Bioresource Technology 150 (2013) 156–162

Contents lists available at ScienceDirect

Bioresource Technology

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

The strengthening effect of a static magnetic field on activated sludge activity at low temperature

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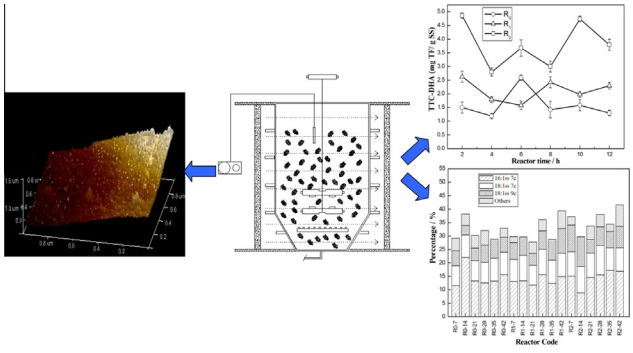
HIGHLIGHTS

- C16:1ω7c, C18:1ω7c and C18:1ω9c were the essential unsaturated fatty acids at 5 °C.
- MF increased the Gram-negative bacteria content to improve the cold adaptability.
- Heteropolar MF enhancement is much more effective than that of the homopolar MF.

GRAPHICAL ABSTRACT

The figure shows the TTC-DHA, main species of unsaturated fatty acids in microbial cell membrane at 5 °C (%), and the AFM image of activated sludge strengthening with magnetic field which provides information about the cell membrane unsaturated fatty acid composition and low temperature response of microorganism enzymatic activity at 5 °C. It is a concise and illustrative application of PLFA analysis to reflect the composition of activated sludge in response to a magnetic field.



ARTICLE INFO

Article history:
Received 26 June 2013
Received in revised form 20 August 2013
Accepted 23 August 2013
Available online 31 August 2013

Keywords:
Low temperature
Magnetic field strengthening
Activated sludge
Phospholipid fatty acid
Microbial diversity

ABSTRACT

The strengthening effects of static (homopolar and heteropolar) magnetic fields (MF) on microorganisms were compared in activated sludge degrading organic matter at low temperature. The TTC dehydrogenase activity improved substantially through external heteropolar MF intensification, and led to the highest COD removal rate of 94.9% at 5 °C. Phospholipid fatty acid analysis showed that C16:1ω7c, C18:1ω7c and C18:1ω9c were the essential unsaturated fatty acids in cell membrane at low temperature (4–15 °C), accounting for the majority of the whole unsaturated fatty acids. The MF effect increased the Gram-negative bacteria content to improve the cold adaptability. Shannon–Wiener diversity analysis demonstrated the samples with heteropolar MF had a higher PLFA diversity index (1.17–1.25) than that with homopolar MF (0.89–1.13). AFM observation showed MF smoothed part of the microbial cell surface, with some remaining distinct protuberances. Heteropolar MF enhancement performance is much more effective than that of the homopolar MF with identical plate distance.

1. Introduction

Low temperature (4–15 °C) inordinately limits the activity of the activated sludge microorganisms, resulting in the irregular operation of biological wastewater treatment. A drop in temperature causes a change in the physical and chemical properties of wastewater, and this can significantly affect the reactor performance (Bandara et al., 2011). Enhancing mechanisms are of interest to attenuate inhibition caused by a cold environment, for example bioaugmentation (Guo et al., 2010), process control (De Kreuk et al., 2005) and external field application (Lebkowska

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http://dx.doi.org/10.1016/j.biortech.2013.08.139

et al., 2013). Magnetic fields can exert an influence on microorganisms, leading to biological effects (Barnothy, 1969), and magnetic field strengthening has become a focus of environmental microbe enhancement research (Liu et al., 2008; Lebkowska et al., 2011).

Many studies have proved that a weak or moderate magnetic field will produce a positive biological impact (Gao et al., 2011), and can influence some biological and biochemical systems (Kovacs et al., 1997). This can be a result of the minimization of external mass transfer limitations, especially with the pulsed type of application, in addition to the increase in microbial activity (Yavuz and Celebi, 2003). Static magnetic field can exert some influence over microorganisms and this effect depends on many factors such as type and magnitude of magnetic field, type of microorganism, temperature, length/duration of exposure, growth media, etc. (Mateescu et al., 2011). Optimal intensity of magnetic field can promote microbial metabolism and further enhance the granulation of nitrifying bacteria (Wang et al., 2012).

At low temperature, the membrane fluidity is reduced and the enzyme activity is inhibited, and increasing the microbial activity is needed to maintain normal physiology (Pambrun et al., 2008). Phospholipid fatty acid (PLFA) composition of cell membrane will show corresponding variation at low temperature. Analysis of this constituent makes it possible to obtain biochemical fingerprints of communities, yielding information about the taxonomy, functions, physiology, and abundance of community members (Green and Scow, 2000). The influence of magnetic field on the PLFA directly reflects the strengthening effect on cold adaptability and species structure variation of activated sludge microorganisms. However, the strengthening effect of the magnetic field on microorganism under low temperature remains uncertain.

