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Continuous cultivation of photosynthetic bacteria for fatty acids production

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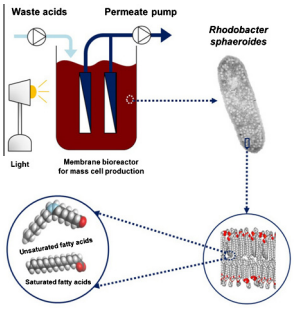
HIGHLIGHTS

- Photosynthetic bacteria cultivation for fatty acids (FA) production from lactate.
- Continuous-flow, stirred-tank reactor and membrane-coupled bioreactor.
- Maximum cell productivity of 1.9 g dcw/L/d and FA productivity of 665 mg FA/L/d.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present work, we introduced a novel approach for microbial fatty acids (FA) production. Photosynthetic bacteria, *Rhodobacter sphaeroides* KD131, were cultivated in a continuous-flow, stirred-tank reactor (CFSTR) at various substrate (lactate) concentrations. At hydraulic retention time (HRT) 4 d, cell concentration continuously increased from 0.97 g dcw/L to 2.05 g dcw/L as lactate concentration increased from 30 mM to 60 mM. At 70 mM, however, cell concentration fluctuated with incomplete substrate degradation. By installing a membrane unit to CFSTR, a stable performance was observed under much higher substrate loading (lactate 100 mM and HRT 1.5 d). A maximum cell concentration of 16.2 g dcw/L, cell productivity of 1.9 g dcw/L/d, and FA productivity of 665 mg FA/L/d were attained, and these values were comparable with those achieved using microalgae. The FA content of *R. sphaeroides* was around 35% of dry cell weight, mainly composed of vaccenic acid (C18:1, omega-7).

1. Introduction

Environmental concerns, energy shortage, and consequent increasing energy costs have emphasized the need to produce sustainable and renewable fuels (Steen et al., 2010). To this end, huge effort is now being focused on the production of lipids using microalgae (Chen et al., 2011). Under unfavorable culture conditions, they are able to store neutral lipids, 20–70% of their body in heterotrophic or autotrophic ways, mainly in the form of triacylglycerol (TAG). TAG is convenient storage compound for carbon and energy, possessing high calorific value, and can be used as industrial chemical and bioenergy feedstock (Alvarez and Steinbuchel, 2002).

On the other hand, although photosynthetic bacteria do not accumulate neutral lipids, they are able to synthesize fatty acids, principally for glycerol-based membrane lipids (Carlozzi et al., 2010). The oil content (20–40% of dry biomass weight) of

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photosynthetic bacteria is generally lower than that of microalgae, and therefore, the research on this subject has been scarce. However, they are simpler to cultivate than microalgae that they do not require stressful environments for lipids production. In addition, bacteria are much more genetically manipulatable than algae. The expression of specific genes could result in the overproduction of fatty acids and their secretion to the broth (Steen et al., 2010).

Until now, the use of purple non-sulfur phototrophic bacteria has been proposed mainly for the production of hydrogen and polyhydroxybutyrate (PHB), as well as for wastewater treatment (Khatipov et al., 1998; Kim et al., 2006; Wu et al., 2012). Electrons contained in organic materials can be released as hydrogen by nitrogenase with the help of light energy, and PHB is formed when PNS bacteria are faced with a suboptimal environment. Recently, the co-production of hydrogen and lipids under anaerobic conditions was suggested (Carlozzi et al., 2010). During the cultivation of *Rhodopseudomonas palustris* under anaerobic light conditions, lipid content of 22–39% of dry biomass weight was observed. However, this study was limited to batch operation: it is important to maximize the lipid productivity by continuous operation. In addition, the effective use and design of a continuous culture are known to lower the production cost (Chen et al., 2011).

During the production of photosynthetic bacteria in a continuous culture system, the ability to maintain a balanced concentration of chemical substances and cells is critical to obtain higher productivity than in a batch culture system. However, there are many difficulties in maintaining a continuous culture for long periods without mutation and microbial contamination. Population changes due to mutation or microbial contamination in the pure culture of a microbial strain have frequently been reported in continuous culture systems (Toda, 2003).

The use of a membrane-coupled bioreactor has recently gained recognition as a solution to the above problems by decreasing contamination risks and cleaning frequencies, and by higher cell concentration compared to a standard stirred tank bioreactor (Glazyrina et al., 2010). In addition, it is possible to reuse the water and nutrients in the permeate from the membrane filter by retaining cells that are larger than the diameter of membrane pore. Recent works employing various microorganisms immobilized in membrane-based culture systems have shown that extremely high, sludge-like densities of ca. 10^{12} cells per ml were possible in such systems and that the productivity of membrane reactors continues at high levels for more than 2 weeks (Lee et al., 2008; Ríos et al., 2012).

