هفدهمین کنگره

ملی مهندسی شیمـــی ایران

دانشگاه فردوسی مشهد - آبان ۱۴۰۰

گواهی ارائه مقاله

بدین وسیله گواهی می شود که مقاله با عنوان

The effect of salinity on microalgae growth in reused culture media

با نویسندگی: یاسمین هامونی، محمود اخوان مهدوی و رضا قشلاقی

در هفدهمین کنگره ملی مهندسی شیمی ایران که در تاریخ ۱۸ تا ۲۰ آبان در گروه مهندسی شیمی دانشگاه فردوسی مشهد برگزار گردید، مورد پذیرش قرار گرفته و ارائه شده است.

اکبر شاهسوند
دبیر علمی کنگره

وحید تقی خانی
دبیر انجمن مهندسی شیمی ایران

مهدی پور افشاری
دبیر کنگره

The effect of salinity on microalgae growth in reused culture media

Y. Hamooni, M. A. Mahdavi*, R. Gheshlaghi
Department of Chemical Engineering, Ferdowsi University of Mashhad, Mashhad, IRAN
mahdavi@um.ac.ir

Abstract
Considering the importance of water and nutrients in microalgae cultivation, reusing media is an outstanding step towards economic viability of algal biomass production. However, biomass harvesting method and post-harvesting conditions greatly influence reusability of culture. In the pH adjustment-sedimentation method, an efficient way of harvesting microalgae, the salinity of the medium increases after biomass separation. In this study, the effect of increasing salinity on cell growth in reused culture medium was investigated. The results indicated cell growth was increased by 24% in fresh medium with higher salinity; but, in subsequent cultures (the second and the third), cell growth decreased due to the increased salinity caused by pH-adjustment harvesting. In high salinity cultures cell density dropped more than low salinity cultures. In the medium with low salinity (3.3% NaCl), the optical density (OD) changed from 2.75 in the first use (fresh) to 2.65, and 2.08 in the second and third uses; respectively, while in the medium with higher salinity (6.8% NaCl) the OD changed from 3.41 in the first (fresh) use to 2.05, and 0.88 in the second and third uses, respectively. The findings reveal that re-nutrient is necessary to maintain reasonable cell growth capacity when the culture medium is reused.

Keywords: Reuse culture medium**,** Salinity, microalgae, growth or biomass

Introduction
Microalgae can be utilized in biofuels, health supplements, and cosmetics because they can be a rich source of carbon compounds [1]. They produce a wide range of bioproducts from biomass, through polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants [2]. Biomass production consumes large amounts of water, nitrogen, and phosphorus [3]. According to a recent study, 1564 L of water is required to produce 1 Kg of microalgal biomass in an open pond [4]. Recycling the culture medium reduces water and nutrient consumption by 84% and 55%, respectively [4], and eliminates the discharge of wastewater [5]. Although reuse of the spent media in microalgal cultivation is economically appealing, the algal biomass harvesting methodology has a great deal of effect on nutritional specifications of the reused medium. One of the less harmful and most economic methods of harvesting algal biomass is pH-induced flocculation–sedimentation [6]. However, it increases the salinity of the spent medium due to the increase of Na^+ and Cl^- ions after pH modulation (pH=12) and subsequent neutralization. Microalgae differ in their adaptability to salinity. Salinity is expressed as abiotic stress and cells develop many adaptive strategies in response

to different abiotic stress such as salinity. Compatible osmolytes such as proline, glycine betaine, sugars, polyols and amino acids are synthesized in response to stress [7].
In this study the effect of medium salinity on microalgal cell growth caused by pH adjustment for cell harvesting from algal broth is investigated. Two high and low saline media are prepared and cell growth in these media at fresh and reused states are evaluated and compared.

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.
Optical density of the medium was measured over the course of cultivation using a spectrophotometer (Unico, UV-VIS2100). At the end of the cultivation (stable optical density) the biomass was separated using pH adjustment followed by sedimentation. For this purpose, the pH of the medium was adjusted to 13 using 10 N sodium hydroxide solution, and the flocs were allowed to settle for 24 hours. After biomass separation, the pH of harvested medium was reduced to normal value of BG11 medium (~7.3) using 12 M hydrochloric acid. This solution was used for the next cultivation as reused medium. Salinity of the medium (%NaCl) was measured using conductometer (ED/TDS Meter, Mi 306 Martini Instruments).
To investigate the effect of salinity, two culture media were used: normal medium with a salinity of 3.3%NaCl and adjusted medium with a salinity of 8.6%NaCl. 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.

Results and discussion

To study the effect of salinity on the growth rate of algal species, two culture media with normal salinity (3.3%) and adjusted salinity (8.6%) were used. After cultivation with the fresh media, each medium was reused twice. In the second and third cultures, salinity was measured after harvest (pH adjustment method) but it was not adjusted. Table 1 indicates the salinity of each medium after three consecutive cultivations. As seen in this table, the salinity of both media increases after each harvest, but the extent of salinity increase depends on the initial salinity. In normal medium, the salinity of the third culture increased 2.5 times compared to the fresh culture. This is while in the adjusted medium, the salinity increase was only 1.5 times. Considering the fact that salinity increase was due to the pH adjustment during harvesting, it was found that salinity increase is limited and does not adds up linearly over the cycles of harvesting and reuse.

