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The effect of salinity on microalgae nitrate assimilation in reused media

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Abstract

Reusing the microalgae culture medium is a significant factor in economy of algal biomass production. However, reusability depends on the microalgae harvesting method and the post-harvesting conditions of the medium. In the pH adjustment-sedimentation method, which is an economical method for microalgae harvesting, the salinity of the environment increases after biomass separation. In this study, the effect of increasing salinity on nitrate assimilation in reused culture medium was investigated. The results showed that most of the nitrate is consumed in the first (fresh) culture, so that in fresh medium with high salinity (EC: 4230 µS) 93% of nitrate and in fresh medium with normal salinity (EC: 1623 µS) 66% of nitrate is consumed. However, in reused cultures (second and third), increasing salinity due to the harvesting method significantly reduces nitrate assimilation so that in the medium with high salinity, nitrate assimilation approaches to zero. Thus, for suitable cell growth in reused culture media, re-nutrition of the constituent elements of culture is necessary.

Keywords: Reuse culture medium**,** Salinity, Microalgae, Growth, Nitrate consumption, Nutrient

1. Introduction

Microalgae are a vital source for production of a variety of biological products. For example, biodiesel is one of the most important products obtained from microalgae biomass in the energy sector. Biodiesel is one type of bioenergy in the subset of renewable energies which play a role in overcoming the energy crisis [1]. Due to the high efficiency of biodiesel which compares to petroleum diesel, it has attracted a great deal of attention. Thus, the third generation of bioenergy based on microalgae biomass was considered [2].

Microalgae biomass production is limited by lack of raw materials and high production costs [3]. Despite many advantages of microalgae, the cost of cultivation and harvesting slows down their industrialization [4]. Water is one of the essential components of microalgae production, which

constitutes more than 95% of the culture medium. Therefore, the reuse of culture medium is considered. According to a recent study, 1564 L of water must be used to produce 1 kg of microalgal biomass in an outdoor pool and recycling of the culture medium reduces water and nutrient consumption by 84 and 55%, respectively [5]. For reuse of culture medium, the harvesting method and its effect on the recycled medium is significantly important. One of the latest and the most cost-effective methods of harvesting algal biomass is the pH adjustment-sedimentation method. In this method, the pH of the spent medium is adjusted to an alkaline level by sodium hydroxide that biomass flocculates and settles in a sufficient amount of time. After separation of biomass, to reuse the culture medium, this solution is neutralize using hydrochloric acid, and its pH restores to its original value. This method (increasing and then decreasing the pH) increases the concentration of sodium and chlorine ions in the medium and ultimately causes salinity in the medium.

The microalgae species response to salinity of the medium in different ways. Salinity causes an environmental stress, and cells undergo many adaptive strategies. Salinity affects not only growth but also the consumption of nutrients. Nitrate is one of the most significant growth factors that salinity affects its consumption. Microalgae cells do not divide in the nitrate-depleted culture medium and stop growing, even if they have enough energy to propagation [6]. Therefore, nitrate deficiency in the reused environment reduces growth. This study aims to investigate the effect of post harvesting salinity on nitrate consumption in microalgae culture as well as cell growth.

2. Experimental

The microalgal species used in this study is *Chlorella sp.* IG-R-96 (accession number MF459966). BG11 culture medium with the following composition was used (in 1 L of distilled water): $NaNO_3$ (1.5 g). $MgSO_4.7H_2O$ (0.075 g); $K_2HPO_4.3H_2O$ (0.04 g); $CaCl_2.2H_2O$ (0.036 g); Na_2CO_3 (0.02 g); $FeCl_3$ (0.006 g); Citric acid (0.006 g); EDTA (001/0 g). The metal solution was added to the culture medium at a rate of 1 mL/L. Metal solution composition is as follows (in 1 L of distilled water): $ZnSO_4.7H_2O$ (222 mg); $Na_2MoO_4.2H_2O$ (39 mg); $CuSO_4.5H_2O$ (79 mg); $MnCl_2.4H_2O$ (1810 mg); H_3BO_3 (2860 mg) and $Co(NO_3)_2.6H_2O$ (4.9 mg). For cultivation of microalgae, a glass bottle was used with a working volume of 1 L at temperature of 26 ± 1°C. For illumination, three sets of SMD LED strips of 30 cm each were used providing light intensity of 327 (µmol photons/m^2s) during 16/8 light/dark cycles. The medium was aerated at the level of 1 vvm containing 6% vol of CO_2.

