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The feasibility of using complex wastewater from a monosodium glutamate factory to cultivate *Spirulina subsalsa* and accumulate biochemical composition

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HIGHLIGHTS

- Complex wastewater (CW) of monosodium factory was used firstly to grow *S. subsalsa*.
- The *S. subsalsa* biomass compositions were promoted by CW.
- The 25% CW was suitable for protein production due to ideal ammonia concentration.
- The 50% CW was recommended for lipid production economically.

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ABSTRACT

This paper is mainly observations on the growth and biomass accumulation of *Spirulina subsalsa* in modified Zarrouk medium supplemented with complex wastewater (CW, from a monosodium glutamate factory) in different concentrations. High ammonia in 75% and 100% CW inhibits algae growth, but maximum biomass production (2.86 mg L^{-1}) was obtained in 25% CW (concentration of CW in medium was 25%). Different CW concentration promoted biomass composition accumulation at different degrees, 41% of protein content in 25% CW and 18% of carbohydrate in 50% CW. In terms of economy, a concentration of 25% CW was suitable for protein production and 50% for lipid and carbohydrate production. These results suggested that CW is a feasible replacement in part for cultivation of *S. subsalsa* to economize input of water and nutrients.

1. Introduction

Spirulina, cyanobacteria, has very high nutritional value for human food supplement, as well as animal feed. As the oldest photosynthesis microalgae *Spirulina* has been applied in many fields like food and medicine in the past and present (Henrikson, 1989; Estefanía et al., 2014). Its nutrient composition and phytonutrients are the most potent in all food, plants, grains or herbs. Due to its highly valuable protein and amino acids, vitamins and minerals *Spirulina* has been called "superfood" (Henrikson, 1989). There are some figures that could hint at the importance of the algae production. In 2003 production of *Spirulina* in China was registered at 19,080 tonnes and rose sharply to 41,570 tonnes in 2004, output valued at about 7.6 million dollars and 16.6 million dollars, respectively (Habib et al., 2008).

Besides its value in food, *Spirulina* has other properties. Compared to unicellular microalgae it is an easy and quick task to harvest the *Spirulina* biomass because of its self-aggregate in hyposaline medium, multicellular and filamentous character (Gabbay and Telor, 1985; Rodrigues et al., 2010). The cost of unicellular algae recovery accounts for 20–30% of general microalgae biofuel manufacturing cost (Lam and Lee, 2012), so *Spirulina* is greatly superior to other unicellular microalgae in harvest cost.

Nevertheless the huge amount of water required restricts the industrial application of algae (Oswald, 1963; Chinnasamy et al., 2010). With regard to this problem, wastewater has been proposed as a solution, serving as a supplement or even complete substitute medium for microalgae cultivation. Phang et al. (2000) cultivated *Spirulina platensis* with digested sago starch factory wastewater in a high rate algal pond and obtained an average specific growth rate

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(μ) of 0.51 d^{-1}, which was comparable to the μ of 0.54 d^{-1} in the inorganic medium. And Park et al. (2013) used municipal wastewater as supplement to a part Zarrouk medium (ZM) to cultivate *Arthrospira* (*Spirulina*) *platensis* in a photobioreactor and obtained 3.29 g kg^{-1}, 2.79 g kg^{-1} biomass in 40% wastewater and ZM, respectively. However for the same species of algae, Markou et al. (2012) obtained less exciting results with olive-oil mill wastewater (OMWW), as OMWW diluted with deionized water without the addition of nutrients could not support algae growth. The results indicate that nutrient bioavailability to algae may be very low even though OMWW contained relatively large amounts of nutrient substance. *Spirulina* has exhibited different potential for assimilating nutritional material from different kinds of wastewater.

The wastewater from monosodium glutamate (MSG) production, which is famous for its high level of contamination, may be a luxuriant nutrient for microalgae growth. There have been many attempts at using this wastewater as a culture medium, such as the cultivation of *Azospirillum rugosum*, *Lipomyces starkeyi*, *Chlorella vulgaris*, and so on (Xue et al., 2008; Singh et al., 2011; Liu et al., 2012; Ji et al., 2014). In their research this wastewater was characterized by high COD (dozens or even hundreds of grams per liter), high BOD_5, high ammonia and low pH (around 3.0). As for making full use of nutrients in wastewater (Ji et al., 2014), it should be noted that the MSG factories have perfected their manufacturing technology, so the parameter of complex wastewater (CW) collected from the new technology has changed a lot compared to that before advanced techniques came into use, with fewer nutrients (COD_{Cr}: 450–950 mg L^{-1}, BOD_5: 300–450 mg L^{-1}, SS: 300–310 mg L^{-1}, sulfate: 140–160 mg L^{-1}) but high free ammonia (NH_3-N: 100–130 mg L^{-1}) in an alkaline environment. In terms of the discharge standard of pollutants for the monosodium glutamate industry (GB19431-2004), additional investment has to be made in treatment of CW. Nevertheless, abundant ammonia and high pH in CW, to a certain extent, are conducive to *Spirulina* growth. This is because ammonia is the optimal nitrogen source for algae, and *Spirulina* could grow in an alkaline environment where most of crop struggles (Habib et al., 2008). So *Spirulina* could be the ideal candidate for CW utilization and wastewater could replace part or all of general medium for algae culture.

