Bioresource Technology 249 (2018) 219–225

Contents lists available at ScienceDirect

Bioresource Technology

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

Mixotrophic *Chlorella* sp. UJ-3 cultivation in the typical anaerobic fermentation effluents

Shuhao Huo[a], Miao Kong[a], Feifei Zhu[b], Bin Zou[a], Feng Wang[a], Ling Xu[a], Cunsheng Zhang[a], Daming Huang[a,*]

[a] School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China
[b] Institute of Life Sciences, Jiangsu University, Zhenjiang 212013, China

ARTICLE INFO

Keywords:
Anaerobic fermentation effluents
Microalgae
Light intensity
Lipid
Pigment

ABSTRACT

The growth of mixotrophic *Chlorella* sp. UJ-3 cultivated in the three typical anaerobic fermentation effluents was investigated in this paper. The results showed that the microalgae grew best under intermediate light intensity for all the types of fermentation effluents. The butyrate type fermentation effluents induced the fastest growth rate for *Chlorella* sp. UJ-3, with a maximal cell concentration of 3.8×10^7 cells/mL. Under intermediate light intensity, the volatile fatty acids (VFAs) were almost depleted on the fifth day of the cultivation for all the three types of fermentation systems. The ratios of chlorophyll *a/b* were all increased for the three systems, indicating enhanced energy-capturing capability of the microalgae for photosynthesis after the VFAs were depleted. The highest lipid content was 25.4%dwt achieved in the butyrate type fermentation, and the fatty acid compositions were found to be considerably different for these three types of fermentation systems.

1. Introduction

Anaerobic hydrolysis and acidification of complex organic wastes is a common method of wastewater treatment. During anaerobic hydrolysis and acidification, hydrogen, carbon dioxide, volatile fatty acids (VFAs), and sometimes alcohols, are simultaneously produced. The three widely recognized fermentation types in mixed culture of acidogenesis are butyric acid type, propionic acid type and ethanol type (Cohen et al., 1984; Ren et al., 1997, 2005). Butyrate type fermentation is characterized by the production of butyrate, acetate, CO_2 and H_2, which usually come from soluble carbohydrates. Propionate type fermentation produces mainly propionate, acetate and some valerate without significant gas production, which usually are degraded from organic compounds containing nitrogen. Ethanol type fermentation generates ethanol, acetate, H_2 and CO_2 which usually come from carbohydrates fermented in a continuous-flow reactor operated at a low pH value of 4.0–4.5. Ren et al. (1995, 1997, 2007). In addition, the end products of anaerobic hydrolysis also depend on the anaerobic, ecological condition relative to the microorganisms in the fermentation system (Ren et al., 2005).

The acidification fermentation effluents also contain substantial amounts of nitrogen and phosphorus that are required to sustain microalgae growth and showed very promising perspectives in terms of production of low-cost microalgae biomass and lipids (Turon et al., 2015a, 2016; Ren et al., 2014; Hu et al., 2013; Cho et al., 2015). Wen et al. (2013a,b) reported that *Chlorella protothecoides* can proliferate in a wastewater hydrolysate and that the lipid content was higher than that in a glucose solution with the same COD concentration. Su et al. (2011) found that the removal rate of VFAs from soybean product wastewater was 96.7% after anaerobic hydrolysis and acidification pretreatment by *C. pyrenoidosa* after 120 h of cultivation, indicating that the algae was able to make full use of acetate, propionate, butyrate, and iso-valerate in soybean product wastewater. Moon et al. (2013) showed that mixotrophic cultivation of *Chlamydomonas reinhardtii* in the presence of VFAs produced excellent yields of biomass ($2.05\ g{\cdot}L^{-1}$ in 5 days) and fatty acids (19.02%dwt of the biomass), indicating that VFAs, which are much less expensive than acetate, are a cost-effective alternative carbon source. VFAs can be produced from various raw materials such as food wastes, sludge, urban and agricultural waste (i.e. manures) and other similar materials (Fei et al., 2011; Ghimire et al., 2015), and can effectively promote algal growth and lipid production.

It is obvious that the contents of VFAs from different anaerobic fermentation effluents are variable (Table 1). The butyric acid and propionic acid types of fermentation were widely used for microalgae cultivation. The ethanol type fermentation is also produces very important organic wastes in the acidogenesis process. However, the ethanol type fermentation effluent is seldom used for microalgae cultivation. Further, the composition of microalgae cultures in the three

* Corresponding author.
E-mail address: danminhuang@126.com (D. Huang).

http://dx.doi.org/10.1016/j.biortech.2017.10.042
Received 31 August 2017; Received in revised form 2 October 2017; Accepted 7 October 2017
Available online 10 October 2017

Table 1
Various microalgae cultivation in the acidification fermentation effluents reported in the literature.