Table.1 Salinity (%NaCl) of fresh and reused culture media

Culture medium	First culture (Fresh)	Second culture	Third culture
Normal medium	3.3	4.38	8
Adjusted medium	8.6	12	13

Figure 1 shows the effect of salinity on biomass production. The results show that increase of salinity from 3.3% NaCl to 8.6% NaCl in the first (fresh) culture improves cell growth by 24% (OD from 2.75 to 3.41) over 64 hours cultivation time. However, this improvement in cell growth is not observed in the second and the third cultures, and the increase in salinity reduces cell growth. In the second use of the normal medium, slight decrease is observed in cell growth (OD from 2.75 to 2.65) in 64 hours of incubation time, while in the third culture the OD reaches to the low value of 2.08 in 64 hours. In the adjusted medium, the decrease in cell growth is more noticeable, so that in the second culture the cell density drops to 2.05 (at 88 hours of growth) and this value reaches 0.88 in the third culture, which is a significant reduction in growth rate.

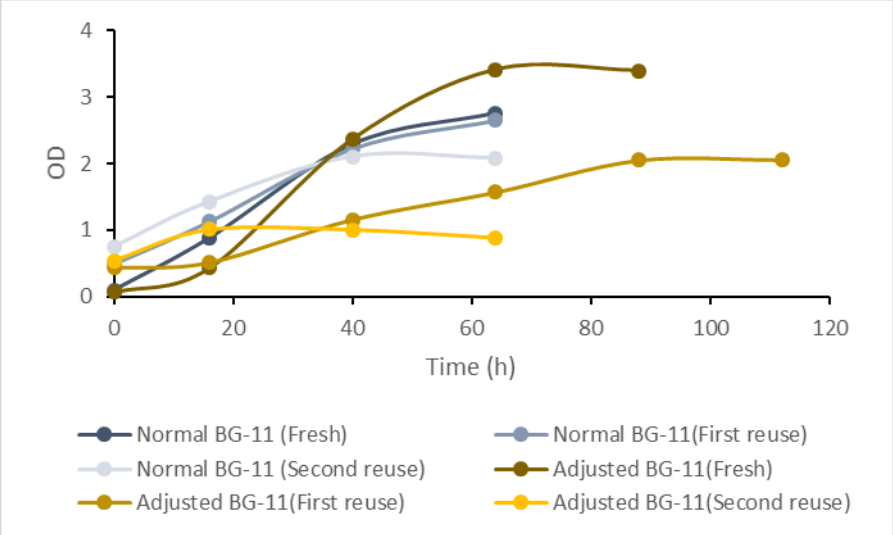

Figure 1. The effect of salinity on cell growth in normal and adjusted media

It is hypothesized that salinity increase in the fresh culture medium makes an environmental and metabolic stress that induce more nutrients consumption and eventually higher growth. Thus, in the second and the third cultures nutrients depletion results in less growth rate. Higher salinity increases nutrients consumption and this is the reason that in the adjusted medium with higher salinity the cell growth in reused media is more descending so that in the third culture cell growth is negligible. Therefore, in case of using pH adjustment technique for cell harvesting, increase in salinity is inevitable and thus reuse of spent culture requires a re-nutrient. The components that have been depleted in the first culture should be added to the medium complimentarily to support cell growth. In this way water, as the most important component of the culture medium, is restored and the lack of nutrients is compensated.

Conclusions

The salinity of fresh culture media increases cell growth, resulting in increased nutrient consumption. Application of the pH adjustment method for cell harvesting increases the

salinity of the culture medium, but when the culture medium is reused, this salinity reduces the cell density. To preserve the biomass production potential of the reused medium it is necessary to re-nutrient the spent culture medium with depleted components.

References

[1] P. Das, S. S. Aziz, and J. P. Obbard.," Two phase microalgae growth in the open system for enhanced lipid productivity", Renew. Energy, 36 (9), 2524–2528 (2011).

[2] L. Brennan and P. Owende, " Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products", Renew. Sustain. Energy Rev., 14 (2), 557–577 (2010).

[3] Z. Lu *et al.*," Identification of auto-inhibitors in the reused culture media of the *Chlorophyta Scenedesmus* acuminatus", Algal Res., 44 (2019).

[4] J. Yang, M. Xu, X. Zhang, Q. Hu, M. Sommerfeld, and Y. Chen, " Life-cycle analysis on biodiesel production from microalgae: Water footprint and nutrients balance", Bioresour. Technol., 102 (1), 159–165 (2011).

[5] M. A. Borowitzka, "High-value products from microalgae — their development and commercialization", 743–756 (2013).

[6] M. Castrillo, L. M. Lucas-Salas, C. Rodríguez-Gil, and D. Martínez, " High pH-induced flocculation-sedimentation and effect of supernatant reuse on growth rate and lipid productivity of *Scenedesmus obliquus* and *Chlorella vulgaris* ", Bioresour. Technol., 128 324–329 (2013).

[7] S. Hiremath and P. Mathad, " Impact of salinity on the physiological and biochemical traits of *Chlorella vulgaris* Beijerinck. ", J. algal biomass Util., 1 (2), 51–59 (2010).