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Extraction of algal chlorophyll(a+b) pigment using adsorption

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Abstract
Pigments are used in various industries such as food, medicine and cosmetics as a dye and a therapeutic agent. Chlorophylls and carotenoids are two large groups of pigments. Generally, chlorophyll a content is highest in plant and algae species. Considering the importance of chlorophylls, extraction and separation of chlorophyll a+b from microalgae *Chlorella vulgaris* was addressed in this study. Extarction was performed using adsorption technique by means of graphene as adsorbent. Loading of adsorbent (mg graphene per ml extract) is an effective factor in adsorption efficiency. It was found that the loading of 30 mg graphene per ml extract was adequate to achieve the adsorption efficiency of 88%. Higher loading were not economically justified. Technically, to reach this efficiency, it was found that the extract solution need to be recirculated through the bed for five times.

Keywords: Microalgae, Chlorella vulgaris, Chlorophyll, Graphene, Adsorption

Introduction
Microalgae biomass is a valuable feedstock due to the macromolecule content and have attracted attention in research [1]. Microalgae produce over 15,000 natural substances including enzyme, carbohydrates, photosynthetic pigments such as chlorophylls and carotenoid, lipids etc [2]. Pigment is a value-added biochemical that play a major role in human and livestock nutrition; for example many carotenoids are precursors to vitamin A while some of them have anticancer properties [3].

To extract chlorophylls and carotenoids from microalgae biomass, different methods of extraction are available including solvent-based methods [1,4], and extractive techniques such as UAE[1],MAE[2] and SFU[3] . However, the yield of extraction depends on the type of algae and experimental conditions, such as temperature and the solvent nature [5].

[1] Ultrasound Assisted Extraction
[2] Microwave-Assisted Extraction
[3] Supercritical Fluid Extraction

Adsorption technique has recently been used to extract pigments from algal biomass. Many studies used adsorbents such as graphene, activated carbon and K10 montmorillonite with the aim of removing pigments in order to achieve better quality biodisel [6, 7]. Vick et al [6] Showed that graphene has a relatively high specificity compared to activated carbon for sequestering chlorophyll as compared to carotenoid.

In this study, the potential of adsorption technique for extraction of chlorophyll(a+b) from biomass of *Chlorella vulgaris* was investigated. Algal cells must initially be disrupted to release intracelluar products including chlorophyll. Ethanol was used as the disrupting and extracting solvent. Then graphene was used as the adsorbent to separate pigment from the extract. One imporatnt feature of this process is adsorbent loading (mg adsorbent to ml extract) that influence the adsorption efficiency. Under different adsorbent loadings the efficiency was examined and the best value was determined.

Experimental

Microalgae cultivation and harvesting

Chlorella vulgaris was grown in BG11 medium at 25±2 ºC under white LEDs (12000 lux) with a 16:8 h light/dark photoperiod in a flat plate photobioreactor (50 L). In order to harvest, pH of the culture was adjusted to 12 using sodium hydroxide and then upon sedimentaion the concentrated solution was dried in oven at 45 ºC. Finally, the dried biomass was grinded and stored in fridge for further experiments.

Cell disruption and Chlorophyll extraction

For cell disruption and extraction, in two 15 ml falcon tubes, 0.25 g ground algal biomass along with 0.025 g magnesium carbonate and 8 ml of ethanol (96% vol) in each tube were mixed. The tubes were vortexed for 3 min followed by sonication at 40 kHz for 30 min at 4°C. Then, tubes were shaked for 18 hr at 40°C inside incubator shaker and centrifuged at 4400 rpm for 10 min at 4°C. The supernatants were collected and then 16 ml of ethanol was added to the pellets then vortexed and centrifuged in two consecutive stpes (8 ml each step). Supernatants were added to the initial supernatant. The concentrations of chlorophyll a (C_a), chlorophyll b (C_b), and total chlorophyll a+b (C_{a+b}) in the final supernatant were determined using Eqs.1-3 [8] upon measurment of absorbance at wavelengths of 470, 664 and 649 nm (UV-VIS spectrophotometer, UNICO, USA).

$$C_a = 13.36\, A_{664} - 5.19\, A_{649} \quad (1)$$

$$C_b = 27.43\, A_{649} - 8.12\, A_{664} \quad (2)$$

$$C_{a+b} = 5.24\, A_{664} + 22.24\, A_{649} \quad (3)$$

Where concentrations are expressed in µg per mL of extract.