In this study, we suggested a novel approach for microbial fatty acids production, which is competitive with a traditional way of using microalgae. Photosynthetic bacteria, *Rhodobacter sphaeroides* KD131, were cultivated for fatty acids production using lactate as a substrate. Organic acids that mostly consisted of lactate can be easily obtained from fermentation products in agricultural and food waste (Kim et al., 2009). To maximize the cell productivity, a continuous-flow, stirred-tank reactor (CFSTR) was operated at various substrate concentrations and hydraulic retention times (HRT). A membrane unit was installed in the CFSTR for further increase of cell concentration and productivity. To our knowledge, this is the first attempt to apply the concept of a membrane-coupled bioreactor aiming at continuous cell harvesting. In addition, the fatty acids profile of the bacteria was assayed and the performance obtained in this study was compared with that using microalgae.

2. Methods

2.1. Inoculum preparation

The phototropic bacterium *R. sphaeroides* KD131 isolated from mudflats along the coast of Daebu Island in the West Sea of South Korea was cultivated for fatty acids production (Kim et al., 2012). The KD131 strain was pre-cultured in a modified Sistrom's broth (Kim et al., 2012) containing 4 mM $(NH_4)_2SO_4$, 0.3 mM L-aspartic acid, and 20 mM lactate at 30 °C for 24 h under 54 W/m^2 irradiance using a halogen lamp (12 V, 50 W).

2.2. Reactor operation

A 1.2 L glass reactor (effective volume of 1.0 L, 200 mm high by 80 mm diameter) installed with a membrane unit was designed for the continuous cultivation of *R. sphaeroides* (Fig. 1). Three halogen lamps were properly located to adjust the light intensity at 54 W/m^2 on the reactor wall. The membranes are made of high density polyethylene (HDPE) with a normal pore size of 0.4 μm and an effective filtration area of 0.006 m^2. A certain amount of centrifuged microorganism was inoculated to reach an initial cell concentration of 0.5 g dcw/L. After purging with Ar gas for 1 h, the reactor was operated for 48 h by batch mode, and then switched to a continuous mode. It was agitated using a magnetic stirrer at 150 rpm. Feedstock contained 30 mM of lactate with nutrient medium as mentioned above. All experiments were conducted in a constant temperature room at 30 ± 1 °C.

First, the reactor was operated by a CFSTR mode (w/o membrane unit operation). Lactate concentration was gradually increased from 30 mM to 70 mM at a fixed HRT 4 d. As the performance failure was observed at 70 mM, lactate concentration was decreased to 40 mM, and the reactor was operated as membrane-coupled bioreactor mode. Once a day, 50 mL of cell broth, which was harvested for fatty acids production, was pulled out corresponding to a cell retention time (CRT) of 20 d, while HRT was kept at 4 d. In order to increase substrate loading, first, substrate concentration was gradually increased up to 120 mM. Afterwards, HRT was gradually shortened to 1 d at a fixed lactate concentration of 100 mM. The CRT/HRT ratio was kept at 5. Permeation of membranes was continuously maintained by using a peristaltic pump according to the HRT. One cycle of membrane filtration consisted of 45 min of filtration and 15 min of releasing. In order to reach the steady-state and to obtain average performance values, the reactor was operated for more than five times of HRT at each operating condition.

2.3. Analysis

Residual lactate was analyzed by a high performance liquid chromatograph (HPLC) (Finnigan Spectra SYSTEM LC, Thermo Electron Co.) with an ultraviolet (210 nm) detector (UV1000, Thermo Electron) and an 100 mm × 7.8 mm Fast Acid Analysis column (Bio-Rad Lab.) using 0.005 M H_2SO_4 as mobile phase. The liquid samples were pretreated with a 0.45 μm membrane filter before injection to both HPLCs. Cell concentration and fatty acids were measured according to the methods described in Kim et al. (2012) and Carlozzi et al. (2010), respectively.

3. Results and discussion

3.1. CFSTR performance

High substrate concentration allows energy-efficient operation and results in concentrated biomass unless substrate inhibition occurs. In microbial oil production, in particular, a high level of biomass concentration is essential for the ease of downstream processing such as harvesting, dewatering, and extracting (Chen et al., 2011).

Fig. 2 shows the daily performance of CFSTR in terms of cell concentration and lactate degradation at HRT 4 d. As lactate