Applied Energy 211 (2018) 296–305

Contents lists available at ScienceDirect

Applied Energy

journal homepage: www.elsevier.com/locate/apenergy

Algal biofuel production coupled bioremediation of biomass power plant wastes based on *Chlorella* sp. C2 cultivation

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HIGHLIGHTS

- Microalgal cultivation is a costly process due to large nutrient substance needed.
- Wastes from biomass power plants can serve as nutrients for *Chlorella* cultivation.
- *Chlorella* cultures produce more lipids and exhibited higher growth rates.
- The residual medium is almost nutrient-free and suitable for recycling.
- An technical strategy for biofuel production coupled bioremediation is proposed.

ARTICLE INFO

Keywords:
Biomass power plant ash
Biofuel
Biological DeNOx
Chlorella sp. C2
CO_2 bioremediation

ABSTRACT

Microalgae have reported to be one of the most promising feedstock for biofuel production. However, microalgal cultivation for biofuel production is a costly process due to the large amounts of water, inorganic nutrients (mainly N and phosphate (P)), and CO_2 needed. In this study, we evaluated whether the nutrient-rich ash and flue gas generated in biomass power plants could serve as a nutrient source for *Chlorella* sp. C2 cultivation to produce biolipids in a cost-efficient manner. When ash was incorporated in the culture medium and photosynthesis was enhanced by CO_2 from flue gas, *Chlorella* cultures produced a lipid productivity of 99.11 mg L^{-1} d^{-1} and a biomass productivity of 0.31 g L^{-1} d^{-1}, which are 39% and 35% more than the control cultures grown in BG11 medium. Additionally, the cultures reduced the nitrogen oxide (NOx) present in the flue gas and sequestered CO_2, with a maximum ash denutrition rate of 13.33 g L^{-1} d^{-1}, a NOx reduction (DeNOx) efficiency of ~ 100%, and a CO_2 sequestration rate of 0.46 g L^{-1} d^{-1}. The residual medium was almost nutrient-free and suitable for recycling for continuous microalgal cultivation or farmland watering, or safely disposed off. Based on these results, we propose a technical strategy for biomass power plants in which the industrial wastes released during power generation nourish the microorganisms used to produce biofuel. Implementation of this strategy would enable carbon negative bioenergy production and impart significant environmental benefits.

1. Introduction

The burning of fossil fuels has contributed to global climate change, environmental pollution, health problems, and an energy crisis associated with the irreversible depletion of traditional sources of fossil fuels [1]. Many countries are thus striving to develop renewable energy sources [2,3]. Among the various potential sources of renewable energy, biofuel, the fuel obtained from biomass (i.e., organic matter derived from plants, animals, and microorganisms), is of great interest and is expected to play a crucial role in the global energy infrastructure in the future [4–6].

Biomass power plants are emerging as important sources of heat and power [7]. Biomass fuels are considered renewable fuel sources and do not affect the overall balance of CO_2 in the atmosphere [8]. Obtaining

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Abbreviations: AL, actinic light; BPPA, biomass power plant ash; Car, carotenoids; Chl, chlorophyll; CLSM, confocal laser scanning microscopy; CR, consumption rate; DeNOx, reduce the NOx; DR, denutrition rate; FCM, flow cytometry; FGFS, flue gas fixed salts; FR, fixation rate; ML, measuring light; N, nitrogen; NO, nitric oxide; P, phosphate; PSII, photosystem II; SP, saturation pulse; TLC, thin layer chromatography

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https://doi.org/10.1016/j.apenergy.2017.11.058
Received 17 August 2017; Received in revised form 7 November 2017; Accepted 8 November 2017
0306-2619/

energy from biomass could reduce the dependency on fossil fuel and energy import, reduce damage to the environment, achieve a zero carbon footprint, and enhance the use of the byproducts of energy production [9]. However, one of the problems associated with biomass combustion in biomass power plants is the ash and flue gas (mainly consisting of CO_2 and nitrogen oxides (NOx)) generated during biomass combustion [10–14], and the environmental management of these pollutants, which can contaminate ecosystems [15–17]. When nitrogen (N) and carbon (C) were oxidized into NOx and CO_2 in flue gas during the biomass combustion, while there are still significant amount of other elements such as Mg, K, and Ca, etc. left over in the ash, both the flue gas and the ash are considered contaminants and need to be further treated before disposal, thus there is an urgent need to develop sustainable technical strategies for dealing with these byproducts, as the volume of industrial wastes and thus the cost for disposal are increasing.

Several technologies, including physical and chemical absorption, cryogenic distillation, and membrane separation, are used to capture CO_2 [18], which is then transported and stored in geological formations. However, these procedures should only be considered as short-term solutions because they are energy consuming and the captured CO_2 needs to be disposed off. Microalgae are the fastest growing photosynthesizing organisms known and, in addition to consuming CO_2 and N-based compounds, are one of the most important producers of oxygen on earth [19,20]. Microalgal biomass contains approximately 50% C by dry weight [21]. During the production of 100 tons of microalgal biomass using natural or artificial light, approximately 180 tons of CO_2 can be fixed [22]. The ability microalgae to fix CO_2 by photosynthesis is 10–50 times that plants [23]. Conventional NOx treatments, i.e., physicochemical DeNOx methods, are expensive and produce secondary wastes that often require further treatment [24]. NOx can serve as a N source for microalgae and can be metabolized by microalgae. Some green algae can utilize NOx as a nitrogen source for biofuel production [25,26]. In addition, biomass ash may be a nutrient source for algal cultivation, but very few studies have evaluated the effects of biomass ash on algal growth [27,28].