The optical density of the medium was measured throughout cultivation using a spectrophotometer (Unico2100, UV-VIS). Also, Residual nitrate in culture medium was measured by Dinesh Kumar et al. method [7]. Briefly, 2 ml of the medium centrifuged and 1 ml of the supernatant was diluted 100 times. Its absorbance was read at 220 and 275 nm and based on Equation 1, the concentration of residual nitrate in mg/L was calculated [8]:

$$Reisdiual\ nitrate = \frac{A_{220} - 2(A_{275})}{0.053} \qquad (1)$$

At the end of culture (stable optical density), the resulting biomass was separated by pH adjustment and then sedimentation. For this purpose, the pH of the medium was raised to 13 using a solution of 10 N sodium hydroxide, and then floc were allowed to settle for 24 hours. After biomass separation, , the pH of remained medium was reduced to normal value of BG11 medium (~7.3) using 12 M hydrochloric acid and prepared for the next culture. Salinity (in terms of electrical conductivity µS) was measured using a conductometer (ED / TDS Meter, Mi 306 Martini Instruments).

To investigate the effect of salinity, two culture media were used: normal culture medium with EC of 1623 µS and adjusted culture medium with EC of 4230 µS. For re-culturing, after biomass separation, each culture medium was inoculated with 10% (v/v) of fresh medium, and cell growth was monitored over the course of cultivation up to the constant optical density.

3. Results and discussion

To study the effect of salinity on nitrate consumption by microalgae, two culture media with normal salinity (1623 µS) and adjusted salinity (4230 µS) were used. After cultivation on fresh media, two more re-cultures were conducted using both media.

Table 1 shows the changes in medium salinity after the end of each culture. As seen in this table, the salinity of the reused media increases compared to fresh media due to the pH-adjustment cell harvesting. However, this increase depends on the initial salinity.

Table.1 Change in medium salinity (EC in µS) after different cultures

Culture medium	First culture (Fresh)	Second culture	Third culture
Normal medium	1623	2214	3950
Adjusted medium	4230	5980	8600

In the normal medium, in the third culture, the EC of reused medium increased almost 2.5 times compared to fresh medium. In the adjusted medium, EC increased 2 times. Considering the increase in salinity of the culture medium was due to the increase and decrease of pH during the microalgae harvesting process, the results show that the increase in EC of the medium is limited and does not increase linearly.

Figures 1 and 2 show that nitrate consumption varies in normal and adjusted media. In the normal medium, nitrate concentration changed from 1500 mg/L to 820 mg/L during three consecutive cultures, this is while in the adjusted medium nitrate concentration changed from 1500 mg/L to 1026 mg/L in the same situation. This comparison reveals that more nitrate is consumed in the normal medium than adjusted medium.

