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7 mT static magnetic exposure enhanced synthesis of poly-3-hydroxybutyrate by activated sludge at low temperature and high acetate concentration

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ABSTRACT

The effect of 7 mT (milliTesla) SMF (static magnetic field) on poly-3-hydroxybutyrate (PHB) production was studied at an acetate concentration of 260 Cmmol l^{-1} and temperature of 10 °C. The SMF decreased the specific acetate uptake rate by 29%, but increased the maximum PHB content and the yield of PHB on acetate by 32 and 28% respectively. The ratio $q_P/(q_S - q_P)$, which described specific PHB production rate over the difference between specific acetate uptake rate and specific PHB production rate, was introduced for evaluation of the ratio of carbon flux into PHB synthesis and into the TCA (tricarboxylic acid) cycle. This value reached 2.3 when activated sludge culture was exposed to magnetic field of 7 mT, which was 1.1 times higher than the $q_P/(q_S - q_P)$ value obtained without magnetic exposure. Therefore, the SMF promoted diversion of more acetyl-CoA towards PHB synthesis and could offset adverse effects of high acetate concentration and low temperature. These results provide evidence that SMF enhances PHB production by activated sludge.

Keywords: Poly-3-hydroxybutyrate; Static magnetic field; Low temperature; High acetate concentration

1. Introduction

Polyhydroxyalkanoates (PHAs) are polyesters formed and accumulated by various bacterial species under unbalanced growth conditions (Lee, 1996). They have been viewed as promising substitutes for petrochemical-derived plastics due to their similar properties and their biodegradability. The major barrier to the wide application of PHAs is their current high cost, and over 40% of the total PHAs production cost is attributed to the carbon source (Salehizadeh and van Loosdrecht, 2004). One attractive alternative for reducing the cost of PHA synthesis is to use carbon wastes such as olive oil mill effluents, paper mill wastewater, and starchy wastewater as carbon sources for activated sludges (Suresh et al., 2004; Dionisi et al., 2005; Bengtssn et al., 2008). In general, raw carbon wastes require prior hydrolysis and acidogenesis to be converted into VFAs (volatile fatty acids) like acetate, propionate, and butyrate, which can then serve as PHA substrates. PHA production with VFAs has been reported at acid concentrations lower than 60 Cmmol l^{-1} and at temperatures in the range of 20–25 °C (Third et al., 2003; Dias et al., 2005; Lemos et al., 2006; Chen and Li, 2008). VFAs can be toxic or inhibitory to microorganisms depending on the pH and acid concentration (Yu et al., 2002). Slightly alkaline pH (8–8.5), when the acetate concentration was more than 200 Cmmol l^{-1}, inhibited cell growth and PHB formation of *Ralstonia eutropha* (Yu and Wang, 2001). At present, little is known about PHA production under conditions of both higher acid concentrations and lower temperatures.

The biological effects of the magnetic fields have attracted the attention of many researchers. Fojt et al. (2004) observed that the viability of *Escherichia coli* decreased with increasing exposure to a magnetic field. In the process of glucose fermentation, the glucose uptake rates and the ethanol production were increased by a magnetic field (Ivanova et al., 1996). Although there have been numerous studies on the effects of magnetic fields on microorganisms, including morphological modifications related to cell shape, cell surface,

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Nomenclature

q_S	specific acetate uptake rate in the feast period (Cmmol S (Cmmol X)$^{-1}$ h^{-1})
q_P	specific PHB synthesis rate in the feast period (Cmmol PHB (Cmmol X)$^{-1}$ h^{-1})
$Y_{P/S}$	yield of PHB on acetate (Cmmol PHB (Cmmol S)$^{-1}$)
$Y_{X/S}$	yield of X on acetate (Cmmol X (Cmmol S)$^{-1}$)
$q_P/(q_S - q_P)$	the ratio of specific PHB production rate over the difference between specific acetate uptake rate and specific PHB production rate

cytoskeleton and plasma membranes, growth, and enzyme activity (Buemi et al., 2001; Miyakoshi, 2005), many gaps still remain in our knowledge.

In our previous work (Chen and Li, 2008), a static magnetic exposure was shown to convincingly influence the biosynthesis of PHA from short-chain VFAs by activated sludge. Maximum PHB (poly-3-hydroxybutyrate, a representative compound of the PHAs) production occurred at 7 mT. In the present study, we have explored this phenomenon in more detail. PHB was again measured and acetate, the most easily assimilated carbon substrate for PHB production, was used as the feed. The activated sludge was collected from the Sibao municipal wastewater treatment plant in Hangzhou, China and was inoculated into SBRs (sequencing batch reactors) under a static 0 mT or 7 mT magnetic field. The aim of this study was to determine the effect of SMF on PHB production at high acetate concentration (260 Cmmol l^{-1}) and low temperature (10 °C) by activated sludge and try to provide an insight into the mechanisms underlying the magnetic field effects.

