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Thermophilic biohydrogen production using pre-treated algal biomass as substrate

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ABSTRACT

Algal biomass is rich in carbohydrates which can be utilized as a promising source of substrate for dark fermentation. It becomes more significant when biomass is produced by capturing atmospheric greenhouse gas, CO_2. In the present study, clean energy was generated in the form of biohydrogen utilizing algal biomass. Biohydrogen production was carried out by thermophilic dark fermentation using mixed culture. The culture of *Chlorella sorokiniana* was cultivated in helical airlift photobioreactor at 30 °C under continuous light intensity of 120 μmol m^{-2} s^{-1} provided by white fluorescent lamps. Biomass reached to stationary phase on 9th day giving maximum dry cell weight of 2.9 kg m^{-3}. Maximum carbohydrate and protein content observed was 145 g kg^{-1} and 140 g kg^{-1}, respectively. Maximum volumetric productivity of 334 g dm^{-3} d^{-1} was observed. Algal biomass was subjected to various physical and chemical pre-treatments processes for the improvement of hydrogen production. It was observed that the pretreatment with 200 dm^3 m^{-3} HCl-heat was most suitable pretreatment method producing cumulative hydrogen of 1.93 m^3 m^{-3} and hydrogen yield of 958 dm^3 kg^{-1} volatile suspended solid or 2.68 mol mol^{-1} of hexose. Growth kinetics parameters such as μ_{max} and K_s were estimated to be 0.44 h^{-1} and 120 g m^{-3}, respectively. The relationship between biomass and hydrogen production was simulated by the Luedeking–Piret model showing that H_2 production is growth associated. The study thus showed the potential of algal biomass as substrate for biological hydrogen production.

1. Introduction

The recent exponential increase in worldwide energy demand caused depletion of energy reserves at greater pace. The combustion of fossil fuels has serious negative effects on environment because of CO_2 emission. Algal biomass cultivation is gaining importance in recent times as they can capture atmospheric CO_2 and can produce carbohydrates rich biomass which can be used for production of biofuels [1]. Unlike other crops such as corn or soybeans, algae can use various water sources ranging from wastewater to brackish water and can be grown in small, intensive plots on denuded land. Hydrogen from algae is possible by two biological processes. The first is the biophotolysis involving light-driven splitting of the water [1,2]. Hydrogen production by biophotolysis had been extensively studied on *Chlamydomonas reinhardtii* or *Anabaena variabilis* [3]. Secondly, dark fermentation of biomass utilizing carbohydrates present in algal cells using thermophilic and mesophilic hydrogen producing bacteria. Thermophilic dark fermentation shows favorable thermodynamics of reaction and with reduced risk of

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methanogenic contamination, higher rate of hydrolysis and higher hydrogen yields [4]. Algal biomass seems to be a potential feedstock source for biofuel production because they have higher growth rate, rich carbohydrate content and simple to harvest. Different processes are available for the production of fuels from algae [5] which includes anaerobic digestion of microalgae biomass to produce methane [6]. Moreover, direct extraction of fuel oils in the form of biodiesel that remains accumulated in certain microalgae [7]. Microalgae can also be used to produce hydrogen photosynthetically by direct or indirect photolysis of water [8]. At this point, there is one report on biohydrogen production from thermophilic dark fermentation using biomass of *Chlorella vulgaris* [9]. In this study, different pretreatments improved hydrogen production. Different physico-chemical pretreatments were employed to increase the accessibility of different complex sugars entrapped in algal biomass into simpler form. Carbohydrates in algal biomass are found as intracellular complex polymeric form bounded with rigid algal cell walls [10,11]. Therefore, it is necessary to break the algal cell wall along with complex carbohydrate to facilitate the release of simple sugar. Cost of pretreatment of biomass adds significantly to overall hydrogen production process. Several methods of pretreatments such as physical (sonication, milling, grinding, pyrolysis), chemical (acid, alkali, thermal, H_2O_2) and biological methods (enzymatic, microbial) have been reported to break algal cell wall, hydrolyze the complex carbohydrates and release fermentable sugars. Each of the method has its own merits and demerits [12]. Physical methods are based on simpler technology but they are energy intensive processes limiting their use for commercial application. Biological based methods are costly and time consuming with low hydrolysis rate. Preference of chemical method such as acid treatment over others is because of higher conversion efficiency of polymeric carbohydrates into simpler sugars in lesser time [10,13]. Some reports are available on use of algal biomass as substrate after pretreatment for hydrogen production using mesophilic microorganisms [14]. In a study, algal biomass was used to produce H_2 using *Clostridium butyricum* and subsequent use of produced organic acids for H_2 production by photo fermentation using *Rhodobacter sphaeroides* KD 131 [14].

