Research Article Open Access

Abstract

In biological wastewater treatment process, the analysis of metabolic compounds that are produced during the process is critical to monitor the performance of microorganisms. The soluble products present in the efþuent directly affect the process efýciency and quality of the water after treatment, and it is also the major reason for fouling in membrane bioreactor. Currently, analytical methods are mainly restricted to the overall measurement of the total amount of polysaccharides, DNA and proteins without any speciýc identiýcation of these compounds. Here we introduce an explorative mass spectrometry based strategy, for the analysis of soluble microbial products and other soluble impurities in the efþuents of wastewater treatments using different digestion process. According to the results from this study, the two stage co digestion process indicated higher treatment efýciency compared with the single stage process, since fewer compounds were detected in the efþuent. For the two-stage process, most of the fatty acids produced in the ýrst stage of digestion by hydrolysis and acidogenesis, were digested in the second stage. The results also indicated that the digestion efýciency of the single stage process was lower than that of the separated two-stage process. This study is one of few exploration of analyzing and identifying unknown compounds using MS based technique from a metabolic analysis perspective. Our novel approach can be applied as an analytical platform to effectively monitor the biological

Keywords: Bioprocess; Wastewater treatment; Mass spectrometry; Soluble microbial products

Introduction

e routine parameters to analyze biological wastewater treatment systems are chemical oxygen demand (COD) and biological oxygen demand (BOC). e presence of organic compounds and biopolymers in the e uent are de ned as soluble microbial products (SMP) when they are soluble, and de ned as extracellular polymer substrates (EPS) when they are insoluble and eventually combine to form ocs or solid colloids together with other impurities [1]. e levels of SMP and EPS correspond directly to the levels of e uent COD and membrane fouling, which in turn a ects e uent discharge levels, treatment e ciency and energy use. Moreover, SMP and EPS are believed to be the main causes of fouling in membrane bioreactors.

Generally, SMPs are produced during substrate metabolism or cell lysis and degradation. ey are classi ed into two groups according to their origins; utilization associated products (UAP) and biomass associated products (BAP). UAP are usually composed of carbonaceous compounds, and are produced during cell growth and metabolism, and substrate utilization. e amount of UAP produced during substrate utilization is proportional to the amount of substrate utilized. BAP are produced from cell lysis, biomass degrade and endogenous decay. BAP are macromolecules produced from cell debris, and the major components are believed to be polysaccharides. e molecular weight of BAP is much larger than that of UAP. e boundary between BAP and EPS is not very clear but it is generally accepted that UAP are soluble hydrolyzed EPS [2].

SMP is critical because they are the main contributors of e uent chemical oxygen demand (COD) and biochemical oxygen demand (BOD). Together with EPS, they a ect COD and the toxicology of the e uent and membrane fouling of the bioreactor systems [3]. Currently, the generation of EPS and SMP, factors a ecting their composition and concentration, and fouling mechanism related with the properties of EPS/SMP are still unknown. Hence, intensive and accurate understanding of SMP and EPS is critical. Furthermore, the generation of EPS and SMP, and the complicated composition of them are the major causes of high COD and membrane fouling resulting in the low e uent quality. erefore, it is essential to analyze SMP and EPS and identify their composition in the e uent, which can facilitate understanding of how to reduce membrane fouling and COD. Especially, based on the understanding of composition of SMP, the SMP generation resource can be further known and relevant e ective strategies can be conducted and developed to reduce SMP resulting in reducing membrane fouling. Hence, analysis and identi cation of SMP composition is meaningful for monitoring bioprocess of wastewater treatment in membrane bioreactor system.

However, the identi cation of SMP and EPS is a challenge as it is a mixture of various unknown compounds. SMP and EPS have a wide range of molecular weights (MW) ranging from 0.5 kilo dalton (kDa) to 50 kDa [4]. e components are believed to include humic substances, proteins, DNAs, lipids, polysaccharides, carbohydrates and small molecules. Over the past years, several groups have been working on

***Corresponding authors:** Wei Ning Chen, School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, 637459, Singapore, Tel: +65 63162870; Fax: +65 62259865; E-Mail: WNChen@ntu.edu.sg

Received March 13, 2014; **Accepted** April 28, 2015; **Published** May 05, 2015

Citation: Feng H, Zhou Y, Zhao G, Lee J, Guo C et al. (2015) Analysis of Soluble Microbial Products by Mass Spectrometry: Potential in Monitoring Bioprocesses of Wastewater Treatment. J Anal Bioanal Tech 6: 241 doi:10.4172/2155-9872.1000241

Citation:

and 35°C). e feed for stage 2 reactor was the e uent from the stage 1 reactor. All samples were centrifuged and pre- ltered with 0.45 µm of nylon membrane a er collection to remove any bulky impurities.

