Enhancement of Lutein Yield from Coagulated Chlorella sp. ESP-6 with Sodium Hypochlorite

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Applying coagulant such as polyaluminum chloride (PACl) or chitosan can improve harvesting efficiency of microalgae from broth. However, the coagulant molecules have affinity to lutein molecules, so coagulation reduces the lutein yield. This work proposed the addition of NaOCl with coagulant for enhancing lutein recovery from Chlorella sp. ESP-6 cells. Adding PACl or chitosan largely enhanced membrane filtration flux but had no effects on residual solid contents of filter cake. NaOCl released insoluble substances from the biomass matrix and deteriorated soluble lutein in water. When both NaOCl and coagulant were presented, the NaOCl helped to release the lutein out of the biomass, while the PACl and chitosan molecules assisted in capturing the OCl– ions. A practical harvest scheme including applying both PACl and NaOCl to optimize coagulation and maximum yield from Chlorella sp. ESP-6 biomass was discussed.

Keywords Coagulant; Lutein; Microalgal harvesting; Oxidant; Recovery

INTRODUCTION

Lutein is a feed or food additive with a market size of USD $233 million in 2010.[1] Lutein is commercially produced from marigold (Tagetes erecta) oleoresin. Since the content of lutein in marigold is lower than some microalgae,[2] and the growth rate of marigold flower is much lower than microalgae,[3,4] microalgae is considered a promising alternative feedstock for lutein production.[5,6] Conversion of cultivated microalgae to lutein requires separation of algal cells from cultivation broth (harvesting),[7] breakage of cell walls to release intracellular lutein (cell disruption), and purification of the released lutein to a desired concentration (purification).[8–11] Harvesting microalgal cells can be difficult since the mass fraction of algae is low in the cultivation broth (0.5–2 gl−1) and the algal suspensions are frequently in a highly dispersed state.[12,13] Coagulation of fine algal cells using coagulants such as polyaluminum chloride (PACl) or chitosan can enhance filtration flux and reduce membrane fouling.[14,15] Utomo et al.[16] showed that the presence of 10 mgl−1 PACl or >30 mgl−1 chitosan could sufficiently coagulate the microalgal cells; however, the presence of coagulants lowers the lutein yield from algal biomass. These authors claimed that the affine adsorption of lutein onto chitosan molecules via hydroxyl–amine interaction likely led to the reduced production of lutein from coagulated microalgal broth.

Sodium hypochlorite is a strong oxidant that can be used as a cleaning agent or a disinfection agent.[17,18] This work proposes a novel way of increasing lutein production from Chlorella sp. ESP-6 cells after PACl or chitosan coagulation by hypochlorite treatment. Turbidity data in a nephelometric turbidity unit (NTU) were used as measures of biomass concentration in the broth. An excitation–emission matrix (EEM) was used to characterize the released substances to water. Filtration flux was used to describe the dewaterability of the broth. Lutein was extracted and isolated for demonstration of the recovery of the pigment from the conditioned broth. The yielded lutein for human use will be purified after extraction stage so as to minimize the adverse effects on human health.

MATERIALS AND METHODS

Microalgae and Culture Medium

Chlorella sp. ESP-6 was cultured at 25°C in a 10-L photobioreactor illuminated at both sides with an external
light source (TL5 fluorescent lamp) at 1945 lux. The growth medium was the Bold’s basal medium (BBM), which comprised the following (mg l⁻¹): K₂HPO₄ (75), K₃HPO₄ (175), MgSO₄ 7H₂O (75), NaNO₃ (250), CaCl₂ 2H₂O (25), NaCl (25), EDTA (50), KOH (31), FeSO₄ 7H₂O (4.98), H₃BO₃ (11.42), ZnSO₄ 7H₂O (1.412), MnCl₂ 4H₂O (2.32), CuSO₄ 5H₂O (2.52), Co(NO₃)₂ 6H₂O (0.8), Na₂MoO₄ 2H₂O (1.92), and contact angle of 60°. The suspension was collected by centrifugation at 9000 g, settled for 30 min. The turbidity, zeta potential, and particle size distribution data for each sample were measured.

Coagulation with or without Oxidant and Filtration

The algal suspensions (1.0 g l⁻¹; or 710 NTUs) were coagulated immediately after sampling using PACl (10% w/w Al₂O₃; Showa Chemical Co., Ltd., Japan) or chitosan (poly[1,4-b-glucopyranosamine], Sigma, USA). One liter of algal broth (720 NTU) in a tank measuring 11.5×11.5×21 cm and equipped with a propeller (PB-700, Phipps & Bird Inc., Richmond, VA, USA) was added with 10 mg l⁻¹ NaOCl, 20 mg l⁻¹ PACl, 20 mg l⁻¹ chitosan, or a mixture. The solution pH was kept at 7.0 with the addition of NaOH. The solution was subjected to rapid mixing at 100 rpm for 3 min, slow mixing at 30 rpm for 20 min, and then the suspension was left still for 30 min. The turbidity, zeta potential, and particle size distribution data for each water sample collected at 2/3 depth from water surface were measured.

