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ORIGINAL RESEARCH ARTICLE

Harnessing of bioenergy from different mixed microalgae consortia obtained from natural ecological niches

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This study investigated the potential and possibility of three mixed microalgae consortia collected from various ecological niches towards bioenergy production of H_2 and CH_4 in batch experiments under mesophilic conditions. Three different consortia collected from wastewater treatment plant, in an open pond system and in the lake bed possess different compositions and characteristics during their growth under a light intensity of 8000 lux and are referred as OP1, OP2 and LB. After 40 days of cultivation, collected wet biomass is directly used for H_2 and CH_4 fermentation and the results indicated that, consortia OP1 is good for H_2 production whereas consortia OP2 and LB showed nearly the similar CH_4 production performances. Peak hydrogen production rate (HPR) and methane production rate (MPR) were achieved as 289 mL/L d and 97 mL/L d, respectively from consortia OP1 and OP2. The energy production from this process could significantly contribute towards CO_2 emission reduction. Besides, this approach could be helpful in choosing the consortia towards which kind of biofuel (either H_2 or CH_4) production.

Introduction

Energy demand and environmental pollution are two major threatening factors that have to be considered the prior to make sustainable and better world. Due to the shortage in fossil fuels supply, and CO_2 emissions caused by burning them have urged environmentalists to find alternative energy sources which are clean and renewable in nature [5,8,13,22,24]. In this regard, algae biofuels are emerging as clean energy source due to their potentials/features such as high biomass productivity, carbohydrate and lipid content, low-cost nutrient medium for growth, and majorly the consumption of CO_2 for their growth [5,4,7–9].

Hydrogen and methane production from various organic sources have been reported to have positive effect on the environment and also economically efficient [9,19,20,23]. Very recently these energy carriers are produced from algal biomass such as micro and macro algal biomasses [2,12,18] and also proved to be the future direction of energy production. Selection of algal biomass for the biofuel production is important, since different algal biomass bearing different characteristics and also the content of lipid, carbohydrate and other fatty acids vary widely [6,21].

Therefore, this work seeks to advance the H_2 and CH_4 production from various microalgae consortia collected from different ecological niche and cultivated in cost-effective photo reactors. Besides, the potential in terms of production rate also determined. Thus, this work would provide some insights to the biofuel production from algal biomass.

Materials and methods

Microalgae consortia collection and characterization

Microalgae consortia were collected from aquatic environments near Kasumigaura Lakeside where the Bio-eco Engineering Laboratory of National Institute of Environmental Studies (NIES) is

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located. 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. In the sampling site the environmental conditions were measured using a portable multi-probe system (pH, dissolved oxygen (DO)). Other conditions such as total solids (TS), dissolved carbon-dioxide (DCO_2), and volatile solids (VS) were performed in lab and the detailed procedures are explained in the analytical methods section. Collected consortia were sieved using a mesh filter in order to remove the impurities such as dust, sand and insect larvae. There forward, the consortia was grown in Bold's basal medium which is a nutrient medium widely used for freshwater microalgae of the classes *chlorophyceae*, *xanthophyceae*, *chrysophyceae*, and *cyanophyceae* [2,3]. The mixed microalgae consortia has been cultivated in plastic bags (25 cm in height and 5 cm in diameter) using bolds mineral medium. The light intensity of 8000–9000 lux was applied and the incubation was followed by 12 h dark and 12 h photo-period using a controller at room temperature of 23°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. The inoculum was 10% (v/v) from the original inoculum and for subsequent transfers the previously grown culture was used. Biomass growth was measured by following the optical density (OD) values at 680 and 750 nm (UV/Visible spectrophotometer Shimadzu). The light microscopic images of the consortia OP1 (*chlorella* and *scenedesmus* predominant species), OP2 (*ulothrix* and *chlorella* dominant species) and LB (*Anabaena* species majorly) are provided in Figure 1. Besides parameters were analyzed as follows: TS (OP1, 8.6 g/L, OP2 8.1 g/L and LB 7.8 g/L), VS (OP1, 5.4 g/L, OP2 5.0 g/L and LB 5.1 g/L) and COD (OP1, 3.2 g COD/L, OP2 5.0 g COD/L and LB 4.8 g COD/L) DO (OP1, 7.4 mg/L, OP2 8.0 mg/L and LB 6.8 mg/L), and DCO_2 (OP1, 1.4 mg/L, OP2, 6.2 mg/L and LB 4.8 mg/L), respectively.