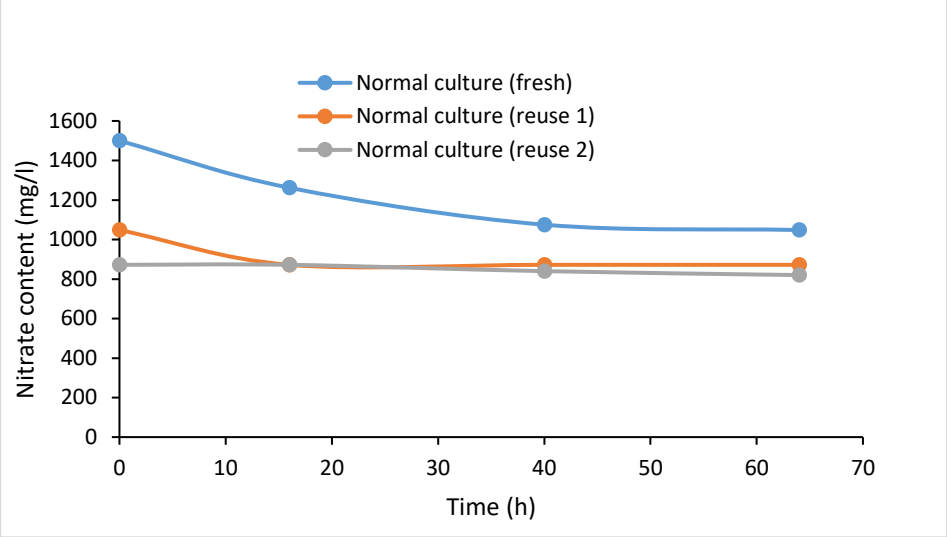

Figure 1. Changes in nitrate content in culture medium with normal salinity

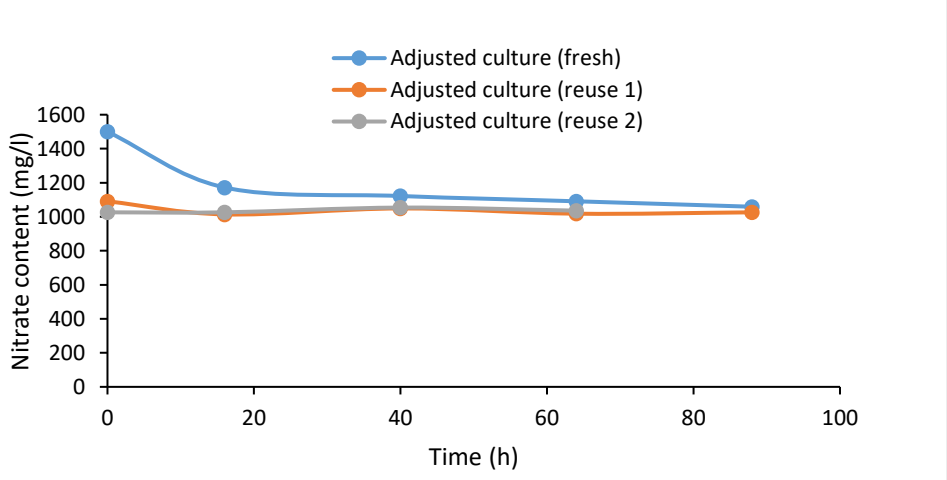

Figure 2 Figure 2. Changes in nitrate content in salinity-adjusted culture medium

Also the results indicate that in the normal medium, the major part of nitrate consumption was related to the first (fresh) culture (66%) and in the second (26%) and the third (8%) cultures, nitrate consumption was remarkably less. The same rate of consumption was observed in the adjusted medium, so that major part of nitrate consumption was related to the first culture (93%), and in the second culture only 7% of nitrate was assimilated and in the third culture, nitrate assimilation reached zero. The trend of nitrate assimilation is also consistent with the rate of cell growth. The optical density of biomass in the first culture of normal medium increased 27.8 times in 64 hours, while in the second and the third cultures accounted for 5.3 times and 2.8 times, respectively. In the adjusted medium, a similar trend is observed so that in 64 hours in the first culture, the optical density of biomass increased 48.5 times. However, in the second culture, cell density increased 4.7 times, and in the third culture 1.6 times. These results show that in medium with higher EC (higher

salinity), the growth rate is higher than that in lower EC medium. However, in high salinity media reused cultures have very limited potential for cell growth. The difference in cell growth potential between fresh and reused environments can be due to the assimilation of a large portion of nutrients in the first culture so that in the second and third cultures, the elements needed for cell growth are not sufficient. Therefore, growth and nitrate assimilation is limited in media with higher EC; this difference is greater because the growth rate is higher. In general, it can be concluded that to reuse the microalgae culture medium, which can have many economic benefits, it is necessary to re-nutrient the spent culture medium.

4. Conclusions

The reuse of culture medium is one of the economic aspects of the microalgae biomass production. However, for reuse, the culture medium should be examined for the elements and compounds that support growth and re-nutrient need to be done if necessary. Since all elements are used in the first culture, elemental analysis is required to effectively re-nutrient the medium.

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