Biodiesel is recognized as an ideal renewable energy carrier [29]. Given their rapid growth and ability to convert solar energy into chemical energy via CO_2 fixation, microalgae have been considered one of the most promising sources of oil for the production of biodiesel [30]. However, algal biodiesel are not produced commercially, as algal oil is more expensive than fossil fuel [31–33]. Microalgal cultivation is expensive due to the large amounts of water and inorganic nutrients (mainly N, phosphate (P), and CO_2) needed [4]. One possible way to reduce these costs would be to use the ash, CO_2, and NOx byproducts of biomass power plants to nourish the microalgae in the system [4,34].

We previously demonstrated the feasibility of coupling *Chlorella*-based production of microalgae-based lipids with the efficient biological DeNOx of flue gas [25,26,35]. In this study, we aimed to establish an integrated technique for algal-based lipid production and bioremediation of biomass power plant wastes (i.e., ash and flue gas). When biomass power plant ash (BPPA) and flue gas are used as the nutrient source, *Chlorella* sp. C2 cultures not only survive and grow, but produce increased levels of lipids. Our study provides an economically viable technical strategy for algal biofuel production coupled bioremediation of biomass power plant waste.

2. Materials and methods

2.1. Microalgal culture

Chlorella sp. C2 used in this study was isolated and autotrophically cultured in BG11 medium as described previously [36]. When the optical density at 700 nm (OD_{700}) reached around 0.8, *Chlorella* sp. C2 was inoculated into a 1 L Erlenmeyer flask containing 500 mL BG11 medium at 25 °C with continuous illumination of $70\,\mu mol\,m^{-2}\,s^{-1}$ and continuously bubbled with filtered air, the initial OD_{700} is 0.05. For culturing in BPPA media, *Chlorella* sp. C2 was harvested at the mid-logarithmic growth phase ($OD_{700} = 0.8$) by centrifuging at 3000 *g* for 3 min. The algal pellets were washed twice with N-free BG11 medium and then re-suspended to an OD_{700} of 0.2 in either regular BG11 or BPPA media in a column photobioreactor with a 750 mL working volume. *Chlorella* sp. C2 was inoculated at 28 °C with continuous illumination of $115 \pm 3\,\mu mol\,m^{-2}\,s^{-1}$ and continuously bubbled with filtered air and 3% CO_2 derived from biomass power plant flue gas.

2.2. Preparation of BPPA media

Eighty grams of biomass power plant ash, the burning wastes of biomass power generation by combustion of majorly rice straw, from the biomass power plant of SUNSHINE KAIDI NEW ENERGY GROUP CO. LTD was lixiviated with 1 L distilled water with a stirring speed of 150 rpm for 5 h. Then the leaching liquor was filtrated and sterilized using a 0.45 μm filter membrane. The main nutrient components (Mg, K, Ca, P, and Fe) in the leaching liquor were detected using Inductively Coupled Plasma-optical emission spectroscopy (ICP-OES, OPTIMA 8000DV, PekinElmer, USA). The nitrate and nitrite concentrations were detected using ion chromatography [37]. According to the main nutrient elements in leaching liquor (Table 1) and BG11 medium, 0.153 mM K_2HPO_4 was replenished in leaching liquor to be BPPA1 medium, 17.65 mM $NaNO_3$ was replenished in BPPA1 medium to be BPPA2 medium, and NOx (flue gas fixed salts, FGFS) was replenished in BPPA1 medium to be BPPA3 medium (Table 2).

2.3. Preparation of CO_2 and NOx

CO_2 was separated from flue gas emitted from the biomass power plant of SUNSHINE KAIDI NEW ENERGY GROUP CO. LTD and stored in a compressed gas vessel.

Nitric oxide (NO), which is the main component of NOx, is sparingly soluble in water, and the dissolution of NO into the microbial culture medium is the rate-limiting step for NO removal. The initial fixation of massive NOx into water and the subsequent cultivation of algal cells using fixed nutrients is possibly an effective way to improve NOx removal efficiency [26]. After desulfurization and dust removal, NOx in flue gas from the biomass power plant was fixed into FGFS as described by Zhang et al. [26] and Chen et al. [25]. The final concentrations of NO_2^- and NO_3^- in the FGFS were 16.77 and 0.88 mM, respectively.

2.4. Algal growth analysis

Cell growth was monitored by measuring OD_{700} and the biomass production. OD_{700} was measured every 24 h with five biological replicates. Biomass production was measured every 24 h with five biological replicates. Biomass collected at a known volume was precisely weighed after 24 h of freeze-drying [26]. Maximum biomass

Table 1
Major nutrient elements in leaching liquor of biomass power plant ash.

Mg (mM)	K (mM)	Ca (mM)	P (mM)	Fe (mM)	NO_3^- (mM)	NO_2^- (mM)
3.284 ± 0.328	2.668 ± 0.297	17.749 ± 1.774	0.022 ± 0.002	0.055 ± 0.006	0	0