2. Materials and methods

2.1. SBR experiment

Two SBRs, with individual working volume of 1 l, were operated under magnetic field intensity of 0 mT (SBR1) and 7 mT (SBR2), respectively. They were connected to a respirometer respectively, with a working volume of 100 ml and an oxygen electrode was introduced. The SBRs were inoculated with activated sludge acclimatized with mixtures of acetate, propionate, and butyrate under an ADF (aerobic dynamic feeding) strategy. The volume ratio of acetate, propionate and butyrate was 2:1:2. The organic load was gradually increased from 10 to 180 Cmmol l^{-1}. The sludge acclimation period lasted about two moths. The SBR system was operated in cycles consisting of influent feeding (10 min), a period of feast phase followed by a famine phase, then final withdrawal (2 min). The ratio of 'feast' to 'famine' time was controlled at 1:3. The sludge retention time (SRT) was kept at 10 d. Oxygen was supplied by an air compressor through a ceramic membrane disperser installed inside the reactor bottom and this maintained dissolved oxygen concentration at around 80% of the saturation value. The reactor was operated without pH control and the temperature was maintained at 10 °C.

The sludge in the SBR was sampled at regular intervals, and acetate, PHB, pH, ammonium, and biomass concentrations were determined. Oxygen uptake rate (OUR) was also determined at the same intervals. Each test was performed in triplicate after a pseudo-steady state had been reached.

2.2. Static magnetic field exposure

The static magnetic field was generated by a pair of magnets. Each magnet was a square prism with two faces of 24 cm × 12 cm and thickness of 2.4 cm. They were placed parallel to each other and fixed onto the laboratory table. The SBR was located in the middle, so that a static magnetic field intensity of 7 mT was produced at the center of SBR.

2.3. Culture medium

The feed composition was as follows (mg l^{-1}): acetate (7800), NH_4Cl (321), K_2HPO_4 (79.5), KH_2PO_4 (64.5), $CaCl_2{\cdot}2H_2O$ (50), $MgSO_4{\cdot}7H_2O$ (100), $FeCl_3{\cdot}6H_2O$ (2), $ZnSO_4{\cdot}7H_2O$ (0.1), $MnCl_2{\cdot}4H_2O$ (0.03), $CoCl_2{\cdot}6H_2O$ (0.2), $NiCl_2{\cdot}6H_2O$ (0.02), H_3BO_3 (0.3), $CuCl_2{\cdot}2H_2O$ (0.01), $NaMoO_4{\cdot}2H_2O$ (0.03), Na_2EDTA (3). The ratio of C:N:P (on molar basis) was 200:5:1. Thiourea (20 mg l^{-1}) was also added to inhibit nitrification. The pH of the solution was adjusted to 7.0 before feeding.

2.4. Analytical techniques

Acetate was measured on filtered sample (0.45 μm porosity) by gas chromatography (Kexiao, GC-1690) equipped with a GDX-103 column (length: 2 m, diameter: 4 mm) and a flame ionization detector (FID). Nitrogen was used as the carrier gas. The temperature of the injector, column and detector were 220, 195 and 220 °C. Ammonium ion was measured by Nessler's reagent colorimetric method (CSEPA, 2002).

PHB determination was performed according to Chen and Li (2008). Briefly, 10 ml of culture was centrifuged, and the pellet was lyophilized and put into vials. Then 2 ml of acidified propanol (10% HCl) containing benzoic acid as the internal standard and 2 ml of 1,2-dichloroethane were added. The vial was closed, mixed completely and heated for 8 h in an oven at 105 °C. After cooling, distilled water was added for extraction, thereafter 1 μl of the sample was injected into gas chromatograph (Kexiao, GC-1690) at 220 °C, which was equipped with a FID and a 30 m × 0.32 mm Chrompack AT.SE-54 column. Nitrogen was used as the carrier gas. The detector (FID) temperature was 220 °C. Calibrations of PHB were done with a standard poly (3-hydroxybutyric-co-3-hydroxyvaleric acid) (12 wt% PHV) of natural origin (Sigma–Aldrich Chem.).

OUR was determined inside the respirometer according to the procedure reported by Dias et al. (2005), with minor modifications: the mixed liquor was continually recirculated between the batch reactor and the respirometer (with a recirculation rate of 300 ml min^{-1}) and, at given intervals, the circulation was suspended for about 2 min and the decrease of DO (Dissolved Oxygen) concentration was registered, enabling the OUR calculation.

The intensity of magnetic field was measured by Teslameter (Model 6010, Bell Technologies Inc.) in the middle of the reactor. The dry weight of biomass was measured as volatile suspended solids (VSS), according to standard methods (APHA, 1999). pH of sample was measured by pH meter (PHS-3C).

2.5. Calculations

The obtained data were used to calculate kinetic parameters. Active biomass (X) was calculated as g X = g VSS − g PHB and assumed to be represented by the typical molecular formula $CH_{1.8}O_{0.5}N_{0.2}$ (Beun et al., 2002). Content of