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Use of Chlorella for the Treatment of the Soft-shelled Turtle Processing Wastewater

Yong-jun Zhang, Dong-yang Su, Gao-fan Zhang, Jia Li, Song-xue Hu, Sheng-hua Han, Ling-tao Zeng and Ting Chen
College of Life Sciences, China Jiliang University, P.R. China

Abstract: The capability of Chlorella vulgaris to remove nitrogen in the form of ammonium ions from the soft-shelled turtle processing wastewater in a local agricultural products limited company (in Hangzhou, China) was studied. The soft-shelled turtle processing wastewater was found to include high concentrations of nitrogen (107.63±4.84 mg/L) in the form of ammonium (NH_4^+) with the small amounts of nitrite (0.32±0.04 mg/L on annual average) at pH 6.7 and to be suitable for growing Chlorella vulgaris. When Chlorella vulgaris was cultivated in a batch mode, a majority of the nitrogen concentration was dramatically removed after a lag-phase period. The total biomass weight gained during the entire cultivation period balanced out well with the nitrogen removed from the culture medium. These results indicate that Chlorella vulgaris has potential to remove nitrogen (i.e., ammonium ion) and nitrite at a reasonable uptake rate from wastewater while being cultivated using the soft-shelled turtle processing wastewater.

Keywords: Chlorella vulgaris, microalgae, nitrogen removal, soft-shelled turtle processing, wastewater

INTRODUCTION

The commercial soft-shelled turtle industry generates large quantities of solid waste and wastewater. Wastewater containing the biological wastes of soft-shelled turtle, which harbors abundant inorganic nitrogen, is one of the most significant causes of eutrophication in waterways (Hwang and Hansen, 1998). Conventional biological treatment methods such as activated sludge involve high energy inputs associated with O_2 supply and do not allow recycling of nutrients present in the wastewater (De Godos *et al*., 2009). Both living and non-viable algae have been used in wastewater treatment. Microalgae are mostly suspension-type microorganisms and very efficient solar energy converters that can produce massive blooms. For decades, they have demonstrated that they can produce a great variety of useful secondary metabolites (Lebeau and Robert, 2006; Moreno, 2008) and are potentially useful as treating agents for wastewater. Microalgae have been utilized as a bioremediature agent in the removal of inorganic nutrients from polluted water to improve short-term quality (Martinez *et al*., 2000). This sort of wastewater has also been used as a microalgal culture medium (Olguin *et al*., 2001, 2003; An *et al*., 2003). Microalgal-based wastewater treatment presents several advantages over conventional technologies, including recovery of nutrients and reduction of CO_2 emissions due to their autotrophic metabolism. Furthermore, when microalgae-containing microbiota are used, symbiotic relationships can be established since microalgae supply oxygen whereas non-photosynthetic microorganisms produce the CO_2 needed for microalgae growth (Molinuevo-Salces *et al*., 2010). These studies have focused primarily on the capacity to remove nitrogen and nitrite, particularly those from soft-shelled turtle's wastewater.

Chlorella vulgaris has been reported to be able to readily uptake nitrogen from ammonium ion and ammonia through the cell membrane from the wastewater (Shi *et al*., 2007; Schlee and Komor, 1986). It was also reported that algae-derived biofuels could compensate for environmental burden using wastewater as a source for CO_2 and fertilizer according to previous Life Cycle Assessment (LCA) studies (Clarens *et al*., 2010; Lardon *et al*., 2009). Different studies have evaluated the growth of microalgae under a variety of wastewater conditions, mainly in municipal and livestock wastewater. To the best of our knowledge, treatment of soft-shelled turtle processing wastewater using Chlorella vulgaris has not been studied. The primary objective of this study was to investigate the use of Chlorella for bioremediation, especially in removing noxious substance such as nitrites and ammonium salt from the soft-shelled turtle processing wastewater.