The entire coagulated suspension was filtered using the surface modified polytetrafluoroethylene (PTFE) membrane (mean pore size of 0.91 μm, surface area of 27.8 cm², and contact angle of 60°) according to procedures by Tu et al.¹⁹ and Huang et al.²⁰ The coagulated suspension was filtered by the stirred ultrafiltration cells model 200 (Millipore, Billerica, MA, USA) at 1 bar.

EEM Analysis

The collected wet cake in the previous section was resuspended in water to make biomass concentration of 26 g l⁻¹ for ultrasound (20 kHz, model VC130, Sonics & Materials, USA) at 0.5 min treating time. The treated suspension was repeatedly extracted with 1.75 vols acetone for 1 min. The suspension was then collected by centrifugation at 9000×g (MiniSpin plus, Eppendorf, Germany) for 3 min. All collected supernatant was then dried in nitrogen flow (model MG-2200, Eyela, Japan). Each gram of the dried powders was mixed with 100 mL of diethyl ether and 100 mL of 4% KOH in methanol. Two mL of 10% w/w NaCl solution was added. Then, the solution was kept at 4°C with its upper, organic phase being collected and dried by nitrogen flow.

Differential Scanning Calorimetry (DSC)

Ten mg of filtered cake was placed into the test cell, and the cell temperature was reduced from room temperature to −30°C at a rate of −1°C/min by DSC (Q20; TA Instruments, USA). The peak areas of the heat flux differences against temperature curves estimated the water–solid binding strength.²¹

Lutein Extraction and Analysis

The EEM spectra of filtered algal samples were recorded by Cary Eclipse (Varian, Palo Alto, CA, USA) with both excitation and emission wavelengths from 200–550 nm. The blank EEM spectrum obtained for double distilled water was the zero. Excitation and emission slits were maintained at 5 nm, and 290-nm emission cutoff filter was used. EEM spectra are illustrated as the elliptical shape of contours with emission spectra from 200–550 nm, excitation wavelength from 200–550 nm, and contour lines at the fluorescence intensity of interval of 5.

Analytical Methods

The dry cell weights of samples were measured via vacuum filtration of suspensions (model V-700, Buchi, Switzerland) followed by weighing before and after 650°C drying.

Lutein at analytical grade was obtained from Sigma–Aldrich for calibration. The absorbance of pure lutein solution was measured at 450 nm by using an UV/Vis spectrophotometer (model V-530, Jasco, Tokyo, Japan). The high-performance liquid chromatography (HPLC) system adopted was Shimadzu LC-20A (Tokyo, Japan) with both column Polaris C18-A 5μ (Varian, Palo Alto, CA, USA) and detector Saphhire 800 (ECOM, Prague, Czech Republic). The mobile phase was a mixture of acetone:methanol:water of 37.5:57.5:5% v/v/v at a flow rate of 1 mL min⁻¹. The lutein concentration of the sample was estimated based on the HPLC peak at 450 nm and the calibration obtained.

RESULTS AND DISCUSSION

Coagulation Tests of the Chlorella sp. Sample

The algal broths after still settling cells were shown in Fig. S1 (available online in the Supplementary Material). The original suspension (RAW) was a green suspension. Adding 10 mg l⁻¹ NaOCl (A), 10 mg l⁻¹ PACl (B), 10 mg l⁻¹ PACl + 10 mg l⁻¹ NaOCl (C) were also green in color. Apparent settlement was observed by 10 mg l⁻¹ PACl + 20 mg l⁻¹ chitosan (D) and 10 mg l⁻¹ PACl + 10 mg l⁻¹ NaOCl + 20 mg l⁻¹ chitosan (E). The turbidity removals were 19.6% (RAW), 23.6% (A), 66.9% (B), 61.9% (C), 99.7% (D), and 99.7% (E). Hence, the natural settling of raw broth was poor. Adding 10 mg l⁻¹ PACl (B) effectively removed 2/3 of initial turbidity, while the addition of an extra 20 mg l⁻¹ chitosan almost completely removed all turbidity. Comparing (A) and (B), and (C) and
we revealed that the presence of NaOCl had no effects on turbidity removal.

**Released Substances-EEM**

The intensities of EEM spectra can be used to characterize the chemical nature of the dissolved substances in waters. As Fig. 1 shows, the raw broth had high intensity of aromatic protein, while the addition of 10 mgl⁻¹ NaOCl released aromatic protein, soluble microbial products, and humic acid. On the contrary, when 10 mgl⁻¹ PACl with or without 20 mgl⁻¹ chitosan was added, the intensities of soluble substances dropped, indicating the adsorption or enmeshment of soluble substances by the coagulants. When 10 mgl⁻¹ NaOCl was also present in the coagulant system, the intensities of the released substances were slightly reduced.

