Bioresource Technology 152 (2014) 471–476

Contents lists available at ScienceDirect

Bioresource Technology

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

Mixotrophic growth and biochemical analysis of *Chlorella vulgaris* cultivated with diluted monosodium glutamate wastewater

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HIGHLIGHTS

- The monosodium glutamate wastewater (MSGW) was used to grow *C. vulgaris*.
- The *C. vulgaris* growth was promoted greatly by MSGW.
- MSGW concentration affects the biomass productivity and biochemical composition.
- The microalga biomass was rich in protein and eight essential amino acids.
- The 100-time diluted MSGW is recommended for *C. vulgaris* cultivation.

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 13 November 2013
Accepted 20 November 2013
Available online 27 November 2013

Keywords:
Monosodium glutamate wastewater
Mixotrophic cultivation
Chlorella vulgaris
Specific growth rate
Biochemical composition

ABSTRACT

Monosodium glutamate wastewater (MSGW) is a potential medium for microbial cultivation because of containing abundant organic nutrient. This paper seeks to evaluate the feasibility of growing *Chlorella vulgaris* with MSGW and assess the influence of MSGW concentration on the biomass productivity and biochemical compositions. The MSGW diluted in different concentrations was prepared for microalga cultivation. *C. vulgaris* growth was greatly promoted with MSGW compared with the inorganic BG11 medium. *C. vulgaris* obtained the maximum biomass concentration (1.02 g/L) and biomass productivity (61.47 mg/L d) with 100-time diluted MSGW. The harvested biomass was rich in protein (36.01–50.64%) and low in lipid (13.47–25.4%) and carbohydrate (8.94–20.1%). The protein nutritional quality and unsaturated fatty acids content of algal increased significantly with diluted MSGW. These results indicated that the MSGW is a feasible alternative for mass cultivation of *C. vulgaris*.

1. Introduction

Microalgae investigation has been focused on in research all over the world. It could be used to produce animal feed, food additives, high-value added products (such as health supplements, cosmetics etc.), especially biofuels (such as biodiesel, bioethanol) (Das et al., 2011; Brennan and Owende, 2010), due to its certain excellent advantages such as rapid growth, the least land demand, high biochemical compositions (protein, lipid) (Tredici, 2010; Rawat et al., 2011). Microalgae could double their biomass within 1 day and their growth rate is 100 times that of terrestrial plants (Tredici, 2010). Nonetheless, compared with heterotrophic or mixotrophic cultivation, the microalgae usually grow slowly in photoautotrophic culture due to the light attenuation (Abreu et al., 2012). The algal biomass is usually not more than 1 g/L in photoautotrophic cultivation (Borowitzka, 1994). Mixotrophic growth occurred when the microalgae are provided with CO_2 and organic carbon sources simultaneously (Wang et al., 2012). That could greatly reduce the dependence on light needed for pure photoautotrophic growth (Cerón García et al., 2005), stimulate the algal growth and increase the cells density significantly (Kong et al., 2012). However, the high cost of adding organic carbon to the medium will make mixotrophic cultivation uneconomical (Bhatnagar et al., 2011). In order to reduce the product cost, many researchers have explored techniques to culture microalgae with organic wastewater (Abreu et al., 2012; Wang et al., 2012). By this means, nutrients from wastewaters are transferred to algal biomass, achieving economical microalgae cultivation and efficient wastewater treatment simultaneously. Because of the good performance of *Chlorella vulgaris* in mixotrophic cultivation (Heredia-Arroyo et al., 2011), it was chosen as the model organism in this study.

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http://dx.doi.org/10.1016/j.biortech.2013.11.047

Monosodium glutamate (MSG) as a flavor enhancer is extensively used in food products throughout east and south-east Asia. The MSG production in China accounts for about half of world's total output (Liu and Zhou, 2010). After extraction of MSG from fermentation liquor the residual dark brown wastewater and effluent have high concentrations of COD, NH_3–N, sulfate and a strong acidity (Yang et al., 2005). Without reasonable treatment the monosodium glutamate wastewater (MSGW) would cause serious pollution to the environment and damage to the ecology (Jin et al., 2013). As MSGW contains abundant nutrient substance, it could be feasible to reuse these organic substances using ecotechnological methods. Jun-Xian Liu (Liu et al., 2012) investigated the association of MSGW treatment by *Rhodotorula glutinis* and *Lipomyces starkeyi* and biodiesel production simultaneously. In addition, MSGW could be used as nutrient source to grow plants, such as Chinese cabbage and maize (Singh et al., 2009). Thus, it would be exciting if the MSGW could also be used for *C. vulgaris* growth. Actually, according to available information there has been no report on microalgae cultivation with MSGW as the medium.

In this paper, the growth of *C. vulgaris* cultivated with MSGW was assessed. As the composition and concentration of nutrients in the medium would influence the algal growth and biochemical composition greatly (Chen et al., 2011; Mitra et al., 2012), therefore, it would be very meaningful to evaluate the biomass productivity and estimate the biochemical composition of *C. vulgaris* cultivated with MSGW diluted different times.