MATERIALS AND METHODS

Culture media: The soft-shelled turtle processing wastewater was collected from Hua Dan Agricultural Products Limited Company in Hangzhou, China and was used as a culture medium. The concentrations of

Corresponding Author: Yongjun Zhang, College of Life Sciences, China Jiliang University, Xueyuan Street No. 258, Xiasha High Education Area, Hangzhou 310018, Zhejiang Province, P.R. China, Tel.: 0571-86835703

ammonium and nitrite on a weekly basis and their annual average concentrations were 107.63±4.84 mg/L and 0.32±0.04 mg/L, respectively. A 30-L sample of Chlorella vulgaris suspended was added to the soft-shelled turtle processing wastewater. The cell density of Chlorella vulgaris in the wastewater was found out to be 5×10^4/mL.

Chemical analysis: Moisture content was determined by gravimetric heating (130±2°C for 2 h) using a 2-3 g sample. Ash and protein were analyzed according to methods 08-01 and 46-13, respectively (American Association of Cereal Chemists (AACC), 2000). Crude fat was analyzed according to the methods of AOAC 15.920.39 (AOAC, 1990). Total carbohydrates (%) = [100-(moisture+protein+ash+crude fat)] %. Total soluble sugars were estimated colorimetrically by the phenol-sulfuric acid method using a standard curve of glucose (Dubois *et al*., 1956).

Determination of cell density of Chlorella vulgaris: The cell density of Chlorella vulgaris was determined by measuring the optical density of a 15-mL sample at 682 nm by using UV-vis spectrophotometer. Here, the absorbance of UV spectrophotometer at 682 nm was calibrated by measuring the weight of dried Chlorella vulgaris. Then, the weight of dried biomass was obtained from the prepared calibration curve.

Determination of nitrate (NO_2^-) concentration: The concentrations of nitrate ions was determined with Dionex DX-500 ion chromatography system equipped with IonPac AS11 anion-exchange column.

Determination of nitrogen concentration in the form of ammonium ion (NH_4^+): Nitrogen concentrations in the form of ammonium ions present in the wastewater was measured with Nessler colorimetric method at 420 nm by using UV-vis spectrophotometer.

RESULTS AND DISCUSSION

The high protein content of certain microalgae was one of the reasons to select these organisms as unconventional protein sources. Chemical composition were shown in Table 1. The main feature was their high protein content (P58.70%).

The soft-shelled turtle processing wastewater was collected from Hua Dan Agricultural Products Limited Company contained large amounts of nitrogen (107.63±4.84 mg/L) in the form of ammonium (NH_4^+) with the small amounts of nitrite (0.32±0.04 mg/L on annual average). In addition, the initial pH of the wastewater effluent was almost constant at pH 6.7. Chlorella vulgaris can grow using carbon dioxide and light, known as the photosynthetic process: $6CO_2+12H_2O+\text{light (energy)}\rightarrow C_6H_{12}O_6+6O_2+6H_2O$. Microalgae can utilize bicarbonate ions (HCO_3^-) as a carbon source for photosynthesis with the help of an enzyme called carbonic anhydrases (CA) as well as carbon dioxide. In addition, inorganic nutrients, especially nitrogen and phosphate are required for their growth.

Table 1: Chemical composition of the *Chlorella* powder (g dry matter)

Constituent	Content
Moisture	6.99±0.24
Crude protein	58.70±1.01
Crude fat	2.27±0.05
Ash	8.05±0.11
Total carbohydrates	23.99
Total soluble sugars	12.10±0.41

