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A simple strategy for the separation and purification of water-soluble polysaccharides from the fresh *Spirulina platensis*

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ABSTRACT

A novel and industrially applicable strategy for separating and purifying the water-soluble polysaccharides from the fresh *Spirulina platensis* is introduced in this study. After hot water extraction, using chitosan flocculation treatment, 86.0% of the particles and 85.9% of the pigments in the crude extract were removed. The purity of polysaccharides could be increased to 94.6% and 96.1% by applying the macroporous resin ADS-7 to remove the impurities in the mode of static adsorption and in a dynamic mode of operation, respectively. The polysaccharides obtained were composed of three major components having molecular weights of 1400, 420 and 2 kDa.

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Introduction

Polysaccharides from seaweed, plants, fungi and algae have many commercial applications in food products such as stabilizers, thickeners and emulsifiers[1] and also potential medical applications.[2,3] Blue-green algae are microscopic plants found in lakes, rivers and brackish waters throughout the world. *Spirulina platensis* is one species of blue-green algae recognized as safe for human consumption. Polysaccharides of *S. platensis* have been shown to reduce the replication of Hepatitis C virus (genotype 4).[2] *In vitro* experiments also found that the polysaccharides from *S. platensis* could decrease lung metastasis of B16-BL6 melanoma cells by inhibiting the tumor invasion of the basement membrane, probably through the prevention of the adhesion and migration of tumor cells.[3] The antioxidant capacity of polysaccharides of *S. platensis* has also been investigated by Chaiklahan *et al.*[4] and was found to scavenge 31.0% of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Hence, the significant potential medicinal applications of *S. platensis* polysaccharides have excited commercial interests.[5]

Water extraction has been widely used in preparing polysaccharides from various natural raw materials, *e.g. Dendrobium nobile* Lindl, *Gynostemma pentaphyllum* Makino, cherries and mushrooms.[6] Kurd and Samavati[7] reported that 13.6% polysaccharides could be extracted by water from dried *S. platensis* powder with the assistance of ultrasonic treatment. Box–Behnken experimental design and response surface methodology have been used to optimize *S. platensis* polysaccharide extraction by water.[4] Following water extraction and removal of the cell debris by centrifugation or filtration, chromatography has been applied for further purification of crude polysaccharides.[2,3,8,9] For example, DEAE–Sepharose CL-6B and Sephadex G-100 column chromatography have been used to fractionate and purify the polysaccharides extracted from *G. pentaphyllum* Makino.[10] Tosoh HW-65, Sepharose 6B and DEAE-cellulose were used in the purification process and in the determination of the molecular weights of polysaccharides from *S. platensis*.[11] However, these chromatographic columns require expensive equipment for scale-up.

Macroporous resins are polar, non-polar or slightly hydrophilic polymers with high adsorption capacity.[12] They can selectively adsorb target molecules from aqueous and non-aqueous systems through electrostatic force, hydrogen bonding interaction and size sieving action.[13] Macroporous resins are widely used in separation of various active compounds (such as saponins and flavones).[14–17] Macroporous resins separation is also a simple method for decolorization and protein removal.[18,19] For instance, macroporous resin (S-8 resin) reportedly removes 84.6% of colored substance and 91.7% of proteins from the crude levan extract of the endophytic bacterium *Paenibacillus*

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polymyxa EJS-3.[19] In comparison with other chromatographic columns (such as DEAE and Sephadex), processes involving macroporous resin are of low cost, high efficiency and easy to scale-up. Based on a literature search, most researchers used dry powder or dried algae for extraction.[8,9,20,21] The cost of drying *S. platensis* contributes an estimated 30% to the total production cost.[22] In the current study, we report the extraction of polysaccharides from fresh *S. platensis* and the application of macroporous resins in purification to reduce the cost and simplify the process. A novel operation of purifying polysaccharides by resin in a "negative" mode of removing impurities was proposed in the study. Compared to the most operations reported for enriching polysaccharides by chromatographic columns, the proposed mode is simple and flexible for industrial operations.

Materials and methods

Materials

Fresh *S. platensis* was collected from Dongtai City Spirulina Bio-engineering Co., Ltd (Dongtai, China). The dry mass content of the fresh *S. platensis* is 11.5% (wt/wt). Chitosan powder (80–95% deacetylated, MW from 235 to 320 kDa) was supplied by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). L-arabinose, D-galacturonic acid, L-fucose, L-rhamnose, D-glucosamine, D-galactosamine, D-galactose, D-glucose, D-xylose, D-mannose, D-ribose and D-glucuronic acid were purchased from Sigma-Aldrich Shanghai Trading Co Ltd (Shanghai, China). Macroporous resins, ADS-7, HPD-100 and S-8 were gifts from Changzhou ZhongNa Chemical Co. (Changzhou, China). AB-8 and D 101 were bought from Anhui Saxin Resin Technology Co. (Saxin, China). The details of physical properties of the resins are listed in Table 1. The other reagents used were of analytical grade and supplied by Shanghai Sinopharm Chemical Reagent (Shanghai, China).

