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The growth characteristics and biodiesel production of ten algae strains cultivated in anaerobically digested effluent from kitchen waste

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ABSTRACT

The growth characteristics and biodiesel production of ten algae strains cultivated in diluted anaerobically digested effluent kitchen waste (KWADE) were studied in this paper. Four microalgae species could tolerate the high NH_3-N concentration in KWADE. Based on the results of PROMETHEE-GAIA, all strains cultivated with KWADE attained better biodiesel properties compared to cultivation in BG11. The most suitable strain was *Scenedesmus* SDEC-8, followed by *Chlorella* SDEC-18 and *Scenedesmus* SDEC-13. The best strain, *Scenedesmus* SDEC-8, achieved an extraordinary lipid content of 33.85% and lipid productivity of 20.27 mg L^{-1} d^{-1}, a desirable fatty acid methyl ester profile of 94.03% and satisfactory biodiesel properties in cetane number (59.39), iodine value (51.58 gI_2/100 g fat) and lower cloud point (13.01 °C) too. In addition, SDEC-8 also exhibited the highest nitrogen average yield coefficient of 53.1 mg g^{-1}, and had an average yield coefficient of 1.8 mg g^{-1} for phosphorous.

1. Introduction

Microalgae, as a new resource for producing many products such as animal feed stuffs, food additives, cosmetics, and agriculture, have been receiving much more attention from researchers all over the world [1,2]. Moreover, they can also be treated as superior candidates for the continuous stable production of biodiesel due to their intrinsic advantageous qualities such as rapid growth, high lipid content, and low requirement for land area [3,4]. Nowadays, the amount of petroleum reserves, the fossil fuel in the highest demand, is sharply reducing, which has caused a global energy crisis. Fortunately, producing biodiesel is one of the most exciting advantages for some algae, making them the third-generation alternative source of biofuel and biodiesel [5]. However, the great demand for growth medium to cultivate microalgae has limited their widespread adoption and development [6].

Recently, because of their tolerance for extreme conditions [7], a variety of microalgae were cultured with diverse wastewater such as monosodium glutamate wastewater [2,8], dairy manure [9], and olive-oil mill wastewater [10]. Their probability to survive in various severe conditions (domestic and industrial wastewater) not only overcomes the problem of huge waste of water, which can cut the costs for cultivation of microalgae, but also removes nutrients from the wastewater and moreover produces much biomass for further uses. Therefore, selecting an appropriate source of wastewater to cultivate high-lipid-yield algae will have positive ecologic effects and considerable economic benefit.

In China, nowadays, the methods for processing anaerobically digested effluent mainly consist of using constructed wetlands, returning effluent back to farmland, and high-cost factory disposal. All of these either can lead to the infiltration of pollution into underground water or have high cost for the overall treatment process. Therefore, biological treatment, with its advantages such as easy to be obtained from secondary pollution and low costs, must figure prominently in researchers' considerations. Shin et al. [11] used three different mixing ratios of anaerobically digested food wastewater effluent and municipal wastewater to culture *Scenedesmus* to determine an appropriate dilution ratio in producing biodiesel. However, the cultivation period was too long (25 d). Ji et al. [12] found that when *Desmodesmus* sp. EJ15-2 was put in anaerobic digestion wastewater for 40-day-fed-batch cultivation, the removal of TN and TP were 94.2% and 95.6%. Yet the lipid content of the microalgae was relatively low. Consequently, anaerobically digested effluent is not a universally beneficial and appropriate choice for algae cultivation currently.

Recently, Kim et al., cultivated four microalgae species with the effluent of anaerobic digester, and achieved high dry cell weight, but failed to achieve high enough FAME productivity [13], and the similar results were found by Koutra et al. [14] who reported that 1 g L^{-1}

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biomass yield of *Acutodesmus obliquus* were obtained in anaerobic digestion effluent, while the lipid content in the microalgae was only 19.5%. Moreover, Cheng et al. [15] used microalgae *Chlorella* PY-ZU1 to treat undiluted anaerobic digestion effluent of food wastes with ozonation pretreatment, and achieved ideal results of nutrient removal, the cost of ozone and aeration, however, were not taken into consideration. Therefore, there still a long way to make it is practical to use KWADE cultivating algae for biofuel production. Especially for the anaerobically digested effluent from kitchen waste with the characteristics of high organic matters, high nutrients and dark color compared with the KWADEs from pig farm biogas plant [16,17]. This kind of wastewater absolutely not only aggravates environmental pollution but also threat to the algae survival when to be used as cultivation media.

In previous studies, in order to evaluate the potential capabilities of different algae for biodiesel production, multi-criteria analysis including lipid production and several biodiesel properties have been widely used [3,18]. However, a multi-criteria process cannot be handled without additional information about the preferences and the priorities of the decision-makers [19]. Therefore, in order to visually obtain a comprehensive and proper conclusion, Anahas et al. [20] and Sinha et al. [21] introduced the Preference Ranking Organization Method for Enrichment Evaluation and Graphical Analysis for Interactive Assistance (PROMETHEE-GAIA), which was firstly established by Brans et al. [19] in the field of statistics. The advantage of PROMETHEE–GAIA is that it provides a decision vector for the analyst, which gives more options to the decision maker directly in the GAIA plane. In this study, considering the growth characteristics, lipid accumulation, biodiesel production and biodiesel properties, we utilized PROMETHEE-GAIA to evaluate four algae strains which can grow well in KWADE to obtain the most suitable strains for biodiesel production.