The overall goal of the research was to assess the feasibility of *Spirulina subsalsa* cultivation in modified ZM (mZM) (Raoof et al., 2006) supplemented with CW and investigate improvement in biomass composition accumulation of the algae. Although CW contains relatively large amounts of nutrients, their bioavailability to algae may not be high as Markou et al. (2012) described. So this major work aims to research the proper proportion of CW in culture for algae growth and biomass composition accumulation, in addition to considering the economic benefits of microalgae cultivation with CW.

2. Methods

2.1. CW from a MSG factory

The CW was sampled at Liangshan Linghua Gourmet Powder factory (Jining, PR China). The wastewater was characterized in high concentration of ammonia, alkalinity (pH: 9.47, COD_{Cr}: 452.73 mg L^{-1}, NH_3-N: 123.40 mg L^{-1}, NO_3-N: 3.31 mg L^{-1}, TP: 0.46 mg L^{-1}, Na: 517.75 mg L^{-1}, Mg: 10.54 mg L^{-1}, K: 24.52 mg L^{-1}, Ca: 52.68 mg L^{-1}). The CW was put into use as supplement of mZM directly without any pre-treatment such as filtering, autoclaving or pH adjusting.

2.2. Microalgae strain

S. subsalsa was bought from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB-Collection), and cultured in *Spirulina* medium (SP) at 25 ± 1°C. In the experiment, the SP was replaced by mZM included (g L^{-1}): $NaNO_3$, 2.5; NaCl, 0.5; $MgSO_4{\cdot}7H_2O$, 0.15; $CaCl_2{\cdot}2H_2O$, 0.04; single super phosphate ($Ca(H_2PO_4)_2{\cdot}CaSO_4{\cdot}H_2O$), 1.25; KCl, 0.898; $NaHCO_3$, 16.8 (Raoof et al., 2006).

To get homogeneous suspension, the microalgae inoculum was eddied before biomass measurement (by OD_{680}). The OD_{680} of algae inoculum was about 0.320 after 10-fold diluted with distilled water.

2.3. Experimental setup

Triangular flasks (250 mL) sealed with parafilm were used as the batch reactors. Prepared seed of 2 mL was inoculated to 50 mL medium in the reactor, and cultivated under the conditions of 25 ± 1°C and light intensity of 60 μ mol m^{-2} s^{-1} provided by daylight fluorescent tubes (Philips, 36 W). The medium of algae culture was the mixture of mZM and CW in different proportions (v/v) (0%, 25%, 50%, 75% and 100%), where 25% CW meant that the medium was made of 25% CW and 75% mZM.

The light intensity was measured at the surface of reactors using an irradiance sensor (ZDS-10, Shanghai Cany Precision Instrument Ltd., China). All the batch experiments were conducted in duplicate.

2.4. Determination of algae growth

Algae growth was measured every 24 h by biomass concentration determination (g L^{-1}, dry weight, DW) as described by Song et al. (2013). Due to self-aggregate and inhomogeneous distribution of *Spirulina* cells in medium, all culture in one triangular flask had to be taken out to measure the day weight.

The specific growth rate (μ, d^{-1}) of microalgae in each medium was calculated according to the following equation (Ji et al., 2014):

$$\mu = (\mathrm{Ln}N_2 - \mathrm{Ln}N_1)/(T_2 - T_1) \quad (1)$$

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 are the dry biomass concentrations at day T_1 and T_2, respectively.

The pH was measured using a pH meter (Shanghai Leici Instrument Co. Ltd., PHS-3C, China) every day.

In consideration of strong volatility of ammonia in an alkaline environment, a control test was conducted simultaneously using complex wastewater without inoculate algae. And NH_3-N was analyzed every day, but the other nutrients (NO_3-N, TP) were only determined after algae harvest as final concentration. The initial concentrations of all nutrients were calculated in the base of the components in mZM and CW.

2.5. Analysis of biomass components

At the end of algae growth, biomass was harvested by filtration through bolting-silk in 200 mesh, dried to constant weight at minus 50°C in lyophilizer (FDU-1200, EYELA, Japan), and then ground to homogeneous powder to analyze its protein, lipid and carbohydrate.

2.5.1. Protein

The amino acid composition of the algae was analyzed through an amino acid analyzer (L-8900, Hitachi, Japan). The total protein content was the sum of all amino acid contents.