Chitosan flocculation treatment of the crude extract from the fresh S. platensis

The extraction was conducted in a 1000-mL round-bottomed flask. Fresh *S. platensis* was mixed with water at a ratio of 1:33.3 (g:mL water), and stirred for 4 h at 80°C. After cooling to the ambient temperature, chitosan (100 mg/L) was added to the mixture and stirred at 200 rpm for 20 min. The pH was adjusted in the range of 5–9 with 0.1 N HC1 or NaOH. The supernatant of crude extract was collected by centrifugation for 15 min at 3800 rpm. The polysaccharide (10-fold concentrated supernatant) was precipitated by adding three volumes of absolute ethanol. The polysaccharides were recovered by centrifugation and dried in a vacuum oven. The flocculation efficiency of chitosan was assessed by comparing the spectroscopic absorbance of supernatant at 600 nm with that of crude extract.[23] Decolorization rate of flocculation (DR) was calculated according to the absorbance intensity changes of samples at 420 nm following flocculation.

Purification of polysaccharides with macroporous resins in shaker operation

In a 100-mL Erlenmeyer flask, 1.0 g of different resins were added to 40 mL of 290.7 mg/L crude polysaccharide solution (86.5% total sugar/total solids) on a rotary shaker at the speed of 150 rpm. Samples were taken to assay the total sugar content. The influence of pH variation (from 4.0 to 9.0) and temperature (from 25°C to 35°C) on the purification was evaluated.

Purification of polysaccharide in a packed column of ADS-7

Based on the results from shaker operation, ADS-7 was selected for packing into a column (10 mm ID × 200 mm length) for polysaccharide purification. The crude polysaccharide solution was loaded with a peristaltic pump into the wet-packed column containing 10 mL of ADS-7 resin. The column operation was conducted at the room temperature and the eluted samples were collected for total sugar content and polysaccharide purity analysis.

Analysis methods

The concentration of total sugar was determined by the phenol–sulfuric acid method.[24] The amount of sulfate residues in polysaccharides was determined by the $BaCl_2$-gelatin method using Na_2SO_4 as a standard.[25]

Table 1. Properties of macroporous resins used in this study.

Resin	matrix	Diameter (mm)	Specific surface area (m^2/g)	Average pore size (nm)	Polarity
ADS-7	Cross-linked Polystyrene with -NRn	0.3–1.25	100–120	25–30	Strongly polar
D101	Polystyrene	0.25–0.84	500–550	10–12	None
AB-8	Polystyrene	0.315–1.25	480–520	13–14	Weak
S-8	Cross-linked- Polystyrene	0.3–1.25	100–120	28–30	Medium
HPD100	Polydivinylbenzene- acrylic ester	0.3–1.2	650–700	8.5–9	Weak

Uronic acid content was determined by the carbazole reaction using D-glucuronic acid as a standard.[26]

The amount of total solids was determined by drying 100 mg sample at 50°C ± 1°C in a vacuum oven for 18 h. The polysaccharide purity was determined based on the total sugar content as a percentage of total solid (% wt/wt).

The analysis of size distribution and zeta potential of the debris particle in the crude extract was carried out on a Zeta PALS Zeta potential and particle size analyzer (Brookhaven Instruments Corporation, USA).

The micrographic images of flocs were obtained using a DMBA400 Inverted Microscope (Motic Instruments Inc., Canada).

Molecular weight distribution of purified polysaccharide was determined with Waters 600 high-performance gel filtration chromatography (HPGFC, Waters Corporation, USA) equipped with an Ultrahydrogel TM Linear (300 mm × 7.8 mm id × 2) 2410 column and a differential refractive index detector at 45°C. After injecting 30 μL of sample, 0.1 mol/L sodium nitrate solution was pumped in as the mobile phase at the rate of 0.9 mL/min. The concentration of dextran standards solutions was 3 mg/mL. Based on the retention times of each standard sample and its corresponding molecular weight, the molecular weight of polysaccharide was calculated using GPC analysis software.

