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Increasing microalgal carbohydrate content for hydrothermal gasification purposes

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ABSTRACT

This research examines the growth of *Chlorella* sp. microalgae under nutrient limitation (10–200 mg $NaNO_3$ L^{-1} and 10–70 mg K_2HPO_4 L^{-1}) and different light intensities (60–450 μmol photons m^{-2} s^{-1}) for achieving maximum carbohydrate content and biomass productivity using Response Surface Methodology (RSM) technique. According to the results, nutrition limitation had considerable effect on carbohydrate accumulation especially phosphorus concentrations; as in constant light intensities, maximum carbohydrate content was obtained in minimum concentration of K_2HPO_4. Under favorable circumstances; i.e. K_2HPO_4 = 10 mg L^{-1}, $NaNO_3$ = 105 mg L^{-1}, and light intensity = 255 μmol photons m^{-2} s^{-1} the highest carbohydrate content by 60.9% was achieved. Moreover, Supercritical Water Gasification (SCWG) of carbohydrate enriched microalgal biomass is able to produce much more hydrogen gas in comparison to the basic microalgal biomass. In addition, a 1.85 times increase in amount of produced gas is appeared as a result of a change in biochemical composition of the microalgal biomass.

1. Introduction

Over recent years, renewable energies have become a key strategy in sustainable development realization which can be attributed to disadvantages of fossil fuels, such as having finite resources and supply insecurity, dependence on petroleum exporting countries, contaminations of environment and concerns about the growth of greenhouse gas (GHG) emissions [1]. Biomass is a renewable source of energy that could be converted into biofuels through multiple processes. The first-generation biofuels which are mainly retrieved from edible plants (such as grains and vegetable oils) need extensive lands. The second-generation biofuels are provided mainly from lignocellulose and forestry and agriculture waste sources which do not fall under the category of food crops. However, their sustainability should be examined concerning criteria like minimum lifecycle, GHG reductions, land use change and social standards [2]. Microalgae cultivation for biofuel production is considered as the source of third-generation biofuels. Microalgae are capable of producing high contents of storage compounds (lipid/carbohydrate) within a few days. In comparison to other biomasses, microalgae have attracted noticeable attention as a feedstock in biofuel production due to following causes: higher photosynthetic rate efficiency, requiring less land compared to terrestrial plants, capability of cultivation on non-arable lands with non-potable waters, cultivation in year-round with short harvesting cycles, bio-fixation of CO_2 and high biomass yields [3–5].

The idea of microalgae cultivation for producing renewable fuels was initially presented in 1950 [6], and examined in state research programs of countries such as US, Japan and Australian 1970s [7]. Since 2000, more than $2billion has been assigned to this research field by private institutions [8]. Various studies have been conducted in the field of algae cultivation for producing biofuels such as biodiesel [9], bioethanol [10], biomethane [11], biohydrogen [12], biochar [13], and similar fuels. Among other fuels, hydrogen has the highest amount of energy per mass unit (142 kJ/g) and is the cleanest fuel as it yields only water in combustion [14]. One of hydrogen production methods is utilizing Supercritical Water Gasification (SCWG) technology of biomass. In supercritical water condition (T > 374 °c and P > 221 bar), gaseous products mainly

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CO_2, H_2, CH_4 and CO are produced from decomposition of organic matters. The mechanism of the process is producing the gaseous products from further decomposition of water soluble oligomers which are derived from hydrolysis of feedstock in supercritical water [15]. Hydrogen gas with high efficiency can be produced from wet biomass especially rich-carbohydrate ones [16]. Algal SCWG was introduced in 1990 for the first time [17] and since then numerous studies have been conducted in this field. The most important advantages of this method is requiring no energy to dry the biomass and also reusing the major part of consumed energy for reaching supercritical conditions [17].

Carbohydrates are considered as substantial constituent of microalgae cells which result from photosynthesis and carbon fixation metabolism. Carbohydrates are either accumulated in plastids as storage elements or in cell walls as the main component [18]. Manipulating the carbohydrate content of cells can be accomplished under environmental stress conditions such as nutrient starvation/limitation, high light intensities or salinity stress, in which culturing in optimum situation would be accompanied by alteration of microalgal metabolic pattern and accumulation of storage compounds [19].

Nitrogen is an essential nutrient for growth of microalgae and is known as principal constituent of structural and functional proteins, the synthesis of which depends on the availability of nonorganic nitrogen in a medium [20,21]. Crucial metabolic consequences of nitrogen starvation are disturbance in photosynthesis process, reduction in synthesizing photosystems reaction center proteins and consequent decrease in photosynthetic pigments [22]. In such situation, Pancha et al. [23] suggested that for providing required intracellular nitrogen content for continuing common growth, the nitrogenous compounds in microalgal cells might be degraded. Therefore the flow of the photosynthetically fixed carbon is switched to the lipid or carbohydrate synthesis pathways [24]. Phosphorous which is another essential macronutrient element in algae cells, plays an important role in cellular metabolic processes [25]. Although Phosphorus is required in very small amounts during algal growth cycle, it must be supplied in excess of basic requirement due to bonding phosphates ions with metals ions [26]. Phosphorous starvation in cultivation medium can stimulate carbohydrate or lipid content [24]. For instance, Douskova et al. [27] reported that cultivation of *chlorella vulgaris* in phosphorus limitation conditions leads to starch accumulation up to 55% dw.