This study focuses on the strengthening efficiency of static magnetic field on the activated sludge of simulated SBR bioreactors, as well as biological magnetic effects under low temperature. The enzyme activity (TTC dehydrogenase activity) and physiological activity (cold adaptability reflected by PLFA) are discussed, and the strengthening law and partial physiological reaction mechanism are acquired. The results will provide a basis for further study on improving microbial activity of activated sludge at low temperature.

2. Methods

2.1. Bioreactor

Three 500 mL beakers as simulated SBRs were applied (R_0, R_1 and R_2). R_0 was control experiment without magnetic field. R_1 was set in the homopolar and R_2 in the heteropolar magnetic field. Two magnet plates were placed in parallel to generate a static magnetic field. The plate interval was adjusted to assure that the magnetic field intensity of R_2 was 13 mT, with the plate interval of R_1 equidistantly set. The three reactors all operated at 5 °C. The synthetic wastewater consisted of $C_6H_{12}O_6$, NH_4Cl and KH_2PO_4, diluted to a final COD concentration of 400 mg/L with tap water, and the mass ratio of C:N:P was 100:5:1. Trace elements were included as well. The cycle time of the reactors was 12 h with 250 mL wastewater and 250 mL activated sludge aerated with an air pump (2.5 L/min). The hydraulic retention time (HRT) was 10 h at a solid retention time (SRT) of 5 days. This operation was batch wise, and the feeding times, reaction times and settling times were 0.2, 10 and 1 h, respectively. The oxygen concentration during the operation was around 8.20 mg/L for the three reactors and no external pH control was applied during this study. The seeding sludge was collected from the aeration tank of a local wastewater treatment plant in Nanjing, China. The seeding concentration of the activated sludge was about 4500 mg/L.

2.2. TTC dehydrogenase activity (TTC-DHA)

The TTC-DHA of microorganisms can reflect the efficiency of substrate degradation, and it was analyzed according to Tracey's with slight modification (Burdock et al., 2011). Activated sludge (15 ml) was placed in a centrifuge tube with stopper, and the supernatant was discarded after 4000 r/min centrifugation for 5 min. Distilled water was added to 15 mL, the mixture was stirred then centrifuged for 5 min with the supernatant discarded again. This step was repeated twice. Tris–HCl buffer solution (2 mL), 0.5 mL TTC solution (4 mg/mL) and 2 mL sludge mixture were added to each of the tubes, and immediately placed into a 5 °C incubator. Two drops of concentrated sulfuric acid were added to each tube, and after 2 h the tubes were carefully shaken to stop the reaction. Toluene (5 ml) was rapidly added after every termination reaction, and samples were extracted at room temperature. The layered solution was centrifuged for 5 min at 4000 r/min, and the absorbance of supernatant organic solvent was measured at 486 nm. The yield was defined as 1 μg TF per hour as a unit of enzyme activity.

2.3. Phospholipid fatty acid (PLFA) extraction and nomenclature

The PLFA extraction method was modified according to Balser's (Smithwick et al., 2005; Kao-Kniffin and Balser, 2007; Pawlett et al., 2013). Briefly, through separation, elution, saponification, methylation and extraction, the resulting fatty acid methyl esters were prepared according to MIDI protocol and detected by Agilent 7890 GC, and the results were analyzed using the MIDI Sherlock Microbial Identification System (MIDI, Newark, DE) (Xue et al., 2008). 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:BωC, 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. Statistical analysis

PLFA data was analyzed with principal component analysis (PCA), which was performed by SPSS 18.0 software (Paasivirta et al., 2005). For PCA analysis, individual PLFA scores were expressed as a percentage of the total PLFA in the sample. After

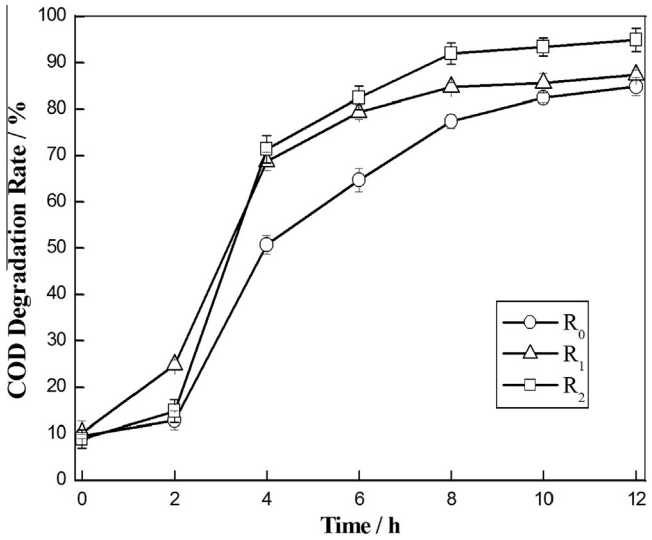

Fig. 1. COD removal efficiency at a single cycle of the reactor stable operation stage.