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Tofu whey wastewater is a promising basal medium for microalgae culture

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ABSTRACT

Tofu whey wastewater (TWW) is an abundant, nutrient riched and safety wastewater and is regarded as an excellent alternative medium in fermentation. In this study, the feasibility of algal cultivation using TWW as the basal medium was investigated. Results indicated that through simple pH adjustment, TWW presented a better culture performance at autotrophic, heterotrophic, and mixotrophic modes compared with that of regular green algae medium, BG-11. The biomass productivities of *Chlorella pyrenoidosa* at each trophic mode were 4.76, 1.97, and 2.08 times higher than that cultured in BG-11 medium, respectively. Although a comparative or even lower lipid and protein content was obtained, much higher lipid and protein productivities were obtained in TWW compared to that of BG-11. The algal biomass accumulated in TWW can be used to produce high-value products. Therefore, TWW is a better alternative medium for efficient algal culture.

1. Introduction

In recent years, microalgae are recognized as a promising source of sustainable biomass feedstock for the production of many valuable added products, including edible nutrition, pharmaceutical and cosmetic products, biofuels, animal and aquaculture feed, and natural dyes etc (Han et al., 2016; Guldhe et al., 2017). For the industrial production of microalgae-based products, it is necessary to accumulate abundant algal biomass at large scale. However, the large scale production of microalgae is severely restricted by high cost. For example, for the pilot or industrial scale production, the cost of fixed assets, energy consumption in cultivation and harvesting process, and raw materials is high (Wang et al., 2014b). In addition, the culture process also needs large amounts of water and nutrients. Sometimes, the cost of culture media even represents 30–40% of the operating costs in the production of microalgae at large scale (Jad-Allah, 2012). It seems that the use of chemical fertilizers and usual water as medium is unsustainable, especially in the production of low-cost products, such as biofuels. Replacing regular medium with wastewater is an effective approach to decrease the cost of large scale cultivation of microalgae.

Wastewater is a freely available, low-cost and promising alternative medium for the culture of various algal species (Ding et al., 2015). It is enriched in various macro- and micro-nutrients, which are necessary for the growth of microalgae, such as carbon source, nitrogen source, phosphate, and various trace minerals (Guldhe et al., 2017). Many different wastewaters, including municipal, piggery/swine, domestic, industrial, and anaerobic wastewater, have been widely investigated in the cultivation of various algal species (Gonçalves et al., 2017; Salama et al., 2017). In most cases, with the accumulation of algal biomass, this culture process simultaneously provides a cost-effective and sustainable means of wastewater treatment (Salama et al., 2017). However, the accumulated algal biomass along with the wastewater treatment can only be utilized to produce biofuels and other low-cost products as these wastewaters usually contain heavy metals and/or toxic organic compounds, which greatly limit the potential to use algal biomass in a biorefinery concept (Matamoros et al., 2015; Podevin et al., 2017). Therefore, the value of the algal biomass obtained in these processes is low. Compared with above mentioned wastewaters, wastewater from food processing may be more suitable for microalgal cultivation because these wastewaters contain significant usable nutrients, and lack toxic compounds and hazardous substances that inhibit the growth of microalgae (Tan et al., 2014). The quality of the biomass cultured from these wastewaters is high and can be further used to produce some high-value products. The starch processing wastewater, dairy wastewater, and food processing wastewater exhibit good culture performance on microalgal culture from lab-scale to bench-scale and pilot-scale studies (Chu et al., 2015; Lu et al., 2015; Ji et al., 2015).

Tofu is a highly digestible and nutritive traditional food. It is popular in worldwide, especially in Asian countries, for many centuries (Yu et al., 2015). Its processing consists of soybean grinding, boiling, protein coagulation, filtration, and preservation. During this processing, it is estimated that about 7–10 tons of wastewater was generated for one ton of processed soybeans (Yu et al., 2002). The tofu wastewater usually contains a high COD (Chemical Oxygen Demand) concentration and is

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considered to be a serious environmental pollutant (Zheng et al., 2010). Due to the enriched nutrition in this wastewater, it can be utilized as a suitable substrate in fermentation industry. Some efforts have been made to utilize tofu whey wastewater (TWW) as the medium to produce various products, such as vitamin B12 accumulated by *Propionibacterium freudenreichii*, lactic acid accumulated by *Streptococcus bovis*, and biohydrogen produced by *Rhodobacter sphaeroides* (Yu et al. 2015; Yuwono and Kokugan, 2008; Zheng et al., 2010). It is also regarded as a potential medium for the cultivation of microalgae. Yonezawa et al. found that the addition of 1–2% TWW greatly enhanced the growth and hydrocarbon accumulation of *Botryococcus braunii*. In addition, the chemical composition of hydrocarbon was also influenced by the addition of TWW (Yonezawa et al., 2012). After being anaerobic hydrolyzed and centrifuged, TWW was investigated in the cultivation of *Chlorella pyrenoidosa*. The treated TWW exhibited good culture performance and a high biomass productivity, 0.64 g/L/day, was achieved (Su et al., 2011). Similarly, current studies on algae culture in TWW are restricted to the preprocessed TWW. For example, the raw wastewater usually needs to be anaerobic digested (Su et al., 2011; Zhang et al., 2012) or dilute to a low concentration before use (Yonezawa et al., 2012).