Seed inoculum

The seed inoculum was collected from the CSTR reactors operated in the Bio-eco Engineering Lab, NIES, Japan. The characteristics were as follows, mesophilic inoculum: TS, 26.2 g/L; VS, 20.4 g/L; TCOD, 28.6 g COD/L; pH, 6.8. Heat treatment (90°C for 30 min in a water bath) was applied as pretreatment to avoid the presence of hydrogen consumers (methanogens) and also to enrich the spore forming hydrogen producers for H_2 fermentation, whereas no such treatment was used for CH_4 fermentation.

Batch H_2 and CH_4 fermentation

Hydrogen and methane fermentation was conducted in batch reactors with 150 mL holding capacity and 60 mL was chosen as working volume. In each vial, 15 mL of seed sludge, 40 mL of wet algal cultures and 5 mL of nutrient solution were added. The nutrient solution contained the following ingredients (g/L): 5.24, NH_4CO_3; 6.72, $NaHCO_3$; 0.125, K_2HPO_4; 0.1, $MgCl_2{\cdot}6H_2O$; 0.015, $MnSO_4{\cdot}6H_2O$; 0.025, $FeSO_4{\cdot}7H_2O$; 0.005, $CuSO_4{\cdot}5H_2O$; 0.00012, $CoCl_2{\cdot}5H_2O$. There forward, the reactors were purged with N_2 gas for 3–5 min in order to remove the headspace oxygen and create strict anaerobic condition. After that, the reactors were kept in an incubator with agitation speed of 120 rpm and mesophilic temperature of 35 ± 0.1°C. Initial pH was adjusted to 5.7 in H_2 reactors, whereas no pH adjustment was done for CH_4 reactors (initial pH was around 6.8–7.1). All the experimental sets were run as duplicates to make sure the reproducibility and data were reported as mean and SD values.

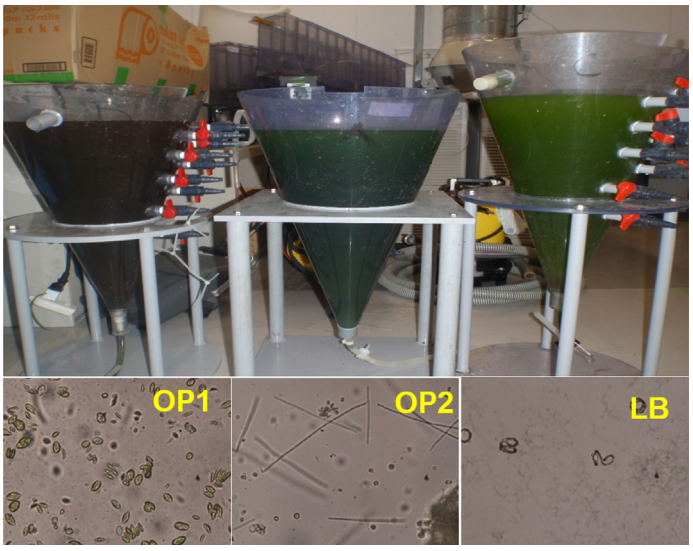

FIGURE 1

Microscopic observation of microalgal consortia OP1, OP2 and LB.

Analytical procedure for gas and liquid products

The compositions of biogas (CH_4, H_2, CO_2, and N_2) were analyzed by a gas chromatograph (SHIMADZU GC-8A) equipped with a thermal conductivity detector (TCD, 80 mA) and a 2 m stainless steel column packed with Shincarbon ST (SHIMADZU GLC). Helium was employed as carrier gas at a flow rate of 30 mL/min. The column temperature was 70°C, and the injector and detector temperatures were both 100°C. The volume of CH_4 and H_2 was measured using glass syringes as per the expected production and then calculated as the values at standard temperature and pressure conditions (STP, T = 273.15 K, p = 100 bar). Other analytical procedures such as COD, TS, and VS were measured by following the standard methods of APHA [1].

Modified Gompertz equation

Kinetic analysis was performed based on the cumulative H_2 or CH_4 production for each experiment from the experimental data. Each graph represents the triplicate values of H_2 or CH_4 production during the reaction time. The modified Gompertz equation (Eqn 1) was used for data analysis [22].

$$H(t) = H_{\max} * \exp\left\{-\exp\left[\frac{R_m \cdot 2.71828}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

where $H(t)$ is the cumulative hydrogen or methane production (mL) at culture time t (h); $H_{\max}$ is the hydrogen or methane production produced maximally (mL); P is the hydrogen or methane production potential (mL), R_m is the maximum hydrogen or methane production rate (mL/h); λ is the lag phase time (h) and t is the cultivation time (h).

Energy analysis

The energy production rate (EPR, kJ/L/d) was calculated as:

$$EPR = \frac{HPR}{22.4 \times HVH_2} \quad (2)$$