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Improving production of volatile fatty acids and hydrogen from microalgae and rice residue: Effects of physicochemical characteristics and mix ratios

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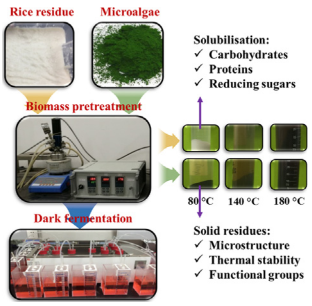
HIGHLIGHTS

- Mixed biomass of rice residue and microalgae was used as co-fermentation substrate.
- Physiochemical characteristics of solid hydrolytic residues were analysed.
- Hydrolysis efficiency of polysaccharide in rice residue was 100%.
- The highest H_2 yield of 201.8 mL/g VS was achieved at a mix ratio of 5:1.
- Energy and carbon conversion efficiencies reached up to 90.8% and 96.8%.

GRAPHICAL ABSTRACT



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ABSTRACT

Dark fermentation may be hindered by insufficient bioavailable carbon and nitrogen sources as well as recalcitrant cell wall structures of substrates. Protein-rich microalgae and carbohydrate-rich rice residue with various mix ratios can optimise biohydrogen and volatile fatty acids production. Optimal pretreatment of the microalgae with 1% H_2SO_4 and the rice residue with 0.5% H_2SO_4 under hydrothermal heating (140 °C, 10 min) achieved reducing sugar yields of 187.3 mg/g volatile solids (VS) (hydrolysis efficiency: 54%) and 924.9 mg/g VS (hydrolysis efficiency: 100%), respectively. Multiscale physiochemical characterisations of solid hydrolytic residues confirmed considerable damage to both substrates. Co-fermentation of pretreated rice residue and microalgae at a mix ratio of 5:1 exhibited the maximum hydrogen yield of 201.8 mL/g VS, a 10.7-fold increase compared to mono-fermentation of pretreated microalgae. The mix ratio of 25:1 resulted in the highest carbon to volatile fatty acids conversion (96.8%), corresponding to a maximum energy conversion efficiency of 90.8%.

1. Introduction

Dark fermentation (DF) is considered as a promising bioconversion technology, in which various types of biomass and organic wastes can be converted to volatile fatty acids (VFAs) and hydrogen by anaerobic microorganisms at relatively low temperature (35–55 °C) and ambient pressure [1,2]. The large amounts of VFAs contained in the DF effluents provide valuable raw materials for the downstream microbial factories

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to produce biofuels (such as biomethane) and biochemicals (such as polyhydroxyalkanoate) [3]. Additionally, compared with energy-intensive hydrogen production methods (such as steam reforming of methane), hydrogen produced via DF is more sustainable and environmentally friendly [2]. Notably, the choice of substrates is the key factor to enhance VFAs and hydrogen production.

Microalgae are a promising biomass substrate due to their fast growth rate, superior CO_2 fixation capacity, and lack of any requirement for arable land for cultivation [4–6]. When microalgae are used as the single substrate during DF, the specific hydrogen yields are generally as low as 7.1–113.1 mL/g VS [7]. This is mainly caused by the following two reasons: (1) the high protein content (10%–84%) in microalgae with low biodegradable carbon sources [7] and, (2) the low release and hydrolysis of intracellular high-molecular compositions [8,9].

Amino acids derived from proteolysis are essential nitrogen sources for microbial growth. Moderate amounts of amino acids can improve the bioactivity of hydrogen producing bacteria (HPB) and enhance the DF performance. However, amino acids are inefficient substrates for DF with negligible hydrogen production (around 0.5 mL/g VS) [10]. Meanwhile, amino acids can be further degraded to ammonia nitrogen (NH_4^+—N); excess proteins would ultimately cause the significant accumulation of ammonia nitrogen, which may inhibit the physiological metabolism of HPB [11–13]. On account of this, co-fermentation of protein-rich microalgae and carbohydrate-rich biomass can increase the bioavailable carbon sources, thereby achieving efficient VFAs and hydrogen production [14]. Rice residue (RR), which is typically the dominant composition of food waste in China, is considered as a potential candidate because of its high biodegradability and high carbohydrate content. In 2015, the amount of rice wasted at the “dinner table” in China was around 4.5 Mt, equivalently to 30% of total food waste [15]. Co-fermentation of microalgae and RR not only can treat the environmental problem of food waste, but also can provide VFAs and carbon-free biohydrogen. However, to the best of our knowledge, such a process has yet to be reported.