This study aimed to investigate the feasibility to use raw TWW as the medium for the culture *C. pyrenoidosa*. On this basis, we further studied the algal growth at heterotrophic and mixotrophic conditions using 100% TWW as the basal medium. In addition, the lipid and protein accumulation of cells cultured in TWW was also measured. This study is meaningful for providing an alternative and cheap medium for microalgae cultivation and decreasing the risk of environmental pollution of TWW.

2. Material and methods

2.1. Microalgal strains and inoculum culture

C. pyrenoidosa FACHB-9 was purchased from Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB), Chinese Academy of Sciences. The stored cells were activated in liquid BG-11 medium and incubated at 25 °C in an orbital shaker at 120 rpm under a 16/8-h light/dark cycle with exposure to 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by cool-white fluorescent lights. After cultured for two weeks, the cells were used as inoculum for following experiments.

2.2. Preparation of TWW media

TWW was obtained from a local tofu workshop (Yangzhou, Jiangsu Province of China) and some key parameters were shown in Table 1. This TWW was the filtrate from filtration step after protein coagulation. The TWW was firstly filtrated using a filter paper to remove the solid matters with large size. Then it was mixed with BG-11 medium at specific percentage if necessary. The pH of the TWW or mixture was adjusted using 1 M NaOH before been autoclaved. The raw TWW or mixture was autoclaved at 121 °C for 20 min and then cooling to room temperature.

Table 1
Chemical parameters of raw TWW.

Parameter	Value
pH	5.12 ± 0.22
Solid suspension (g/L)	2.77 ± 0.31
Total sugar (g/L)	4.43 ± 0.19
Reducing sugar (g/L)	1.40 ± 0.06
Total nitrogen (g/L)	0.59 ± 0.02
NH_4^+-N (g/L)	0.078 ± 0.004
Nitrate (g/L)	0.26 ± 0.01
Total phosphorus (g/L)	0.049 ± 0.002

To investigate the growth of microalgae at heterotrophic and mixotrophic conditions using TWW, 10 g/L glucose was added in pH adjusted TWW. BG-11 medium added 10 g/L glucose was set as control.

2.3. Microalgal culture

The autotrophic and mixotrophic cultures were performed at 25 °C in an orbital shaker at 150 rpm under continuous illumination with 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity. Heterotrophic cultures were performed at same conditions except under dark environment.

2.4. Analytic methods

2.4.1. Determination of microalgal growth

Because the wastewater contained abundant suspended solids and its turbidity was much higher than usual medium, the cell concentration biomass was difficult to be measured by gravimetrical measurement or spectrophotometry. Therefore, the cell growth was determined by monitoring the cell density using a microscope (Olympus BX53, Japan) and a hemacytometer. The dry cell weight (DCW, g/L) of the cells was calculated based on the linear correlation between cell density and dry cell weight.

The biomass productivity (P_b, g/L/day) was calculated as Eq. (1) (Wang et al., 2014b):

$$P_b = (DCW_x - DCW_0)/t_x \quad (1)$$

where DCW_0 and DCW_x are the initial dry cell weight (g/L) and the dry cell weight (g/L) at time t_x (day), respectively.

2.4.2. pH measurement

pH of culture broth was measured using a pH meter (METTLER TOLEDO SG8, Switzerland).

2.4.3. Measurement of lipid and protein content

Lipids were extracted and quantified as reported by Morales-Sánchez et al. (2013). Briefly, cell pellet was collected by centrifuging (14,000 × *g*, 10 min, 4 °C). The cell pellet was resuspended in methanol/dichloromethane (2:1, v/v) containing 0.5 mg of butylated hydroxytoluene after washing twice with deionized water. The mixture was stored at 4 °C overnight. The supernatant was collected and transferred to another tube after centrifuging the mixture at 4 °C, 14,000 × *g* for 10 min. Then the residue was extracted twice with 4 mL of methanol/dichloromethane (1:1; v/v) in an ultrasonic cleaner for 10 min. The solvents in the combined organic phase were removed using an evaporator at 40 °C and atmospheric pressure. After drying under nitrogen atmosphere, the lipids were gravimetrically quantified.

The protein was quantified using the Kjeldahl method as previously described (Wang et al., 2014a).

The lipid and protein productivity (P_p, g/L/day) was calculated as Eq. (2):

$$P_p = (DCW_x * C_x - DCW_0 * C_0)/t_x \quad (2)$$

where C_0 and C_x are the initial lipid/protein content (w/w, %) and the lipid/protein content (w/w, %) at time t_x (day), respectively.

2.5. Statistical analysis

Data were presented as means ± standard error of the mean based on three parallel experiments. The statistical significances were analyzed by one-way analysis of variance (ANOVA) ($P < 0.05$) using Origin 9 (OriginLab, USA).