So far, various researches have been conducted on nutrients stresses, including nitrogen, phosphorus or Iron starvation stress and also increase or decrease in light intensity aiming to increase microalgal carbohydrate content. However, the resultant stress from simultaneous effect of two macro nutrients in cultivation media (nitrogen and phosphorus) in the presence of light intensity stress and its effect on microalgal carbohydrate content has not been examined yet. This study aims at assessing the increase in carbohydrate content of *Chlorella* microalgal cells under nutrition stress circumstances (reduction in nitrogen and phosphorus concentrations) and increasing light intensity, in order to produce the suitable feedstock in SCWG process. By designing the experiments, changes in range of variables (10–200 mg NaNO3 L^{-1}, 10–70 mg K2HPO4 L^{-1} and light intensities of 60–450 μmol photons m^{-2} s^{-1}) on carbohydrate content and biomass productivity will be evaluated. Since nutrient limitation is accompanied by decreasing in growth of the algae, carbohydrate productivity was also examined. The levels of each designing parameter were selected based on pre-experiment. At the end, the SCWG of microalgal biomass rich in carbohydrate content was investigated in comparison to the basic one in batch reactor operating at temperature and pressure of 380 °C and 225 bar respectively and in the reaction time of 30 min.

2. Material and methods

2.1. Growth condition

In this study, Chlorella sp. (PTCC 6010, Persian Type Culture Collection) was obtained from Iranian Research Organization for Science and Technology (IROST). This microalga is native to the Persian Gulf, resistible and easily adaptable to the environment.

Pre-cultivation of the microorganism was carried out in a 500 ml glass bubble column photobioreactor with 50 μmol photons m-2 s-1 light intensity in Rodik medium with the following composition (per liter): 0.30 g $NaNO_3$, 0.08 g K_2HPO_4, 0.02 g KH_2PO_4, 0.02 g NaCl, 0.047 g $CaCl_2$, 0.02 g $MgSO_4 \cdot 7H_2O$, 0.1 mg $ZnSO_4 \cdot 7H_2O$, 1.5 mg $MnSO_4 \cdot H_2O$, 0.08 mg $CuSO_4 \cdot 5H_2O$, 0.3 mg H_3BO_3, 0.3 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 17 mg $FeCl_3 \cdot 6H_2O$, 0.2 mg $Co(NO_3)_2 \cdot H_2O$, and 7.5 mg EDTA. In order to reach the Persian Gulf sea-water condition, the salinity of cultivation medium was adjusted to 33 g L^{-1}.

Prior to the main experiments, the broth at the late exponential phase of the growth curve was centrifuged (5000 rpm) for 12 min and washed with distilled water twice. Then the pre-cultured microalgae were inoculated into 5 L bubble column bioreactor (3 L working volume) to reach an approximate inoculum size of 0.028 g L^{-1}. *Chlorella* sp. Microalgae was exposed to different concentrations of NaNO3 (10–200 mg L^{-1}) and K_2HPO_4 (10–70 mg L^{-1}) in a new medium.

The temperature was adjusted to 27 ± 1.5 °C and two to eleven 40 W fluorescent lamps mounted on both sides of the bubble column bioreactor with suitable distance to reach the illumination of 60–450 μmol photons m^{-2} s^{-1} which measured by light meter (TES-1330 A, Taiwan). During the cultivation, the bioreactor operation performed with 2.0% v/v CO_2 aeration rate of 0.2 vvm continuously. 4 ml liquid sample was collected daily to determine biomass concentration.

2.2. Harvesting

Electro-coagulation was applied as a rapid and cost effective approach to separate microalgae from the cultivation medium. In this process, coagulants form during the electro-oxidation of the sacrificial anode and formation of flocs appears as a result of destabilization and charge neutralization of the suspension [28]. In this research, two parallel aluminum electrodes with effective surface and distance of each other equal to 96 cm^2 and 4 cm respectively were placed inside the plexiglas reactor with dimensions of 15 × 15 × 20 cm (useful volume of 2700 cm^3). Initial pH of each experiment was adjusted to 6.5 and DC power supply (DAZHENG PS-305D) with a production capacity of 1.4 A was utilized. In order to have mixing during the reaction, the reactor was placed over the magnetic stirrer (Alpha stirrer, made in Iran) with 100 rpm.

At the end of cultivation time, biomass was harvested, washed twice with distilled water and dried in 65 °C oven over the night to analyze the carbohydrate content.

2.3. Determination of biomass concentration

For determining Cell concentration of the culture, optical density was used and the absorbance was measured at wavelength of 550 nm with UV/VIS spectrophotometer (GBC-Model 911). In wavelength of 550 nm, the absorption by chlorophyll and other pigments is the least and negligible [29,30]. These values were converted to *Chlorella* sp. dry weight by using the following equation (equation (1)) which comes from the correlation curve between biomass dried weight per liter (X, mg L^{-1}) and the culture absorbance. Biomass productivity (mg $L^{-1}d^{-1}$) was calculated