment technologies such as membrane bioreactors (MBR) and forward osmotic membrane bioreactors (FO-MBR) (Fenu et al., 2010; Yuan et al., 2015).

A certain level of microbial activity must be warranted in AS

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https://doi.org/10.1016/j.biortech.2018.01.113
Received 30 November 2017; Received in revised form 19 January 2018; Accepted 22 January 2018

treatment processes to achieve the desired effluent quality. However, extreme conditions of pH, temperature, or salinity in influent wastewater may impede the normal microbial activity by rendering AS microorganisms exposed to unusual stress. The extent of such disturbances could even overwhelm those caused by the alternation in the operational parameters of hydraulic retention time (HRT), sludge retention time (SRT), and organic loading rate (OLR) (Shahzad et al., 2015; Wang et al., 2013; Yan et al., 2013). Accumulated salt tends to force microorganisms to release greater amounts of extracellular polymeric substances (EPS) than usual, along with substantial cell lysis and excretion of intracellular constituents (Reid et al., 2006). Higher salinity may also change the chemical composition of EPS and SMP produced from AS microorganisms. For example, Wang et al. (2013) reported the increased content of polysaccharides and proteins in EPS with higher salinity levels in sequencing batch reactor. Chen et al. (2014a) observed the maximum 88.9% increment of protein content in SMP with the salinity changing from 1.2 to 17.3 ms cm^{-1} in submerged anaerobic OMBR. Several previous studies indicated that changing salinity might affect the microbial performance to degrade organic matter in wastewater (Ng et al., 2005; Peyton et al., 2002; Uygur, 2006). Elevated salt concentrations may induce the outflow of the intracellular water to cause cell dehydration, substantially lowering the microbial activity to degrade organic matter (Uygur, 2006). Lay et al. (2010) have proposed a salt tolerance range (< 10 g/L) for AS microorganisms during saline wastewater treatment. Below the salinity level, however, there seems to exist stimulatory effects on organic carbon degradation (Uygur, 2006). The above-mentioned studies have successfully demonstrated a higher production of EPS and SMP, and a decreased removal efficiency for organic carbon. However, the information is lacking regarding the mechanistic variations and subtle changes in SMP constituents upon a higher osmotic stress, which is essential for further understanding of salinity effects on SMP production. In addition, those studies failed to capture the shift of SMP composition, which might abruptly occur upon the changes in osmotic stress, because of the long sampling intervals (in days).

SMP is a complex pool of dissolved organic matter (DOM) with origins from biomass growth to its decay, which are called utilization associated products (UAP) and biomass associated products (BAP), respectively (Jarusutthirak and Amy, 2007). It is easily inferred that osmotic stress condition could make physical or metabolic changes to AS microorganisms, leading to the variation in the relative abundance of UAP and BAP. However, it is not clear which factor plays a more critical role in UAP or BAP production at high salinity: osmotic pressure or metabolic activity. The question could be partially answered by examining the relative sequences of UAP and BAP production in relation with the microbial activity changing by elevating salinity.

It is difficult to distinguish among different organic constituents such as UAP, BAP, and remaining organic substrates using the conventional methods of chemical and biochemical oxygen demand (i.e., COD and BOD). On the other hand, using advanced spectroscopic and chromatographic tools for DOM characterization can open a new analytical window to discriminate different SMP constituents. For example, Maqbool et al. (2017) proposed a range of spectroscopic indices to represent the SMP produced at three different microbial growth phases (i.e., exponential, pseudo endogenous and endogenous phases) via the fluorescence excitation-emission matrix (EEM). Jiang et al. (2010) utilized size exclusion chromatography coupled with organic carbon detector (SEC-OCD) to identify the SMP size fractions responsible for membrane fouling. EEM combined with parallel factor analysis (EEM-PARAFAC) enhances the applicability of EEM spectroscopy in tracking SMP compositional changes along treatment processes by deconvoluting the EEMs into several independent components representing different chemical nature or sources (Cohen et al., 2014; Shutova et al., 2014).

Two-dimensional correlation spectroscopy (2D-COS) is a very useful mathematical tool for identifying subtle changes in DOM upon external perturbations. 2D-COS generates two types of maps (i.e., synchronous and asynchronous maps), which enables one to identify the sequential order of spectral variations as well as their extents. Many examples are found in the application of 2D-COS for DOM studies, in which the external perturbations tested were mostly related to chemical reactions such as metal binding, effects of pH and temperature, or adsorption (Chen et al., 2017; Chen et al., 2014b; Xu et al., 2013). Recently, this technique has been utilized in exploring the effects of heavy metals and organic pollutants on SMP production by AS microorganisms (Wei et al., 2016).

In this study, two advanced spectroscopic tools, namely, EEM-PARAFAC and 2D-COS were used to track the changes in SMP composition under the stressful conditions with increasing salts in influent wastewater. The objectives of the study were (1) to assess microbial responses in AS bioreactors toward elevating osmotic stresses in terms of substrate consumption, microbial activity, and the production of SMP, (2) to explore the production mechanisms of different SMP constituents at dissimilar salinity levels in feed using the advanced spectroscopic and chromatographic tools, and (3) to determine the sequential order of different SMP constituents using 2D-COS for inter- and intra-bioreactor comparison.

2. Material and methods

2.1. Experimental setup and sampling

Four parallel batch type bioreactors with a same volume of 1.6 L were operated at different salinity levels. Seed sludge (MLSS, 3 g/L) was collected from an aeration tank of a municipal wastewater treatment plant located in Seoul (South Korea). Air was equally supplied to each reactor at a rate of 1L/min to maintain aerobic conditions. All the reactors were fed with synthetic wastewater solution consisting of glucose ($\sim$400 mgC/L) as sole carbon source, and NH_4Cl and KH_2PO_4 as nitrogen and phosphorus source, respectively, along with trace amounts of micro-nutrients (i.e. $MgSO_4$, $FeCl_3$, and $CaCl_2$, Supplementary Data). In this study, the AS microorganisms were not acclimated to the feed wastewater to avoid the rapid microbial consumption of the carbon source upon the start of the operation, which may cause the failure to observe prolonged impacts of salinity on microbial activity (Karahan et al., 2010). Four salinity levels were tested in the bioreactors by adding different amounts of NaCl to the feed. The salinity levels were classified into (i) 0% NaCl (i.e., control reactor), (ii) a low level with 1% of NaCl, (iii) a high level with 3% of NaCl, and (iv) an extremely high level with 5% of NaCl. The salinity ranges were selected based on the previous literature (Uygur, 2006; Yogalakshmi and Joseph, 2010; Zhang et al., 2017). Eight hours was chosen as a typical operation time to complete the cycle of substrate degradation based on the preliminary performance of the control bioreactor. An aliquot (12 mL) of the AS sample was collected from the reactors every one hour of operation. Supernatant samples containing SMP (i.e., DOM samples) were separated by centrifuging the AS samples at 4000 rpm followed by the filtration through 0.45 μm cellulose triacetate (CTA) membrane filter.

2.2. Dissolved organic carbon measurement and oxygen uptake rate (OUR) test

Dissolved organic carbon (DOC) of the supernatant was measured using TOC-analyzer (TOC-L, Shimadzu, Japan). Oxygen uptake rate (OUR) was measured to estimate the microbial activity in AS samples. Dissolved oxygen (DO) probe was inserted into the AS samples contained in 60 mL-BOD bottles without any air space available, and it was connected with a data logger for recording. The OUR values were finally calculated by a slope through linear regression (Ni et al., 2010).