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Favorable energy conversion efficiency of coupling dark fermentation and microalgae production from food wastes



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long-term operation.

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Keywords: Energy conversion efficiency Food waste Hydrogen production Lipid accumulation Long-term operation Microalgae	A coupled system of dark fermentation and microalgal cultivation was applied to investigate the hydrogen and lipid production potentials from three main substrates of food waste. Compared with waste proteins and waste fats, waste carbohydrates were more appropriate for energy production, which exhibited the highest cumulative hydrogen volume of $668.3 \pm 36.5 \text{ mL L}^{-1}$ and hydrogen yield of $133.66 \pm 7.31 \text{ mL g}^{-1}$ substrate. Most by-products of dark fermentation were consumed in algal culture process, and the maximum lipid content and lipid productivity reached $33.2 \pm 1.8\%$ and $52.6 \pm 1.1 \text{ mg L}^{-1} \text{ d}^{-1}$, respectively. The energy conversion efficiency significantly enhanced from 10.14% (dark fermentation) to 24.06% (coupled system). In continuous operation, energy production was steady and efficient, and the average hydrogen production rate of $741.4 \text{ mL L}^{-1} \text{ d}^{-1}$ and average lipid concentration of 0.4 g L^{-1} were obtained. This study provides a promising and sustainable route for efficient energy recovery from waste substrates by using coupled hydrogen and lipid production system in

1. Introduction

Hydrogen is widely considered as a promising alternative energy carrier due to its clean, efficient and non-polluting characteristics [1-3]. Generally, hydrogen gas can be generated from steam reforming of hydrocarbons, electrolysis of water and auto-thermal processes [4,5]. However, these methods are cost-intensive owing to high-energy requirements. Much attention has recently been paid to produce hydrogen through dark fermentation because this process is renewable and environmentally friendly [6-8]. In addition, dark fermentation can utilize low-value materials as fermentation substrates, which can contribute to reducing the cost of biohydrogen production.

The quantity of food waste collected and disposed in China amounts to 65,000 tons per day, and the main disposal method for food waste is landfill (90.5%) [9]. Food waste contains high contents of volatile solids (85–95%) and moisture content (75–85%), which can create a series of problems such as putrid smells and leachate polluting underground waters in the landfill treatment process [4]. As a result, food waste has become one of the most serious environmental problems [10–12]. Main compositions of food waste, such as carbohydrates, proteins and fats, are potential sources for hydrogen production [13,14]. In some studies, the feasibility of dark fermentative hydrogen production from food waste has been reported [6,15].

Food waste consists mainly of starch and protein which make food

waste to be an economical source for biofuels production. However, nutrients stored in food waste are in the form of macromolecules (such as starch and protein) which have to be broken into utilizable forms (glucose and free amino nitrogen) before utilized by microorganisms for fermentative hydrogen production. Han et al. developed a novel combined bioprocess based on enzymatic hydrolysis and dark fermentation for hydrogen production (and ethanol production) from food waste (and bakery waste) which could effectively accelerate the hydrolysis speed, improve nutrient conversion efficiency and increase hydrogen production [16]. Nevertheless, dark fermentation application has been limited by the low energy conversion efficiencies since many soluble metabolites, such as volatile fatty acids (VFAs), are produced during the hydrogen production process from food waste.

Compared with dark fermentation alone, a two-stage process of coupled dark fermentation and algal cultivation is more promising to improve the energy conversion efficiency (Fig. 1). Microalgal biomass can be produced by using simple organic compounds (e.g., VFAs) as both carbon and energy sources through heterotrophic culture [17–19]. Moreover, VFAs can be converted into acetyl-CoA, which is a direct precursor for lipid synthesis in algal cells [20–22]. The effluent of dark fermentation contains large amounts of VFAs and can act as suitable feedstock for algal growth and lipid accumulation. To date, little information is available on the hydrogen and lipid production characteristics from food waste by coupling dark fermentation and

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Fig. 1. Schematic diagram of the coupled system of dark fermentation and algal culture from food waste.

microalgal culture.

Therefore, this study investigated the energy production potentials of three main types of food waste in coupled system, including waste carbohydrates (WCS), waste proteins (WPS) and waste fats (WFS). The waste substrates were utilized in dark fermentation to produce biohydrogen and short chain organic acids, which were further converted into microalgal biomass containing lipids. The energy conversion efficiencies of various waste substrates were examined. Furthermore, the performance of the coupled system in long-term operation was evaluated.

