Bioresource Technology 211 (2016) 494–501

Contents lists available at ScienceDirect

Bioresource Technology

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

Effect of low temperature on highly unsaturated fatty acid biosynthesis in activated sludge

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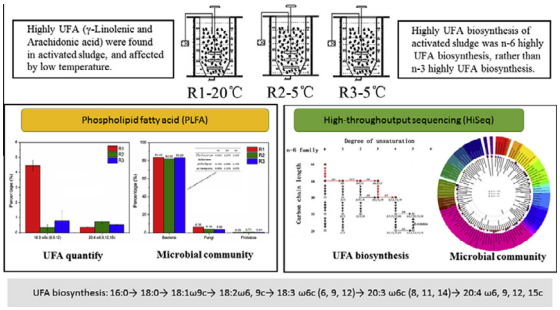
HIGHLIGHTS

- UFA biosynthesis of activated sludge was n-6 highly UFA biosynthesis at low temperature.
- Highly UFA biosynthesis metabolism of activated sludge was established.
- The microbial community structure was analyzed with HiSeq and PLFA simultaneously.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form 9 March 2016
Accepted 13 March 2016
Available online 15 March 2016

Keywords:
Low temperature
Activated sludge
Unsaturated fatty acid
High-throughput sequencing
Highly UFA biosynthesis

ABSTRACT

Low temperature is a limiting factor for the microbial activity of activated sludge for sewage treatment plant in winter. Highly unsaturated fatty acid (UFA) biosynthesis, phospholipid fatty acid (PLFA) constituents and microbial structure in activated sludge at low temperature were investigated. Over 12 gigabases of metagenomic sequence data were generated with the Illumina HiSeq 2000 platform. The result showed 43.11% of phospholipid fatty acid (PLFA) in the activated sludge participated in UFA biosynthesis, and γ-Linolenic could be converted to Arachidonic acid at low temperature. The highly UFA biosynthesis in activated sludge was n-6 highly UFA biosynthesis, rather than n-3 highly UFA biosynthesis. The microbial community structures of activated sludge were analyzed by PLFA and high-throughput sequencing (HiSeq) simultaneously. *Acidovorax*, *Pseudomonas*, *Flavobacterium* and *Polaromonas* occupied higher percentage at 5 °C, and genetic changes of highly UFA biosynthesis derived from microbial community structures change.

1. Introduction

As one of the main limiting factors of activated sludge (AS) bioactivity, low temperature can lead to many problems in wastewater treatment plants (WWTPs), such as, worse effluent quality (Cirja et al., 2008), higher wasted activated sludge production (Reyes et al., 2014), lower sedimentation rate of activated sludge (Winkler et al., 2012) and higher likelihood of filamentous bulking (Pajdak-Stos and Fialkowska, 2012). The microbial community analysis is commonly used to explain the mechanisms behind the problems in water treatment at low temperature (Li et al., 2012). The microorganisms regulate the membrane lipid composition to keep the membrane fluidity and phase structure to have better adaptability (Margesin and Schinner, 1994). Membranes could incorporate specific lipid constituents to maintain fluidity and the critical ability to transport substrates and nutrients at low temperature (Chattopadhyay and

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http://dx.doi.org/10.1016/j.biortech.2016.03.069
0960-8524/

Jagannadham, 2001). Unsaturated fatty acid (UFA) in cell membrane could improve the cold adaptability of microorganisms in activated sludge by lowering of lipid melting point of membrane to maintain good mobility at low temperature (Niu et al., 2012).

The significant approaches to improve the cold adaptability include increasing UFA, extending of the average fatty acid chain length, head group restructuring and changes of the cholesterol-to-phospholipid ratio (Kostal, 2010). The composition of membrane phospholipids is highly important for the temperature at which the phase transition occurs, and it is often found that cold adaptation or cold acclimation is associated with changes in the membrane phospholipid composition. More UFAs increase membrane fluidity, which partly is due to the lower melting temperature of unsaturated phospholipid fatty acids (PLFA) compared to saturated fatty acids (SFA). PLFA can be used as a marker for microorganism, and PLFAs characterizing psychrophilic bacteria are 20:5 and 22:6 (Joergensen and Potthoff, 2005). Highly UFAs might help microorganisms resist the cold environment, but how activated sludge microorganisms regulate highly UFA from the perspective of biosynthesis at low temperature was rarely reported.

The special DNA topology could produce cold shock protein at low temperature with bacterial RNA thermometers regulation (molecular zippers and switches) (Jens and Franz, 2012), whose functions counteract membrane rigidification (increase UFA) and the formation of stable RNA structures (Lim, 2011). The way of low temperature sensation is through local changes in DNA topology, followed by transcriptional and translational events that exert regulatory effects. To our knowledge, studies on the biosynthesis of UFA were relatively rare reported in activated sludge system. Recently, a metagenome of activated sludge from a full-scale WWTP generated by Illumina sequencing was reported (Albertsen et al., 2012). The results showed that high diversities of microbial species and functional genes exist in activated sludge. The next generation high-throughput sequencing technologies developed recently created several new approaches to analyze the microorganisms in activated sludge. Sequencing a huge amount of metagenomic data has been applied to analyze the microbial populations, functions and metabolisms of activated sludge (Ye et al., 2012). At present, the methods for the microbial structures of activated sludge at low temperature include Microplates (Li et al., 2012), PLFA (Niu et al., 2012), and high-throughput sequencing (Ye et al., 2012). These methods greatly improved our understanding of the microbial communities in activated sludge.