**Filtration Flux and Cake**

For microalgal harvesting, high filtration flux and low cake moisture after filtration are desired. The filtration curves of the tested samples declined over time, suggesting the occurrence of membrane fouling (Fig. 2). The raw sample and the NaOCl-treated samples had a similar filtration decline. On the contrary, the addition of 10 mgl⁻¹ PACl reduced the filtration flux. The presence of 20 mgl⁻¹ chitosan significantly mitigated the membrane fouling potentials. On average, the filtration flux over the testing period was around 2 m³/m²-min for the RAW and sample A, 1.6–1.9 m³/m²-min for samples B and C, and >7.5 m³/m²-min for samples D and E.

After 1-bar filtration, the cake on the membrane surface generally had >23% w/w solid cake. Coagulation or NaOCl oxidation had no significant effects on the residual moisture contents in the membrane cake. The specific energy for the moisture-solid bond by applying methods proposed by Lee et al. [22] ranged 160–320 J/g (Fig. 3). Hence, coagulation or oxidation did not markedly affect the binding strength of water on solid cake.

**Lutein Contents from Coagulated-Treated Chlorella sp.**

The lutein contents in the isolated Chlorella sp. ESP-6 by coagulation followed by extraction are shown in Fig. 4. The original broth had lutein concentration of 0.91 mg/g after 20 kHz treatment. Coagulation using 10 mgl⁻¹ PACl or 10 mgl⁻¹ PACl + 20 mgl⁻¹ chitosan reduced the lutein yield to 0.69 and 0.43 mg/g, respectively. The NaOCl oxidation largely reduced the lutein content to about 0.4 mg/g. Therefore, coagulation or oxidation reduced lutein yield from the microalgal broth.

Conversely, the 10 mgl⁻¹ NaOCl + 10 mgl⁻¹ PACl or 10 mgl⁻¹ NaOCl + 10 mgl⁻¹ PACl + 20 mgl⁻¹ chitosan increased the lutein yields to 1.31 or 1.90 mg/g, respectively.

**Discussion**

The above experimental results revealed the different roles of coagulant and the oxidant on the microalgal broth. The increased lutein yield with 10 mgl⁻¹ NaOCl + 10 mgl⁻¹ PACl + 20 mgl⁻¹ chitosan or 10 mgl⁻¹ NaOCl + 10 mgl⁻¹ PACl + 20 mgl⁻¹ chitosan
PACL is worthy of attention. The benefit of chitosan was apparent, since it could greatly increase the membrane flux, which was likely attributable to the relatively rigid cake structure with chitosan. The drawback of using chitosan was its high cost. Applying PACl or PACl $+$ chitosan could adsorb released lutein as proposed by Utomo et al.\cite{16} The use of NaOCl had adverse effects on lutein yield, likely owing to the strong oxidation reaction on the lutein molecules. However, when both PACl and/or chitosan and NaOCl were present, lutein yield was increased. A possible interpretation on this observation is that the NaOCl helped release the lutein out of the biomass, while the PACl and chitosan molecules assist capturing the OCl$^-$ ions for reactions by adsorbing the released lutein from waters.

In application, the present study revealed a welcome scenario for lutein production from \textit{Chlorella} sp. ESP-6. Applying cheap PACl with NaOCl can lead to sufficiently coagulated broth with satisfactory filtration flux with minimum loss of lutein yield from microalgal biomass.

**CONCLUSIONS**

This study tested a novel scheme on \textit{Chlorella} sp. ESP-6 cells for reaching sufficient coagulation of microalgal broth for filtration and high lutein yield with minimum adsorption loss. Coagulation using PACl or PACl $+$ chitosan reduced the suspension turbidity, while NaOCl oxidation did not. Filtration flux followed PACl $+$ chitosan $>$ PACl = RAW with no role of NaOCl. The addition of NaOCl released insoluble substances into water, and the released substances could be captured by the coagulants (EEM results). Nonetheless, neither coagulation nor oxidation affected the solid contents or moisture-to-solid binding strength of filter cake. The lutein yield was according to the following order: 10 mgl$^{-1}$ NaOCl $+$ 10 mgl$^{-1}$ PACl $+$ 20 mgl$^{-1}$ chitosan $>$ 10 mgl$^{-1}$ NaOCl $+$ 10 mgl$^{-1}$ PACl $>$ RAW $>$ 10 mgl$^{-1}$ PACl $+$ 20 mgl$^{-1}$ chitosan $>$ 10 mgl$^{-1}$ NaOCl. Based on experimental observation, a practical harvest scheme including applying PACl and NaOCl to treat \textit{Chlorella} sp. ESP-6 biomass was suggested.

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**SUPPLEMENTAL MATERIAL**

Supplemental data for this article can be accessed on the publisher’s website.

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