2. Materials and methods

2.1. Bacteria and microalgae

The seed sludge used in the present study was obtained from a wastewater treatment plant in Harbin, China. For hydrogen production, the sludge was heat-treated at 100 °C for 60 min to inhibit the hydrogen-consuming microorganisms and to enrich the hydrogen-producing bacteria [23]. The microalgal strain *Scenedesmus* sp. R-16 with high lipid production capacity was adopted in all experiments [24]. The microalgal cells were cultured on glucose supplemented BG11 medium at temperature of 25 ± 1 °C in dark environment [24]. Methanogenic bacteria were enriched from the sludge and used as the inoculum for methanogenesis [8].

2.2. Batch and continuous operations

2.2.1. Batch culture mode

Hydrogen production in batch experiments were conducted in 250 mL reactors with a working volume of 150 mL. According to previous studies [13,25], three substrates (potato powder, peptone, and glycerol) were used to simulate WCS, WPS and WFS, respectively. The initial concentration of WCS, WPS and WFS in each experiment was set at 5.0 g L^{-1} . Other mineral and vitamin solution were prepared according to the methods described in the literature [25,26]. The initial pH of the culture medium was adjusted to 6.5 \pm 0.2 and nitrogen gas was applied to create anaerobic environment in dark fermentation. Temperature of dark fermentation was kept at 35 \pm 1 °C using a temperature-controlled shaker (130 rpm). Effluents from dark fermentation were collected and used to cultivate microalgae. Prior to use, the effluents were centrifuged at 9000g for 10 min to remove the solids and the pH of supernatant was adjusted to 7.0 \pm 0.2. The reactors for microalgal cultivation were shaken at 130 rpm at constant temperature of 25 ± 1 °C in darkness [27]. Each test was run in triplicate. To compare the mean of hydrogen and lipid yields, the data were statistically analyzed using Analysis of Variance (ANOVA) [28,29].

2.2.2. Continuous culture mode

Continuous hydrogen production was conducted in 1.0 L anaerobic sequencing batch reactor (ASBR) and the working volume was 0.6 L. The operation temperature, feedstock concentration, and hydraulic retention time (HRT) used in continuous mode were 35 ± 1 °C, $5.0 \,\mathrm{g \, L^{-1}}$, and 24 h, respectively. In every cycle of operation, half (0.3 L) of culture medium was replaced by fresh medium of 0.3 L. Sequencing batch reactor (SBR) with total volume of 1.0 L and working volume of 0.6 L was applied in the continuous algal lipid production. The SBR was started with batch culture period of 96 h. After that, half of the medium (0.3 L) was removed and the same volume of dark fermentative effluent medium was supplied. This process was repeated every 48 h in the total cultivation period and the HRT was 96 h. On the other hand, the waste bread used in this study contained 46.5% starch, 11.5% protein, 3% fat, 37% water, and 2% ash. The initial concentrations of TN and TP in waste bread medium were 100 and 20 mg L^{-1} , respectively. The dark fermentative effluent from waste bread was used as the feed for methane production. The HRT and temperature for methane fermentation were 288 h and 35 \pm 1 °C, respectively.

2.3. Energy conversion analysis

The specific heat values of hydrogen, lipids, WCS, WPS, and WFS were 142.0, 36.3, 16.7, 23.0, and 17.28 kJ g^{-1} , respectively [20,30–32]. The energy conversion efficiency (ECE) and total energy conversion efficiency (TECE) were calculated according to Eqs. (1) and (2), respectively.

$$ECE (\%) = \frac{\text{Heat value of hydrogen (kJ) or Heat value of lipids (kJ)}}{\text{Heat value of substrate (kJ)}} \times 100\%$$
(1)
$$TECE (\%) = \frac{\text{Heat value of hydrogen (kJ) + Heat value of lipids (kJ)}}{\text{Heat value of substrate (kJ)}}$$

2.4. Analytical methods

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The pH value of the culture medium was adjusted with 1 M NaOH or 1 M HCl solution to obtain the desired initial pH, and the pH was monitored with a digital pH meter (Mettler FE20K, Switzerland). The produced gas was sampled from the head space of the reactors by using a gas-tight glass syringe. Hydrogen and methane contents were determined by a gas chromatograph (Agilent 4890D, USA) equipped with thermal conductivity detector using argon as the carrier gas at the flow rate of 30 mL min⁻¹. Temperatures of injection, oven and detector were 120, 35, and 120 °C, respectively. Biomass concentration was measured by filtering samples through 0.22 μ m Millipore filter, drying at 105 °C,

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