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Cultivation of microalgae using anaerobically digested effluent from kitchen waste as a nutrient source for biodiesel production

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ABSTRACT

Anaerobically digested effluent from kitchen waste (ADE-KW) is a potential medium for microalgae cultivation because it contains abundant nutrients. In this study, ADE-KW was utilized to cultivate the isolated microalgae *Chlorella sorokiniana* SDEC-18 and *Scenedesmus* SDEC-8, which showed optimal biomass productions of 0.42 g/L and 0.55 g/L, respectively, with 1/15 diluted ADE-KW. The lipid contents of *Chlorella sorokiniana* SDEC-18 (30.27–41.69%) and *Scenedesmus* SDEC-8 (35.97–47.39%) in this medium had obvious advantages over the controls grown in BG11 medium. The reason is that unbalanced nutrient and organic carbon levels in ADE-KW stimulate microalgae to turn more photosynthetic flow of carbon and energy from protein and carbohydrate into the biosynthesis of lipid than that in usual media. Moreover, this study showcased a proof-of-concept for the production of ideal biodiesel with desirable properties from microalgae under the cultivation of ADE-KW. In a large-scale experiment with K_2HPO_4 addition, the biomass and lipid productivity of *Scenedesmus* SDEC-8 were both promoted significantly (by factors of 2.27 and 1.08, respectively) by adding K_2HPO_4 to the ADE-KW.

1. Introduction

Currently fossil fuels are the main energy source in China [1]. The energy crisis caused in part by increasing global energy demand and depleting fossil fuel resources is attracting increasing concern worldwide [2]. In response to the challenges, biological research for energy feedstock production is a promising scientific direction to help solve the global energy issues and work towards sustainable development, and bioenergy is considered to have the highest potential to satisfy these energy needs [3]. Moreover, microalgae have been regarded as one of the most promising feedstocks for biofuel production due to the advantages of higher oil content, higher rate of photosynthesis, no direct competition for agricultural land and easy cultivation [4,5]. However, many challenges that hinder the commercialization and scaling up of microalgal biofuel manufacturing processes are still existed. Microalgae cultivation as feedstock for biomass production still needs a great deal of freshwater and high-cost nutrients [6]. In 2009, the annual worldwide freshwater consumption was approximately 3908.3 billion m^3 [6], and serious shortage of water resource occurred. It is necessary to find an alternative low-cost medium for microalgae cultivation.

Benefiting from its abundance and enrichment of nutrients, wastewater could be utilized to cultivate microalgae, which as one way to substantially reduce the nutrient expenses and water resources could contribute to successful large scale microalgal-based biofuel production [6,7]. Currently, there have been wide applications of wastewater to microalgae cultivation, with the variety of wastewater including municipal wastewater [8–10], domestic wastewater [11], urban wastewater [12] and animal manures (piggery wastewater and aquaculture wastewater) [13,14], the nutrient concentrations in which were in the range of 80–1000 mg/L for COD, 50–1500 mg/L for total nitrogen and 5–100 mg/L for phosphates. Additionally, anaerobically digested effluents have also been regarded as a choice. Anaerobic digestion (AD) is a technology utilized to decompose organic matter under oxygen-free conditions and produce biogas, while the residual effluent from the digestion process contains abundant nutrients, such as ammonium and

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phosphate [2]. The superfluous amounts of anaerobically digested effluent may cause challenges of environmental pollution or economic issues if additional treatment is adopted prior to discharge. Fortunately, nutrients in anaerobically digested effluent could be a good source for the microalgae production. There have been a few applications of anaerobically digested effluent to cultivate microalgae, with the feedstocks of anaerobic digestion including activated sludge [15], animal manures [16–18] and municipal wastewater [2]. Tan et al. [15] demonstrated nutrients in anaerobically digested activated sludge effluent can be remediated through assimilation into algal biomass with 2.43 g/L biomass concentration and 29.76% lipid content. Passero et al. and Levine et al. [16,18] presented *Chlorella vulgaris* and *Neochloris oleoabundans* grew well in anaerobically digested dairy effluents, while the lipid accumulations were only 7.2% and 9.9% respectively. For current several kinds of anaerobically digested effluents that have been confirmed to be feasible alternatives for algae feeding, the lipid accumulation of algae was unsatisfactory. Consequently, there is still a long way to search for a kind of anaerobically digested effluent to be beneficial and appropriate algae growing media.

