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Evaluation of different pretreatments on organic matter solubilization and hydrogen fermentation of mixed microalgae consortia

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ABSTRACT

This study investigated the effects of pretreatment methods (such as autoclave, ultra-sonication and electrolysis) of mixed microalgae consortia (predominantly composed of *Scenedesmus* followed by *Chlorella* species) from natural ecological niche. In addition, the cultivated biomass (wet) was subsequently utilized for fermentative H_2 production in mesophilic regime. The results showed that peak hydrogen production rate (HPR) and hydrogen yield (HY) were achieved from electrolysis pretreated algal consortia as 236 ± 14 mL/L-d and 37.7 ± 0.4 mL/g (volatile solids) VS_{added}, whereas the untreated algal consortia resulted in the turnout as 64 ± 5 mL/L-d and 9.5 ± 0.0 mL/g VS_{added}, respectively. The significant increment observed in HPR and HY values were nearly 4 times higher. The solubilization of organic matter during the pretreatment showed positive correlation with the H_2 production. The energy generation rate and yield of the corresponding pretreatment methods were as follows, 1.44, 1.79 and 2.65 kJ/L-d for autoclave, ultra-sonication and electrolysis, the corresponding yields also fell in the range of 0.32, 0.41 and 0.43 kJ/g VS_{added}, respectively.

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Introduction

The demand for sustainable clean energy sources and the depletion of fossil fuels are growing side by side in the last few decades. Recent trend in the prospecting of renewable energy sources has found that algal biomass could be a feasible feedstock than terrestrial and lignocellulose biomass. Algal biomass is abundant, bear high protein, lipid and carbohydrate content [1–4]. Besides, bio-H_2 production from algae biomass, particularly for the species rich in carbohydrate via a process called dark fermentation could contribute to the future energy demand and also carbon neutral society owing to easy cultivation methods, high CO_2 fixation ability and the nutrient values of the various algae species [5–10].

Algal biomass can be cultivated on vast tracts of photobioreactors by using sunlight as source of energy. However, the design of photobioreactor is expensive, thus limits the bioenergy utopia of algal biomass [8]. An efficient photobioreactor design could enhance the research niche of algal biomass to energy carriers such as H_2, CH_4 and ethanol [6]. In a recent study, using plastic bags, conical flask and plastic bottles were investigated and proved that plastic bags are cost effective photo reactors and also suggested as efficient strategy [2]. However, during large scale operations the possibilities in the outdoor environment should be accounted. Conversion of pure strains of microalgae species to H_2 by pure cultures of hydrogen producing bacteria has been reported earlier [9,10]. However, in terms of industrial perspective using pure cultures of algal species would be economically not feasible due to the sterilization issue to avoid the contamination by alien species. Thus, using mixed culture biotechnology and also mixed microalgal species for H_2 production has been suggested to be a suitable operation [11–14].

The cell envelope of algae is considered as rigid and not easily destructible. Pretreatment of algal biomass is in need to degrade the recalcitrant cell wall and get access to the sugars which would be turned into biofuels [15–17]. Many studies have reported that pretreatment has increased the organic matter, protein, carbohydrate content and also the energy production [16,17]. For example, a study by Passos et al., compared the different pretreatments such as thermal, hydrothermal, microwave and ultrasound increased the energy production up to 72% compared to non-treated biomass [17]. In another study by Batista et al., wet and dry *Scendesmus obliquus* biomass has been fermented by *Clostridium buytricum* to 113.1 mL H_2 and by *Enterobacter aerogens* to 56.7 ml H_2 while pretreated by autoclave (121 °C) for 15 min [9].

Passos et al., reported that an increase of 20.6% in terms of volatile solids (VS) solubilization while exposing the biomass to thermal treatment (Temperature range 55–95 °C, and exposure time 5–15 h) [18]. Cho et al., investigated various degradation methods like ultrasonication, thermal, and alkali on a mixed microalgal biomass and found that thermal treatment was effective with the enhancement in methane yield of 20%, which accounted 0.40 L CH_4/g VS [19]. A recent investigation also reported that hydrothermal pretreatment has increased the VS solubilization to 15% while the best condition is adapted as 130 °C for the duration of 15 min [20].

Therefore, this work seeks to advance and investigate the efficient cultivation of mixed microalgae consortia in cost effective photo reactors (plastic bags) and subsequent utilization of the cultivated biomass for biohydrogen production. Three major pretreatment methods were opted to increase the production performances, such as autoclave, ultrasonication and electrolysis. The hydrogen production from mixed microalgal biomass using electrolysis as pretreatment has been reported first time here.

Materials and methods

Collection and cultivation of microalgae consortia and seed inoculum

Microalgae consortia was collected from aquatic niches near Kasumigaura Lakeside where the Bio-eco Engineering Laboratory of National Institute for Environmental Studies (NIES) is located, in Japan. The consortia was collected by a mesh net (10–20 μm) in a plastic container and then stored in vials prior to inoculation at room temperature. Collected microalgae consortia was sieved using a mesh filter in order to remove the impurities such as dust, sand insect larvae others. There forward, the consortia was grown in Bold's basal medium as mentioned earlier [21,22]. The mixed microalgae consortia were cultivated in plastic bags (25 cm in height and 5 cm in diameter) with the light intensity of 8000–9000 lux using normal lamps, besides, the incubation period was followed by 12 h dark and 12 h photo period using a controller at room temperature of 23 ± 1 °C. The reactors were aerated at a flow rate of 2 L/min (air contains 0.035% CO_2 in the inlet) using air spargers (stone diffusers) and keeping the same culture conditions. Biomass growth was measured by following the optical density (OD) values at 600 nm (UV/Visible spectrophotometer Schimadzu) [14].

The seed inoculum was collected from a reactor which was fed with *Egeria densa* as feedstock, thus it is adapted well for the cellulose biomass. The parameters are provided in Table 1. Heat treatment (100 °C for 1 h in an incubator) was opted as

Table 1 – Parameters of inoculum and mixed microalgae consortia used in this study.

Parameter	Unit	Inoculum	Microalgae consortia
pH	–	7.2 ± 0.5	8.7 ± 0.6
TS	g/L	20.6 ± 3.8	20.4 ± 1.6
VS	g/L	11.7 ± 2.5	13.6 ± 1.2
T-COD	g/L	24.7 ± 3.1	26.7 ± 1.5
S-COD	g/L	1.1 ± 0.2	1.3 ± 0.6
Total protein	g/L	2.7 ± 0.5	3.4 ± 0.9
Total carbohydrate	g/L	3.5 ± 0.6	6.5 ± 1.5
Elementary analysis			
C	–	ND	39.2 ± 1.4
H	–	ND	5.8 ± 0.9
N	–	ND	5.6 ± 1.0
S	–	ND	0.3 ± 0.1
O	–	ND	17.8 ± 2.6
C/N ratio		ND	7.1 ± 0.8

ND: Not detected/determined.