2. Methods

2.1. Monosodium glutamate industrial wastewater (MSGW)

The MSGW was supplied by Sanjiu Monosodium Glutamate Industry, Chiping County, Liaocheng City. The brown–black acidic liquid, MSGW, used in this work was the residual fermentation broth after glutamate extraction. Detailed characteristics of MSGW were as shown in Table 1. The MSGW was diluted and sterilized at 120 °C for 30 min before being used as the culture medium.

2.2. Microalgae and inoculum preparation

The microalgae *C. vulgaris*, provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB-Collection), was cultured in BG11 medium at 25 ± 1 °C and 30 μ mol/m^2 s light intensity under 12:12 h L/D cycle for preparing inoculum. The contents of BG11 were as follows: $NaNO_3$ 1.5 g/L, K_2HPO_4 0.04 g/L, $MgSO_4{\cdot}7H_2O$ 0.075 g/L, $CaCl_2{\cdot}2H_2O$ 0.036 g/L, Citric acid 0.006 g/L, Ferric ammonium citrate 0.006 g/L, EDTANa 0.001 g/L, $NaCO_3$ 0.02 g/L, A5 trace metal solution 1 ml/L. The A5 trace metal solution was composed of H_3BO_3 2.86 g/L, $MnCl_2{\cdot}4H_2O$ 1.86 g/L, $ZnSO_4{\cdot}7H_2O$ 0.22 g/L, $Na_2MoO_4{\cdot}2H_2O$ 0.39 g/L, $CuSO_4{\cdot}5H_2O$ 0.08 g/L, $Co(NO_3)_2{\cdot}6H_2O$ 0.05 g/L. The light intensity was measured by opto-meter (Gigahertz Optik P9710).

2.3. Experimental setup

Triangular flasks (1000 mL) with an additional sample tap on one side were used as the batch reactors. These were sealed with 8-layer gauze and incubated on the shaker at the speed of 150 rpm. The MSGW diluted 25-, 50-, 100-, 200-, 400-, 600- and 800-times with distilled water was used as the experimental media. A control test was conducted simultaneously using BG11 medium. Prior to operation all the media were primarily adjusted to an initial pH value of 7.0 and then sterilized at 120 °C for 30 min using autoclave (SANYO, MLS-3750). To eliminate the effect of the contaminating microorganism during the incubation, antibiotic (levofloxacin hydrochloride tablets, final concentration of 20 mg/L) was added to the MSGW media and BG11 medium. The final *C. vulgaris* concentration was 0.2×10^6 cell/mL after inoculum with prepared seed. All experiments were operated at room temperature (25 ± 3 °C), under continuous illumination with an intensity of 30 μ mol/m^2 s (mean light intensity of the reactors walls). All the batch experiments were carried out in triplicate.

Daylight fluorescent tubes (Philips, 36 W) were used as a light source and the light intensity was measured at the surface of the reactors using an irradiance sensor (ZDS-10, Shanghai Cany Precision Instrument Ltd., China).

2.4. Determination of C. vulgaris growth

Microalgae growth was measured every 24 h by cells count with a light microscope using a Neubauer Hematocytometer (Gonzalez and Bashan, 2000) and biomass concentration determination (g/L, dry weight) as described by (Song et al. (2013)). The specific growth rate (μ, d^{-1}) of microalgae in each medium was calculated according to the following equation (Lim et al., 2010):

$$\mu = (LnN_2 - LnN_1)/(T_2 - T_1) \quad (1)$$

where N_1 and N_2 are the dry biomass concentrations at day t_1 and t_2 respectively. The biomass productivity during the incubation was calculated according to the following formula:

$$P_b = (X_2 - X_1)/(T_2 - T_1) \quad (2)$$

where X_1 and X_2 (g/L) are the dry biomass concentrations at day T_1 and T_2 respectively.

2.5. Analysis of biomass components

At the end of culture, biomass was harvested by centrifugation at 4000 rpm for 10 min and then dried at 60 °C to constant weight. The amino acid composition of algal cells was analyzed using amino acid analyzer (L-8900, Hitachi, Japan). The essential amino acid score (EAAS) was calculated by the following formula (according to FAO/WHO, 1973):

$$\mathrm{EAAS} = C_t/C_r \times 100\% \quad (3)$$

where C_t is percentage content of one essential amino acid (EAA) in the test protein, and the C_r represents the percentage content of the same kind of EAA in the reference protein. The essential amino acid having the lowest EAAS was the first limiting amino acid of the test protein. And this lowest EAAS was regarded as the chemical score (CS) of this protein. The essential amino acid index (EAAI) was computed as follows (according to (Rakowska et al., 1978)):

$$\mathrm{EAAI} = \sqrt[n]{\left(\frac{a}{A}\times 100\right)\times\left(\frac{b}{B}\times 100\right)\times\cdots\times\left(\frac{j}{J}\times 100\right)} \quad (4)$$

Table 1
Characteristics of the MSGW used as nutrient medium in this study.

COD (g/L)	BOD_5 (g/L)	TN (g/L)	NH_3–N (g/L)	TP (g/L)	TS (g/L)	VS (g/L)	pH
496	162	7.2	1.01	2.41	491.3	419.2	2.5 ± 0.1

Note: COD, chemical oxygen demand; BOD_5, biochemical oxygen demand; TN, total nitrogen concentration; NH_3–N, amino nitrogen; TP, total phosphorus concentration; TS, total solid content; VS, volatile solid content.