High-molecular weight substrates in biomass (such as polysaccharides and proteins) are surrounded by a rigid cell wall structure that is hard for HPB to access and degrade [16,17]. In order to release and hydrolyse the intracellular organic matter, the biomass cell wall should be disrupted prior to DF. Various pretreatments such as thermochemical, physical, and biological technologies have been employed to hydrolyse biomass [18]. Among them, hydrothermal acid pretreatment is considered as a simple and efficient method with a high hydrolysis rate of converting high-molecular weight substrates to low-molecular ones (such as glucose and amino acid) [7,14,19]. Notably, most of these previous studies just focused on the yield of organic matter solubilisation, while the physicochemical characteristics of solid biomass hydrolytic residues were rarely investigated. Mendez et al. found that about 30% of carbohydrates and 45% of proteins still remained in the solid residues [19]; these organic materials can be also effectively used to produce VFAs and hydrogen via DF. Additionally, solid residues with different chemical composition can directly affect the bioaccessibility of biomass hydrolysates [20], and the changes of functional groups can be used to analyse fermentation inhibitors (such as furans and phenols) produced by pretreatment [21].

Table 1
Characteristics of rice residue pulp and *Chlorella pyrenoidosa* powder.

Parameters	Rice residue	*Chlorella pyrenoidosa*
Proximate analysis		
Moisture (wt%)	80.25 ± 2.38	5.96 ± 0.22
TS (wt%)	19.75 ± 2.38	94.04 ± 0.22
VS (wt%)	19.67 ± 0.00	79.83 ± 0.35
VS/TS (%)	99.61 ± 0.01	84.89 ± 0.32
Ultimate analysis		
C (VS%)	43.28 ± 0.00	50.19 ± 0.14
H (VS%)	6.01 ± 0.05	6.18 ± 0.34
O (VS%)	48.74 ± 0.02	33.14 ± 0.11
N (VS%)	1.69 ± 0.00	9.22 ± 0.07
S (VS%)	0.28 ± 0.02	1.27 ± 0.02
C/N molar ratio	29.81 ± 0.01	6.35 ± 0.03
Energy value (kJ/g VS)	16.92 ± 0.06	21.28 ± 0.39
tCOD (mg/g VS)	1124.59 ± 52.31	1439.63 ± 47.15
tCarbohydrates (mg/g VS)	913.76 ± 33.16	347.94 ± 16.35
tProteins (mg/g VS)	123.88 ± 14.27	480.86 ± 16.59
sCOD (mg/g VS)	26.31 ± 3.06	121.45 ± 7.86
sCarbohydrates (mg/g VS)	4.22 ± 0.95	54.31 ± 9.64
sProteins (mg/g VS)	12.55 ± 1.82	30.27 ± 6.13
Reducing sugars (mg/g VS)	1.91 ± 0.02	2.94 ± 0.36

The abbreviation referred to total (t) and solubilised (s) matters.

In this study, microalgae *Chlorella pyrenoidosa* (CP) and RR separately pretreated by diluted sulphuric acid catalysis under a hydrothermal environment were used as mixed substrates to improve the performance of fermentative VFAs and hydrogen production. The objects of this study are to:

- Assess the effects of pretreatment parameters on the hydrolysis characteristics of microalgae and rice residue.
- Compare the changes of surface microstructures, thermal stability, chemical composition and functional groups between raw biomass and solid hydrolytic residues.
- Evaluate the effects of mix ratios of microalgae and rice residue on VFAs and hydrogen production during dark fermentation.
- Analyse the carbon conversion efficiency (CCE) and energy conversion efficiency (ECE) of dark fermentation.

2. Materials and methods

2.1. Substrates and inocula

RR was collected from a dining hall located in Chongqing University, China. The collected RR was washed with deionized water to remove the attached greases and then blended into pulp in a blender. RR pulp was stored in a refrigerator at −20 °C before use. CP powder was purchased from a microalgae production plant located in Shandong Province, China. The purchased CP powder was stored in a cool and dry place at room temperature before use. The characteristics of RR pulp and CP powder are outlined in Table 1.

HPB were sourced from a rural household digester in Chongqing, China. The original sludge was heated in an autoclave at 100 °C for 30 min to inactivate methanogens and to obtain the spore-forming HPB. Subsequently, the spore-forming HPB were acclimatised 3 times (3 days each time interval) using a modified culture medium [22] at 35.0 ± 0.5 °C under an anaerobic environment. Total solids (TS) and VS of the activated HPB were 106.21 and 61.56 g/kg fresh weight, respectively.

2.2. Pretreatment of substrates

The separate pretreatment of RR or CP was conducted in triplicate in a reaction kettle (QN-WCGF, Taikang, China) with a working volume of 50 mL. The effects of reaction parameters, including reaction temperature (80–180 °C), concentration of sulphuric acid (0–5%), reaction time (0–120 min), and substrate concentration (12.5–150 g TS/L), on the yields of organic matter solubilisation including solubilised chemical oxygen demand (COD), carbohydrates, proteins, and reducing sugars in the supernatants of hydrolysates were evaluated. Raw RR and CP without any pretreatments were also assessed. During the experimental process, the time at which the desired temperature was reached in the reaction kettle was considered as time 0. The substrate concentration was defined as the required substrate level of RR or CP to 50 mL deionized water. After pretreatment, the hydrolysates composed of solubilised matters and solid residues were neutralised using 3 M