Values are means of three replicates ± standard error

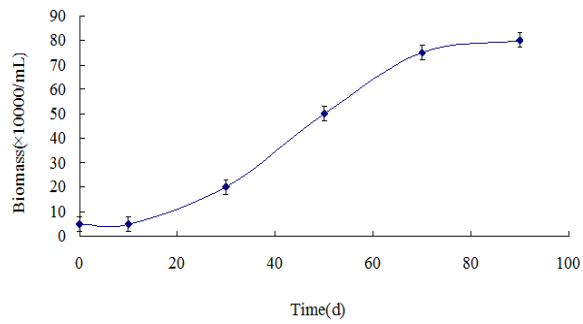

Fig. 1: Growth rate of Chlorella vulgaris

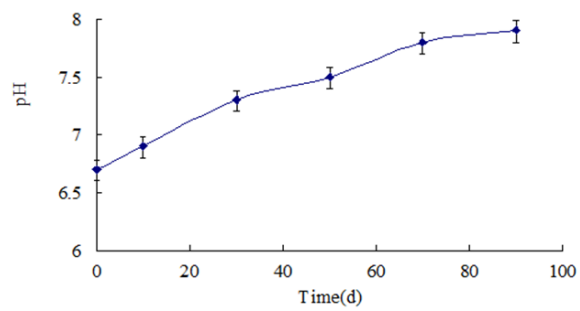

Fig. 2: pH of wastewater increase

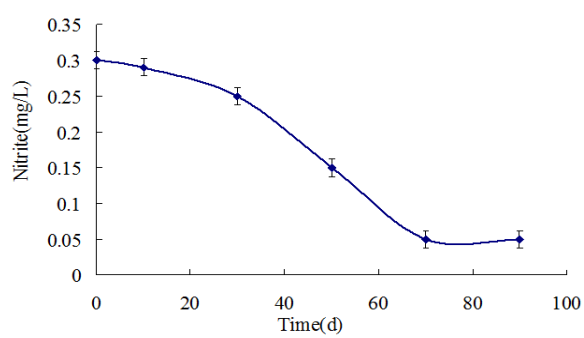

Fig. 3: Removal of nitrite from the wastewater by Chlorella vulgaris

The growth rate of Chlorella vulgaris was shown in Fig. 1. The lag-phase period for adaptation to the wastewater condition was be found to be 10 d. During the growth phase, the cell density of Chlorella vulgaris dramatically increased until 70 d, indicating active

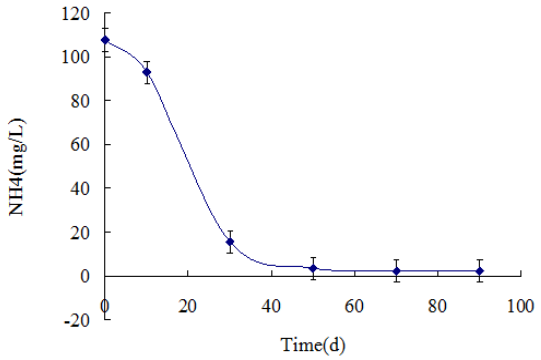

Fig. 4: Removal of nitrogen in the form of NH4+ from the wastewater by Chlorella vulgaris

photosynthetic reaction. The pH of the wastewater continued to increase from the lag phase through the growth phase as shown in Fig. 2. Microalgae are known to produce hydroxyl ions (OH^-) when a bicarbonate ion is consumed for photosynthesis within the algal cell by following the reaction: $HCO_3^- \rightarrow CO_2 + OH^-$ (Shiraiwa *et al*., 1993).

The removal of nitrite is shown in Fig. 3. During the lag phase, the nitrite removal efficiency varied little from 0.32±0.04 mg/L to 0.30±0.03 mg/L matched with no growth of the Chlorella vulgaris. The nitrite concentration did not decrease during the initial lag phase, but then sharply decreased from 0.32±0.04 mg/L to 0.06±0.01 mg/L during the rapid growth-phase.

The removal of nitrogen (ammonium ion (NH_4^+)) is shown in Fig. 4. During the lag phase, the nitrogen removal efficiency varied from 107.63±4.84 mg/L to 92.86±5.36 mg/L matched with no growth of the Chlorella vulgaris, but then sharply decreased from 107.63±4.84 mg/L to 3.48±0.47 mg/L during the rapid growth-phase. It is interesting to note that the total biomass weight gained during the entire cultivation period is well balanced by nitrogen removed from the culture medium as clearly shown in Fig. 1.

CONCLUSION

In this study, Chlorella vulgaris was used to remove nitrogen in the form of ammonium ions from the soft-shelled turtle processing wastewater collected from the Hua Dan Agricultural Products Limited Company, Hangzhou, China. The mass balances taken for nitrogen show that the weight gained by biomass is inversely proportional to the amount of nitrogen removed from wastewater. The experimental results indicated that Chlorella vulgaris has potential to remove nitrogen (i.e., ammonium ion) and nitrite at a reasonable uptake rate from wastewater while being cultivated using the soft-shelled turtle processing wastewater. As a result of consuming bicarbonate ions, the pH of the culture medium increased. This can open up an opportunity to utilize wastewater in order to cultivate microalgae for the dual purpose of removing noxious substance and producing nutrients.

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