Sample preparation for soluble compounds analysis with LC-MS/MS

All of the collected e uents samples were ltered with a 0.22 µm membrane for detection of soluble compounds. e supernatant then was subjected to a modi ed Bligh and Dyer extraction method. Brie y, 800 µl methanol-chloroform with 3:5(v/v) was added to 300 µl wastewater sample. A er vortexing, the mixture was centrifuged at 12000 rpm for 10 min. e supernatant aqueous phase containing the soluble compounds was transferred to a clean tube. e extracts were vacuum-dried, and the pellet dissolved with 60 µl H_2O-methnol, 1:1 (v/v), and centrifuged at 12000 rpm for 2 min to remove the insoluble part. e supernatant was ready to be injected.

Compounds identification with LC-MS/MS

e supernatant fraction from sample preparation step was analyzed using Agilent 1200 HPLC system (Waldbronn, Germany) equipped with a 6530 Q-TOF mass detector managed by a MassHunter workstation. e column used for the separation was an Agilent rapid resolution HT Zorbax SB-C18 (0.5 × 50 mm, 1.8 mm; Agilent Technologies, Santa Clara, CA, USA). e gradient elution involved a mobile phase consisting of (A) 0.1% formic acid in H_2O and (B) 0.1% formic acid in acetonitrile. e initial condition was set at 2% B. A linear gradient to 98% B was applied in 25 min, held for 2 min, then quickly returned to starting conditions for over 1 min and held for another 2 min. Flow rate was set at 20 µl/min, and 2 µl of sample was injected. e electrospray ionization mass spectra were acquired in positive ion mode. Mass data were collected between m/z 100 and 2000 at a rate of 3.35 spectra per second. e ion spray voltage was set at 3,500 V, and the heated capillary temperature was maintained at 350°C. e drying gas and nebulizer nitrogen gas ow rates were 9.0 L/min and 45 psi, respectively. Two reference masses were continuously infused to the system to allow constant mass correction during the run: m/z 121.0509 ($C_5H_4N_4$) and m/z 922.0098 ($C_{18}H_{18}O_6N_3P_3F_{24}$).

	Name	Formula	m/z	Score (DB)
1	Undecanoic acid, 3-hydroxy-, (S)-	$C_{11}H_{22}O_3$	225.146	86.55
2	Sugetriol	$C_{15}H_{24}O_3$	253.1808	81.89
3	Sapelin A	$C_{30}H_{50}O_4$	475.3792	76.7
4	Proclavaminic acid	$C_8H_{14}N_2O_4$	203.1029	78.55
5	Phosphatidyl glycerol	$C_6H_{15}O_8P$	269.0385	81.53
6	PGH2-EA	$C_{23}H_{39}NO_4$	416.2772	89.45
7	Lauryl hydrogen sulfate	$C_{12}H_{26}O_4S$	267.1628	75.88
8	Ivermectin B1b	$C_{47}H_{72}O_{14}$	861.5023	90.92
9	Dihydrojasmonic Acid, Methyl Ester	$C_{13}H_{22}O_3$	227.1631	82.7
10	dexpanthenol	$C_9H_{19}NO_4$	223.1647	78.87
11	Decanoic acid, 9-hydroxy-, (R)-; (-)-(R)-9-Hydroxydecanoic acid; D-9-Hydroxydecanoic acid	$C_{10}H_{20}O_3$	211.1296	86.46
12	Cycluron	$C_{11}H_{22}N_2O$	221.1626	87.43
13	Chrysanthetriol	$C_{15}H_{26}O_3$	255.1958	82.66
14	Arachidic acid(d3)	$C_{20}H_{37}D_3O_2$	316.3285	92.13
15	AAPH	$C_8H_{18}N_6$	199.1671	90.99
16	9-Tridecynoic acid	$C_{13}H_{22}O_2$	211.169	85.89
17	9-Methyl-undecanoic acid	$C_{12}H_{24}O_2$	223.1679	82.59
18				

the compounds of the three samples SD, TD1 and TD2 are identi ed and list in the Tables 1-3 respectively.

From the Tables 1-3, it can be seen that the largest number of compounds were detected in TD1, and the number reached only 42 and some compounds had low match relationship with the mass feature of compounds in the database. In fact, the fragmentation pattern of a compound by MS/MS analysis can further help to validate or negate the search hits. According to preliminary results obtained, fragmentation