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Short Communication

Enhanced energy conversion efficiency from high strength synthetic organic wastewater by sequential dark fermentative hydrogen production and algal lipid accumulation

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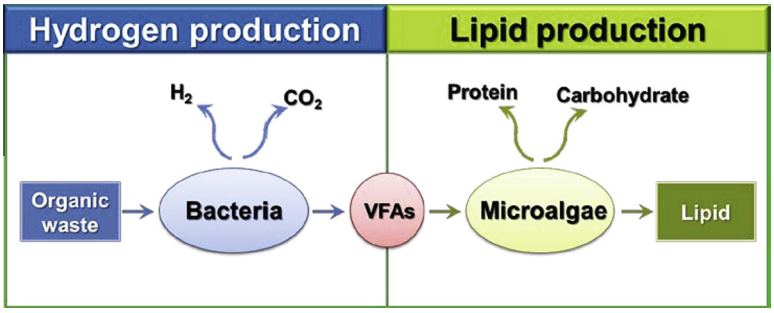
HIGHLIGHTS

- Dark fermentation and algal cultivation were combined to produce H_2 and lipid.
- The sequential system successfully converted wastewater into bioenergy.
- The performance of the two-stage process was evaluated for the first time.
- Energy conversion efficiency raised from 18.6% (single stage) to 37.4% (two-stage).

GRAPHICAL ABSTRACT



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ABSTRACT

A two-stage process of sequential dark fermentative hydrogen production and microalgal cultivation was applied to enhance the energy conversion efficiency from high strength synthetic organic wastewater. Ethanol fermentation bacterium *Ethanoligenens harbinense* B49 was used as hydrogen producer, and the energy conversion efficiency and chemical oxygen demand (COD) removal efficiency reached 18.6% and 28.3% in dark fermentation. Acetate was the main soluble product in dark fermentative effluent, which was further utilized by microalga *Scenedesmus* sp. R-16. The final algal biomass concentration reached 1.98 g L^{-1}, and the algal biomass was rich in lipid (40.9%) and low in protein (23.3%) and carbohydrate (11.9%). Compared with single dark fermentation stage, the energy conversion efficiency and COD removal efficiency of two-stage system remarkably increased 101% and 131%, respectively. This research provides a new approach for efficient energy production and wastewater treatment using a two-stage process combining dark fermentation and algal cultivation.

1. Introduction

The combustion of fossil fuels can result in the emission of greenhouse gases, causing the environmental pollution and global warming (Ren et al., 2013b; Williams and Laurens, 2010). Recently, some new technologies that can generate clean and effective energy alternatives to fossil fuels have been paid increasing attention. Microalgae-based biodiesel is one of the most promising alternatives to fossil fuels. Microalgae can accumulate 30–60% of their dry weight in the form of lipids that can be converted into biodiesel through transesterification reaction (Harwati et al., 2012). Further, microalgae grow much rapidly compared with terrestrial oilseed crops and can be cultured on land unsuitable for agriculture (Vandamme et al., 2014). To date, the major challenge in

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microalgae-based biodiesel production remains its relatively high costs of production, especially the cost of algal cultivation (Zhang et al., 2013). Therefore, cultivation of oleaginous microalgae with proper waste materials containing short chain organic acids is a promising way to overcome the bottleneck. In particular, the effluent from dark fermentative hydrogen production happens to satisfy the demand.

It is well known that hydrogen production by dark fermentation can convert the feedstock at a high rate, but the substrate conversion efficiency is low and the accumulation of volatile fatty acids (VFAs) induces a sharp drop in pH of fermentation medium that limits the production of hydrogen (Ren et al., 2012a; Singhania et al., 2013). More importantly, these VFAs may bring bad smell to the air and become a potential threat to the environment if they are not properly treated (Yang et al., 2010). Microalgae-based system grown in dark fermentative effluent might be a promising alternative to solve above problems. Heterotrophic microalgae can utilize a variety number of organic substrate as both carbon and energy sources and accumulate substantial quantities of lipids (Williams and Laurens, 2010). In this way, both the cost of algal cultivation and the potential threat of dark fermentative effluent to the environment can be lowered, and this also improves the energy conversion efficiency. At present, information about the integration of dark fermentation and microalgal lipid production with wastewater as feedstock is rather limited, and the energy conversion efficiency of the sequential system has not yet been evaluated.

In this study, a sequential process of dark fermentative hydrogen production and algal cultivation for lipid production was employed for conversion of wastewater into bioenergy. To simply investigate the factors related to this two-stage process, preliminary results were achieved using artificial wastewater rather than real wastewater. The synthetic wastewater was used by dark fermentative bacteria to produce hydrogen, and then algal lipid production using the dark fermentative effluent was studied. Furthermore, changes in the microalgal biomass, substrate utilization and biochemical compositions during the operation process were also investigated. Finally, the performance of the two-stage system was evaluated.