Currently, kitchen waste has become a severe problem for its environmental pollution. The daily Chinese urban waste production exceeded 65 million tons in 2013, containing about 30% of food waste [19]. After the AD process, anaerobically digested effluent from kitchen waste (ADE-KW) can still seriously pollute the environment because of its high concentration of pollutants, mainly ammonium and phosphate [20]. If ADE-KW was applied to microalgae cultivation, microalgae can not only reduce the concentration of pollutants in ADE-KW, but also can utilize the nutrients effectively to grow for biodiesel production. Moreover, there are important infrastructures existed in the plant of anaerobic digestion of kitchen waste including oil transesterification section (applying to transesterification of the oil separated from the kitchen waste) and anaerobic digestion tanks, while the former could be employed to produce algae-based biofuel from transesterification of algae lipid and the latter for tackling algae residue after oil extraction. Therefore, cultivating microalgae in ADE-KW would reduce not only the cultivation cost, but also transesterification cost combined with algae residue treatment for recycling. Additionally, ADE-KW was unique due to its characteristics of dark color, high organic matter levels (COD: 6000–8000 mg/L, TOC: 3000–5000 mg/L), high nitrogen concentration (1000–3000 mg/L) and limited phosphorus concentration (10–20 mg/L). In fact, there are limited studies on applying anaerobically digested effluent from kitchen waste to microalgae cultivation, and it is necessary to confirm whether it is feasible to use ADE-KW with special nutritional composition to cultivate algae. Furthermore, microalgae can convert atmospheric CO_2 along with light and water into organic matter. The excess of fixed carbon is commonly allocated into carbohydrates and lipids in stressful environmental conditions, like nitrogen starvation, phosphate limitation, high salinity and carbon source concentration [21–23], and the properties of biodiesel produced from algae lipid could be changed in that case. Therefore, it is worthwhile to investigate the carbon allocation and biodiesel properties of microalgae under the special environment (high organic matter levels and phosphorus limitation) caused by ADE-KW so that the mechanisms of lipid accumulation could be revealed and the suitability of biodiesel for direct use in engines could be evaluated. However, previous studies on microalgae cultivation with wastewater were restricted to the investigation of nutrient removal, biomass and lipid accumulation: Jiang et al. [8] explored the effects of different ratios of municipal wastewater and 15% CO_2 aeration on the growth of *Nannochloropsis* sp., and lipid accumulation of microalgae was also studied under nitrogen starvation and high light; analogously, the development of a culture system with piggery wastewater for *Chlorella* sp. GD to efficiently produce biomass and oil for biodiesel production was investigated by Kuo et al. [13]; the study of Arbib et al. [24] has signified that microalgae growth can simultaneously remove inorganic constituents of wastewater and produce energy rich biomass. Nevertheless, rare attempts have been presented on biodiesel properties and carbon allocation of microalgae cultivated with wastewater.

According to the statistics from previous studies, *Chlorella* sp. and *Scenedesmus* sp. are the most widely applied microalgae species in wastewater treatment [25]. In this study, we selected *Chlorella sorokiniana* SDEC-18 and *Scenedesmus* SDEC-8 to process ADE-KW. For one thing, *Chlorella sorokiniana* SDEC-18 and *Scenedesmus* SDEC-8 were isolated from the local environment, namely Quancheng Lake in Shandong Jinan; for another thing, it has been proved that *Chlorella sorokiniana* SDEC-18 and *Scenedesmus* SDEC-8 have high growth rates and lipid contents [26].

Thus, this study has three specific objectives: (1) to find out the optimum dilutions of ADE-KW for growth of *Chlorella sorokiniana* SDEC-18 and for *Scenedesmus* SDEC-8; (2) to investigate the biochemical compositions of the microalgae, especially their lipid properties; (3) to study the carbon partitioning under the stress caused by ADE-KW.

2. Methods

2.1. Microalgae strain

The freshwater microalgae strains used were *Chlorella sorokiniana* SDEC-18 and *Scenedesmus* SDEC-8 isolated previously from the local environment, namely Quancheng Lake in Jinan [26], which showed desirable performance in terms of growth and lipid content. Under a microscope, SDEC-18 shapes in sphere in a diameter of 2–6 μm, with a characteristic emerald-green color and without flagellum (Fig. 1(a)). The ultrastructure indicated that each cell contained a whole cup-shaped chloroplast which contained a noticeable pyrenoid surrounded by a starch envelope of two halves. Many different sizes of vacuoles with oil-like content were found between chloroplast and the cell wall. The result of 18S rRNA gene sequence and its alignment indicate that the closest relative of the strain is *C. sorokiniana* strain SM6, with over 99% similarity, which confirms the SDEC-18 as *Chlorella* sp. (Accession No.: KY355143). As depicted in Fig. 1(b), SDEC-8 was composed of 2–4 cells in oblong or ovate shapes. And it bore large spines with the length of about 6 μm at the four poles of colonies. The length and width of colonies ranged from 9 to 26 μm and 8–14 μm, respectively, depending on different growth phase. The ultrastructure showed that each cell contained a single chloroplast that occupied half of the cell besides nucleus and mitochondrion. The chloroplast contained a noticeable pyrenoid surrounded by a starch envelope of two halves. A different type of vacuoles with oil-like content were visible. The 18S rRNA sequence from *Scenedesmus* SDEC-8 confirmed its identification as *Scenedesmus* sp. (Accession No.: KF999643). They were precultured in BG11 medium which contains: 1.5 g/L $NaNO_3$, 40 mg/L K_2HPO_4, 75 mg/L $MgSO_4 \cdot 7H_2O$, 36 mg/L $CaCl_2 \cdot 2H_2O$, 6 mg/L citric acid, 6 mg/L ferric ammonium citrate, 1 mg/L EDTA-Na_2, 20 mg/L Na_2CO_3, and 1 mL/L A_5. A_5 is a trace metal solution containing 2.86 g/L H_3BO_3, 1.86 g/L $MnCl_2 \cdot 4H_2O$, 0.22 g/L $ZnSO_4 \cdot 7H_2O$, 0.39 g/L $Na_2MoO_4 \cdot 2H_2O$, 0.08 g/L $CuSO_4 \cdot 5H_2O$, and 0.05 g/L $Co(NO_3)_2 \cdot 6H_2O$. BG11 medium also contains 247.06 mg/L TN, 7.12 mg/L TP, 3.06 mg/L TOC.

2.2. Anaerobically digested effluent from kitchen waste (ADE-KW)

The ADE-KW was provided by Shandong Shifang Environmental