Surface chemistry of biomass changes with different pretreatments. X-ray diffraction (XRD) has been used to study the changes in crystallinity with respect to different pretreatment processes [15]. Effect of acid and enzymatic pretreatment on surface chemistry of cornstalk for improvement of biohydrogen production was studies by using X-ray diffraction [16].

Similarly, fermentation of algal biomass by a mixed culture of lactic acid bacterium *Lactobacillus amylovorus* and photosynthetic bacterium *Rhodobium marinum* was used in the single-step process to convert algal starch to H_2 [11].

Monod model and Luedeking-Piret unstructured models were used for the determination of the kinetic parameters. These models allow us to relate microbial growth rate and product formation in an aqueous environment to the concentration of a limiting nutrient. These kinetic parameters helps us to understand the growth characteristics of the microorganism, design the bioprocess based experiment and scaling up the process.

Thus, the present study aimed to investigate the effect of different pretreatment methods on saccharification of complex carbohydrates present in algal biomass; *Chlorella sorokiniana*. Cell morphology was studied to investigate the extent cell wall rupture using microscopic and X-ray diffraction (XRD) with respect to different pretreatment methods. Optimized concentration of pretreated algal biomass was further used as carbon source in H_2 production medium for thermophilic mixed culture. Attempts were also made to determine the kinetics of hydrogen production using conventional Monod, Luedeking-Piret and modified Gompertz equations.

2. Materials and methods

2.1. Cultivation and harvesting of microalgae

The culture of *C. sorokiniana* was cultivated in helical airlift photobioreactor at 30 °C under continuous light intensity of 120 μmol m^{-2} s^{-1} provided by white fluorescent lamps. The helical photobioreactor was constructed of four parts: i) the helical photostage; ii) the gas riser; iii) the degasser and iv) the downstream tube. The construction material used was plexiglass (polymethacrylate). The helical photostage had tube dimensions of 2.0 cm ID and 3.0 cm OD wound on a 15.0 cm imaginary cylinder with a gap of 1.0 cm between the spirals. It had 8 spirals in total with a height of 32.0 cm. The gas riser consisted of a T-shaped joint which had a top diameter of 8.0 cm and a bottom diameter of 2.0 cm. The degasser was cylindrical in shape with a height of 12.0 cm. The downflow tube connected the bottom of the degasser with the end of the helix via a T-piece which also served as a sampling port. Inside the reactor, air sparging had a space velocity of 0.33 min^{-1} for proper circulation and mixing of the culture. The *C. sorokiniana* was grown in mTAP [-acetate] medium which was prepared by substituting the nitrogen source NH_4Cl by 1.5 kg m^{-3} of $NaNO_3$ [17]. Algal biomass was harvested at stationary phase and centrifuged at 4053× g RCF for 5 min. It was washed thrice with distilled water and further air dried to get a powdered algal biomass.

2.2. Microbial consortium

An enriched thermophilic mixed culture capable of producing hydrogen was used in this study. Media used for this study consist of Na_2HPO_4 (4.2 kg m^{-3}), KH_2PO_4 (1.5 kg m^{-3}), NH_4Cl (1.95 kg m^{-3}), $MgCl_2$ (0.18 kg m^{-3}), yeast extract (2.0 kg m^{-3}), glucose (10 kg m^{-3}), cysteine HCl (1 kg m^{-3}), vitamins solution (DSMZ medium No141, German Collection of Microorganisms and Cell Cultures). This mixed culture derived from a distillery industry anaerobic digester [18] had predominantly *Thermoanaerobacterium* sp. genus and was mostly related to *Thermoanaerobacterium thermosaccharolyticum* which have hydrogen production ability.

2.3. Algal biomass pretreatment

Dried algal biomass with initial concentration of 14 kg m^{-3} was subjected to physical and chemical pre-treatments such as