2. Methods

2.1. Microorganisms and culture conditions

Ethanoligenens harbinense B49 (AF481148 in EMBL) was obtained from Research Center of Environmental Biotechnology (HIT) and used in the dark fermentative hydrogen production (Wang et al., 2007). Synthetic wastewater with chemical oxygen demand (COD) of about 15.4 kg m^{-3} was used to simulate the high strength wastewater. Detailed composition of synthetic wastewater was described previously (Liu et al., 2009; Wang et al., 2007). *E. harbinense* B49 was cultured anaerobically at 35 °C for 35 h in a constant oscillator (130 rpm). Dark fermentation was performed in 100 ml reactors with a working volume of 80 ml in batch experiments. Inoculum of 10% (v/v) was added to each reactor and nitrogen was used to maintain the anaerobic condition. Green microalga *Scenedesmus* sp. R-16 was isolated from soil and employed for lipid production (Ren et al., 2013a). After dark fermentation, the effluent was firstly centrifuged at 12,000 rpm for 5 min, and filtered through a 0.22 μm membrane to remove the bacteria. The supernatant was collected and the pH was adjusted to 7.0. The 250 ml Erlenmeyer flasks containing 150 ml supernatant were used as reactors. *Scenedesmus* sp. R-16 in the stationary growth phase was added to reactors with the inoculation ratio of 10%. The flasks were shaken at 130 rpm for 144 h at constant temperature of 25 °C. All experiments were run in triplicate.

2.2. Hydrogen production and cell growth kinetics

Modified Gompertz equation was used to describe the cumulative hydrogen production progress in batch cultures. The time–course data of cumulative hydrogen production was simulated by modified Gompertz equation (Eq. (1)) to determine the kinetic parameters in dark fermentation.

$$H = H_{max} \exp\left\{-\exp\left[\frac{R_{max}e}{H_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where H denotes cumulative hydrogen production (ml H_2 L^{-1} of culture), H_{max} denotes maximum cumulative hydrogen production (ml H_2 L^{-1} of culture), R_{max} denotes maximum hydrogen production rate (ml L^{-1} h^{-1}), t denotes culture time (h), λ denotes duration time of the lag-phase (h), e = 2.71828.

Logistic equation was applied to interpret the relationship between the microalgal growth and cell concentration as shown in Eq. (2).

$$N = \frac{K}{1 + e^{a - rt}} \quad (2)$$

where N (g L^{-1}) denotes the algal cell concentration at time t (h); K (g L^{-1}) denotes the carrying capacity (the maximum algal cell concentration obtained in the culture); r (h^{-1}) denotes the specific growth rate; a is a constant.

2.3. Analytical methods

Biogas was sampled from the head space of the reactor with a gas-tight glass syringes. The hydrogen content was measured by a gas chromatograph (Agilent 4890D, USA) equipped with a thermal conductivity detector (TCD). The operational temperatures of injection, oven and TCD were 120, 35, and 120 °C, respectively. Argon was adopted as the carrying gas at a flow rate of 30 ml min^{-1}. The biomass concentration in dry weight (DW) was determined by filtering samples through a 0.22 μm Millipore filter. Afterwards, the samples were dried at 105 °C until constant weight was obtained. The lipid, carbohydrate and protein contents of microalgae were determined as the methods in the literature (Lv et al., 2010; Ren et al., 2013a; Wen et al., 2013). The pH value was monitored using a pHS-3C pH meter (Shanghai Leici Instrument Factory, China). COD was measured according to the method described previously (Ren et al., 2006). Residual VFAs in the dark fermentative effluent were determined by a second gas chromatograph (Agilent 7890A, USA) equipped with a flame ionization detector (FID). Before the analysis, the liquor samples were centrifuged at 12,000 rpm for 5 min, and filtered through a 0.22 μm membrane. The operational temperatures of injection port, column and FID were 250, 300, and 300 °C, respectively. Nitrogen was adopted as carrier gas at flow rate of 50 ml min^{-1}.

3. Results and discussion

3.1. Hydrogen production by dark fermentation

It has been demonstrated that ethanol-type fermentation was the optimal fermentation type for hydrogen production and has high hydrogen production capacity, and thus the typical strain of hydrogen–ethanol coproducing fermentation, *E. harbinense* B49, was used in this study (Liu et al., 2009; Ren et al., 2006). Fig. 1a presents cumulative dark fermentative hydrogen production curve fitted by the modified Gompertz equation. Kinetic analysis showed that hydrogen production by strain B49 was well correlated to the modified Gompertz equation ($R^2 > 0.99$). The maximum cumulative hydrogen production (H_{max}) and maximum hydrogen