In this study, production analysis of UFA biosynthesis and possible metabolic pathways with gene prediction were utilized to confirm the highly UFA biosynthesis in activated sludge and the low temperature effect on the biosynthesis of highly UFA. Meanwhile, both PLFA and HiSeq sequencing were used for analyzing microbial community structures diversity. HiSeq sequencing was used to investigate the genetic changes in activated sludge between 20 °C and 5 °C.

2. Materials and methods

2.1. Laboratory-scale sequencing batch reactor (SBR)

Three reactors (Reactor 1, 2 and 3) made of plexiglass were operated with the working volume of 2 L. Activated sludge from the aeration tank (suspended solids concentration about 4400 mg/L) of a municipal wastewater treatment plant in Nanjing, China was taken and used as seeding sludge for the reactors. Activated sludge source of three reactors were different in sampling time. Reactor 1 and 2 used the seeding sludge taken in September 2013 (summer sludge), and Reactor 3 used the seeding sludge taken in February 2012 (winter sludge). The working temperature of Reactor 1 was 20 °C, and Reactor 2 and 3 worked at 5 °C. The cycle time of the reactors was 12 h and the feeding time, reaction time and settling time were 0.2 h, 10.5 h and 1 h, respectively. The oxygen concentration during the operation was about 8.2–8.4 mg/L for the three reactors and no external pH control was applied during over half-year operation time. The reactors were generally operated with fixed MLSS concentration (2700–3400 mg/L) after stabilization. The excess sludge was extracted from the reactor once a week. SRT was calculated according to the volume of waste activated sludge (R1-87 mL, R2-140 mL and R3-147 mL).

2.2. Synthetic wastewater

The synthetic wastewater took glucose, NH_4Cl and KH_2PO_4 as carbon, nitrogen and phosphorus source, respectively, and the mass ratio of C:N:P was 100:5:1 (COD concentration of 400 mg/L). The synthetic wastewater contained (per litre): 400 mg $C_6H_{12}O_6$, 96 mg NH_4Cl, 11.96 mg KH_2PO_4 and 0.6 mL of a metals solution. One litre of the metals solution contained: 11 g $CaCl_2{\cdot}2H_2O$, 1.5 g $FeCl_3{\cdot}6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4{\cdot}5H_2O$, 0.18 g KI, 0.12 g $MnCl_2{\cdot}4H_2O$, 0.06 g $Na_2MoO_4{\cdot}2H_2O$, 0.12 g $ZnSO_4{\cdot}7H_2O$, 0.15 g $CoCl_2{\cdot}6H_2O$ and 10 g EDTA.

2.3. PLFA extraction and nomenclature

The PLFA were extracted from activated sludge after six months acclimatization according to the method described by Niu et al. (2012) 5 mL of static settlement activated sludge was extracted with a chloroform–methanol–phosphate buffer mixture (1:2:0.8), and the phospholipid were separated from other lipids on solid phase extraction cartridges (HT30323, LabTech) filled by silicic acid. The phospholipid fraction was transesterified by mild alkaline methanolysis followed immediately by addition of C19:0 methyl ester internal standard to each sample. Saponification was carried through with a sodium hydroxide–methanol–distilled water (45 g: 150 mL: 150 mL). Tethylation needed a mixture of hydrochloric acid–methanol (32 mL: 275 mL). After extraction, the resulting fatty acid methyl esters were prepared according to MIDI protocol and detected by Agilent 7890 Gas Chromatography with flame ionization detector (FID), and the results were analyzed using the MIDI Sherlock Microbial Identification System (MIDI, Newark, DE).

Fatty acids were designated in terms of total number of carbon atoms, with the number of double bonds given after a colon, following the form $A{:}B\omega C$, where A is the number of carbon atoms, B is the number of double bonds, and C is the position of the first double bond from the ω or the molecule's aliphatic end. In branched chain fatty acids, iso and anteiso present homotype and heterotype chain fatty acid, respectively.

2.4. DNA extraction and HiSeq sequencing

DNA were extracted from 0.5 g of each activated sludge sample of three reactors after six months acclimatization by using a Fast DNA® SPIN Kit for soil (MP Biomedicals, OH, USA) following the manufacturer's protocol (Zhu et al., 2015). The amount and purity of DNA were determined by using NanoDrop® Spectrophotometer ND-1000 (Thermo Fisher Scientific, MA, USA) based on the absorbency of A260 and the ratio of A260/A280, respectively. Extracted DNA of activated sludge from three reactors was stored at −80 °C before sending to a commercial company (Wuxi, Jiangsu, China) for Illumina HiSeq sequencing. Detailed description of HiSeq sequencing is available in the Supplementary Material.