In this paper, the usage of anaerobically digested effluent from kitchen waste to cultivate ten algae strains was investigated with the following goals: (1) To select the algae strains feeding on nutrients with high concentration in the digested effluent. (2) To check the influence of digested effluent on lipid content and fatty acid profiles. (3) To select the most suitable algae for biodiesel production using the soft PROMETHEE-GAIA. (4) To assess whether the nitrogen and phosphorus in KWADE reduced rapidly by analyzing the TP, TN and NH_3-N concentrations.

2. Materials and methods

2.1. Microalgae strains

During the experiments 10 algae species were used: *Scenedesmus* SDEC-8 (Accession No.: KF999643), *Chlorella* SDEC-10, *Chlorella* SDEC-11, *Scenedesmus* SDEC-13 (Accession No.: KF999644), *Golenkinia* SDEC-16 (Accession No.: KT180320), *Monoraphidium* SDEC-17 (Accession No.: KT180321), *Chlorella* SDEC-18, *Anabaena* (freshwater algae culture collection of the Institute of Hydrobiology in China, FACHB-Collection), *Skeletonema* and *Stichococcus bacillaris* Nag. Among them, SDEC-8, SDEC-16 and SDEC-18 were isolated from a local freshwater lake (Quancheng Lake in Jinan), SDEC-10, SDEC-11 and SDEC-13 were screened from an artificial lake in Shandong Jianzhu University, *Skeletonema* and *Stichococcus bacillaris* Nag. were obtained from Qingdao, and SDEC-17 was from a local freshwater lake (Nansi Lake in Jinan). Before the experiments, most of the algae were cultivated in BG11 medium at 25 ± 1 °C, except *Stichococcus bacillaris* Nag which was cultured in artificial sea water.

The algae inocula were centrifuged and resuspended before measuring biomass (by OD_{680}) to gain equal absorbance. The OD_{680} values of algae inocula were nearly 0.26 after 10-fold dilution with tap water.

2.2. Anaerobically digested effluent and pretreatment

The experiments were carried out using digested effluent from an environmental protection company, Jinan Shifang Environmental Protection Co. The wastewater contained 2213.54 ± 47.77 mg L^{-1} of ammonia nitrogen (NH_3-N), 28.14 ± 0.11 mg L^{-1} of total phosphorous (TP), 2814.97 ± 53.67 mg L^{-1} of total nitrogen (TN), and 6096.10 ± 40.66 mg L^{-1} of COD, and had a pH of 8.3 ± 0.4, and after 20-fold dilution with tap water, it contains 115.31 ± 2.13 mg L^{-1} of TN, 1.02 ± 0.02 mg L^{-1} of TP, 296.68 ± 1.98 mg L^{-1} of COD and 8.0 ± 0.4 of pH.

At the beginning of the experiments, the wastewater was centrifuged (6000 rpm for 10 min) to remove impurities, namely insoluble particles in the liquid. There was no further pretreatment (*e.g.* sterilization) before the usage of wastewater.

2.3. Experimental design

After activation of these algae strains, they were sampled (80 mL) and inoculated into the anaerobically digested effluent that had been diluted by a factor of 20 with tap water in ten 1 L Erlenmeyer flasks. The total volume of algae solution and diluted digested effluent was 800 mL. The algae strains were cultivated at 25 ± 1 °C with a continuous light intensity of almost 60 μmol m^{-2} s^{-1} provided by daylight fluorescent tubes (Philips, 36 W). The original optical density was measured using a UV spectrophotometer (ZDS-10, Shanghai Cany Precision Instrument, China). Two sets of parallel experiments were conducted under the same conditions and at the same time.

At the end of the experimental stage, the algal solution was centrifuged at 4000 rpm for 10 min [3]. In order to obtain dry biomass pellets of these algae, they were freeze-dried using a lyophilizer (FDU-1200, EYELA, Japan). After that, the pellets were ground for the next analysis procedure. The algae strains were harvested when they came into the stationary phase, as the maximum accumulation of lipid often occurs during that phase.

Through several days of experiments, four algae strains showed better growth trends than the others. Thus, control studies using BG11 medium were then set up for these four strains, under the same culture conditions.

2.4. Algae analysis

2.4.1. Determination of microalgae growth

The biomass concentration of algae was calculated by OD_{680} according to the linear relation for each algae species between the dry weight of algae cells and optical density which had been measured before the experiments. During the experiments, the cell morphology (length, width and diameter) of the ten strains was observed by microscope (CX31, Olympus, Japan).

The specific growth rate (μ, d^{-1}) of the 10 strains in each Erlenmeyer flask was calculated as follows [8]:

$\mu = (\mathrm{Ln}N_2 - \mathrm{Ln}N_1)/(T_2 - T_1)$where N_1 and $N2$ are the dry biomass concentrations (g L^{-1}) on days T_1 and T_2, respectively.

Besides the optical density, the pH of the algae strains was also measured every 24 h using a pH meter (PHS-3C, Shanghai Leici Instrument Co., China).

2.4.2. Measurement of total chlorophyll content

The total chlorophyll content of microalgae was measured by the method of methanol extraction [22]. Firstly, a 2 mL sample of culture was transferred into a 2 mL centrifuge tube and centrifuged at 10000 rpm for 10 min. The supernatant was then replaced by 2 mL of pure methanol. Next the mixture was shaken and incubated at 45 °C for 24 h in the dark. The content was calculated by the following equations:

Chlorophyll a: Chl-a (mg L^{-1}) = 16.72 $A_{665.2}$ – 9.16 $A_{652.4}$;

Chlorophyll b: Chl-b (mg L^{-1}) = 34.09 $A_{652.4}$ – 15.28 $A_{665.2}$;

Carotenoids = (1000 A_{470} – 1.63 Chl-a – 104.9 Chl-b) / 221;

Total Chlorophyll content (mg L^{-1}) = Chl-a + Chl-b.