Type of wastewater	VFAs in COD (g COD/L)	Type of anaerobic fermentation	Microalgae	Reference
Waste activated sludge	Acetic acid: 1.2; Propionic acid: 0.45; Butyric acid: 0.23; Iso-butyric acid: 0.24; Valeric acid: 0.36; Iso-Valeric acid: 0.13	Propionic acid	*Chlorella protothecoides*	Wen et al. (2013b)
CSTR effluent	Ethanol: 0.25; Acetic acid: 0.64; Propionic acid: 0.46; Butyric acid: 0.69; Valeric acid:0.37	Butyric acid	*Chlorella protothecoides*	Wen et al. (2013a)
Dark fermentation effluent[a]	Acetic acid: 1.5; Butyric acid: 0.85	Butyric acid	*Micractinium reisseri* YSW05	Hwang et al. (2014)
Sewage sludge	Acetic acid: 0.7; Propionic acid: 0.66; Butyric acid: 0.09; Iso-Valeric acid: 0.55	Propionic acid	*Chlorella vulgaris*	Cho et al. (2015)
Dark fermentation effluent[a]	Acetic acid: 1.09; Butyric acid: 1.43	Butyric acid	*Chlorella sorokiniana*	Turon et al. (2015b)
Sewage sludge	10.8 g COD/L of total VFAs: 33.6% Acetic acid, 20.9% Propionic acid, 19.9% Iso-valeric acid, 11.2% Butyric acid, 8.1% Valeric acid, and 6.3% Isobutyric Acid	Propionic acid	Microalgal bacterial consortium	Turon et al. (2015c)
Starch wastewater/alcohol wastewater[a]	Acetic acid: 2.9; Propionic acid: 2.8; Butyric acid: 4.3; Iso-butyric acid: 0.3; Valeric acid: 0.21; Iso-Valeric acid: 0.37	Butyric acid	*Chlorella pyrenoidosa*	Yang et al. (2015)

Note:
[a] The real concentration.

types of acidification effluents, with the same organic carbon loading conditions, has not yet been determined. The aim of the present study is mainly to investigate the growth, VFAs removal, and lipid production by *Chlorella* sp. UJ-3 in the three typical anaerobic acidification effluents (butyrate type, propionate type and ethanol type fermentation) with the same organic carbon loading. The effect of light intensity on the growth rate and biomass productivity of *Chlorella* cultures is also investigated.

2. Materials and methods

2.1. Microalgae

Chlorella sp UJ-3 was obtained from the School of Food and Biological Engineering, Jiangsu University (Zhenjiang, Jiangsu, China). A BG11 medium [$NaNO_3$ (1.5 g·L^{-1}); K_2HPO_4·$3H_2O$ (40 mg·L^{-1}); $MgSO_4$·$7H_2O$ (75 mg·L^{-1}); $CaCl_2$·$2H_2O$ (36 mg·L^{-1}); $NaCO_3$ (20 mg·L^{-1}); $FeCl_3$·$6H_2O$ (3.15 mg·L^{-1}); citric acid (6 mg·L^{-1}) and 1 mL of microelements (H_3BO_3 (2.86 mg·L^{-1}), $MnCl_2$·$4H_2O$ (1.81 mg·L^{-1}), $ZnSO_4$·$7H_2O$ (0.22 mg·L^{-1}), Na_2MoO_4·$2H_2O$ (0.39 mg·L^{-1}), $CuSO_4$·$5H_2O$ (0.08 mg·L^{-1}) and $Co(NO_3)_2$·$6H_2O$ (0.05 mg·L^{-1}) diluted in 1000 mL acidified water)] was used to pre-cultivate the inoculum. The culture was placed at 25 °C under a light intensity of 100 μmol/(m^2·s). After 5 days of cultivation, the culture was used to further inoculate (10% v/v) the different culture media mentioned below.

2.2. Characteristics of typical anaerobic acidification effluents

Commercial fatty acids were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), and were all analytical grade unless otherwise noted. The compositions of the VFAs in the three anaerobic acidification effluents used in the experiment are listed in Table 2. For each type of the effluent, the desired composition of VFAs were formulated to the BG11 medium to for a final COD concentration of 2718.8 mg·L^{-1}. VFA concentration was converted to COD concentration by multiplying the respective conversion factors: 2.09 for ethanol, 1.07 for acetic acid, 1.54 for propionic acid, 1.82 for butyric acid, and 2.04 for valeric acid (Wang, 2015). The effluents were neutralized with 1 N NaOH to a pH 7.3. The media were sterilized at 121 °C and 120 kPa for 20 min before use.

2.3. Microalgae cultivation in flasks

Mixotrophic *Chlorella* sp. UJ-3 cultivation were cultured in 500 mL Erlenmeyer flasks containing 200 mL of the typical anaerobic acidification effluents. Triplicate algal treatments were maintained at 25 °C and continuously shaken at 150 rpm while under light intensities of either 50 μmol/(m^2·s) (low light) 100 μmol/(m^2·s) (intermediate light), and 150 μmol/ (m^2·s) (higher light). Fresh algae cells were inoculated into the medium with the average initial cell concentration of about 5×10^5 cells/mL.

Table 2
Compositions of VFAs/ethanol in the three anaerobic acidification effluents used in the experiment.

Composition	Ethanol type (mg·L^{-1})	Propionic acid type (mg·L^{-1})	Butyric acid type (mg·L^{-1})
Ethanol	710.2	123.7	138.6
Acetic acid	726.8	557	450.3
Propionic acid	88.6	959	80.1
Butyric acid	125.7	113.5	938
Valeric acid	43.8	86.5	55.9