Chlorophyll Adsorption

Adsorption was carried out using a packed bed of graphene. 15 ml of the extract was taken and passed through the packed bed containing 15 mg graphene per ml of extract. The extract was recirculated through the bed for five times and then chlorophyll (a+b) concentration at the outlet stream was measured using Eq. (3). Then, adsorption efficiency Y (%) was calculated using Eq. (4) as follows:

$$Y = \frac{Ci - Co}{Ci} \times 100 \quad (4)$$

Where C_i is concentration of chlorophyll (a+b) in extract and C_o is concentration of chlorophyll (a+b) in packed bed outlet stream.

Results and discussion

Experiments were performed under three adsorbent loadings of 15, 30 and 45 mg graphene per ml of extract. The concentration of chlorophyll (a+b) in extract was 164.6 µg per mL of extract. The number of recirculation affects the concentration of chlorophyll (a+b) in the outlet and eventually the adsorption efficiency. Table 1 indicates how concentration reduces as the number of passages through the bed increases. In the first pass, the concentration drops 62%, 72%, and 78% in 15, 30, and 45 mg graphene per ml of extract loading; respectively. After 5 passes it drops 71%, 88%, and 98% in three loadings; respectively. Further passes did not reduce the concentration significantly (data not shown). Thus, five recirculation was found to be sufficient to achieve low concentration in the outlet.

Table 1. Chlorophyll (a+b) concentrations (µg per mL of extract) in the outlet stream of each pass.

Number of pass	Loading of Graphene (mg graphene per ml of Extract)		
	15	**30**	**45**
1	62.58	46.01	36.07
2	62.08	38.05	14.78
3	58.52	23.75	3.62
4	53.7	23.49	2.75
5	47.1	19.08	2.54

Adsorption yield in three different loadings were calculated and reported in Fig.1. As seen in this figure as adsorbent loading increases, the absorption efficiency increases. The increase in efficiency could be due to an increase in the active sites available for absorption of pigment. Comparing the efficencies at 15 and 30 mg graphene per ml of extract loadings it was improved from 71% to 88%. Further loading to 45 mg graphene per ml of extract increased the efficiency to 98%. The results indicate that using 15 mg graphene per ml of extract the adsorption efficiency is not high enough and approximately 30% of the pigment still remanis in the outlet. When the adsorbent is doubled to 30 mg graphene per ml of extract the efficiency improves 24%. With 50% increase in adsorbent consumption, the efficiency increases only 10% that is not appealing from the economic point of view. Therefore, the bed loading of 30 mg graphene per ml of extract was found to be optimal for pigment extarction from algal extract.

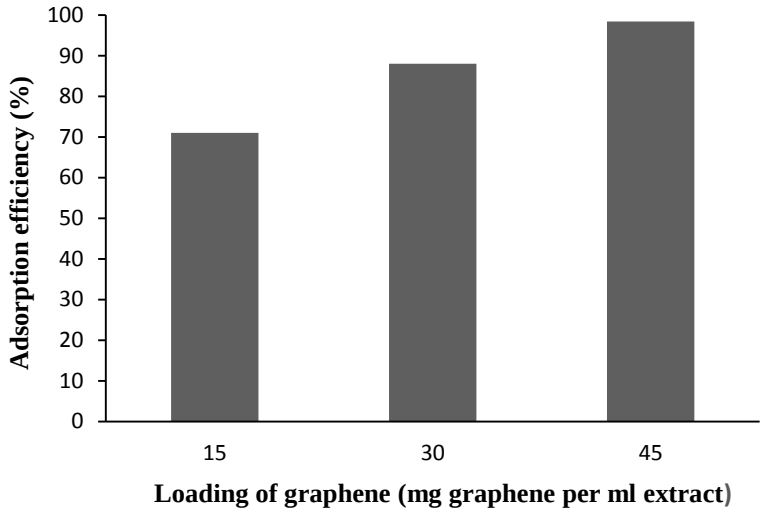

Fig. 1. Adsorption efficiency of extarcting chl (a+b) from microalgae in different loadings of adsorbent.

Conclusions

Extraction of chlorophyll (a+b) from algal biomass using adsorption was investigated. The results indicated that the initial extract should be recirculated through the bed five times to reach the adsorption effieciecy of 88% in the outlet. The proper loading of the bed was found to be 30 mg graphene per ml extract to achive this efficency.

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