Monosaccharide compositional analysis: polysaccharide (20 mg) was dissolved in 1 M H_2SO_4 (2 mL) and hydrolyzed at 108°C for 8 h. After the hydrolysis, the solution was cooled to room temperature and neutralized by the addition of $BaCO_3$. The sample was centrifuged at 15,000 rpm for 2 min to remove precipitation. The monosaccharide composition of the hydrolyzed polysaccharide was analyzed by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD; Dionex ICS-5000, Sunnyvale, CA, USA).[27] A CarboPac PA20 column was used, and the eluent flowed at a rate of 0.5 mL/min. The elution program consisted of isocratic elution with water for 11 min, a linear gradient of NaOH from 0 to 100 mM for 1 min, isocratic elution with 100 mM NaOH for 1 min, a linear gradient of sodium acetate from 0 to 100 mM in 100 mM NaOH for 1 min, and isocratic elution with 100 mM sodium acetate in 100 mM NaOH for 11 min. The monosaccharide standards used included L-arabinose, D-galacturonic acid, L-fucose, L-rhamnose, D-glucosamine, D-galactosamine, D-galactose, D-glucose, D-xylose, D-mannose, D-ribose and D-glucuronic acid. A standard mixture (3.33 mg/L each sugar) was run to verify the response factors before analysis of samples.

Results and discussions

Physical properties of crude polysaccharides

The crude polysaccharide extract of fresh *S. platensis* was thick containing many insoluble green debris particles. We measured the particle number/size distribution (Figure 1a) and zeta potential (Figure 1b) of debris particles to design a proper protocol to separate debris from the crude extract. The diameter of debris particles ranges from 160 nm to 1.9 μm and 90.9% of particles is <1 μm (Figure 1a), suggesting the crude extract of fresh *S. platensis* can be considered as colloidal particle suspension.[28] Based on theoretical calculations, we expected that the suspension of debris in the crude extract would be stable and not separable by the force of gravity.

Coagulation of particles is another important factor influencing the suspension stability of the debris particles in the crude extract. According to the DLVO theory,[29] the dispersion or coagulation of colloidal particles is determined by the balance between van der Waals attractive and electrical double layer repulsive forces when particles closely approach one another. Zeta potential is measured at or near the boundary of diffuse layer on the surface of the particle; hence the stability of colloidal suspension is closely related to the zeta potential of suspended particles.[30,31] The higher the absolute value of zeta potential, the thicker

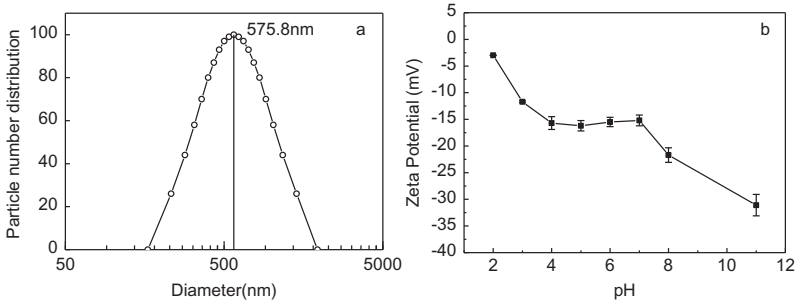

Figure 1. Particle number/size distribution (a) and zeta potential (b) of debris particles in the crude extract of fresh *S. platensis*.

the double layer, resulting in suspended particles more stable against coagulation.[29,32] In the crude extract of the fresh *S. platensis*, according to the results shown in Figure 1b, the absolute value of potential on the surface of the debris particles increased with pH, which enhanced the electrostatic repulsive forces between particles. By reducing the pH, the zeta potential of particles became zero at pH 2.0, minimizing the mutual electrostatic repulsion between particles; however, no precipitation or coagulation of particles was observed, even when the crude extract was allowed to stand for 24 h at pH 2.0. The reason for this may be Brownian forces and the high viscosity of the fluid greatly decreasing the coagulation or the precipitating velocity of the debris particles. Furthermore, low pH can result in partial hydrolysis of polysaccharides, thus, neutral pH is more suitable for extraction, by maintaining polysaccharides stability. At the neutral pH, the zeta potential of debris particles dispersed in the crude extract was −15.2 mV. This electrostatic repulsion between particles prevented the coagulation and precipitation of debris particles in the crude extract of fresh *S. platensis*. This was supported by our experimental trial showing the debris could not be separated by the high-speed centrifugation at 15,000 rpm for 40 min. In addition, the membrane filtration was not useful since the filter fouled with by debris soon after the initial loading of crude extract.

Flocculating treatment of crude extract with chitosan

Flocculation can promote the aggregation of colloidal particles and facilitate the separation of solid particles by filtration or configuration.[33] Flocculants such as clay, aluminum sulfate, polyferric sulfate, chitosan and praestol have been tested for harvesting algae.[34–36] Among these flocculants, chitosan was selected for our study because of its renewability and ecological acceptability.[37] The effective flocculation process of crude extract with addition of chitosan is shown in Figure 2.

Next, the effect of chitosan treatment of the crude extract from fresh *S. platensis* was investigated and the efficiency of flocculation, decolorization and polysaccharides recovery was determined as a fraction of chitosan concentration, pH, time and temperature (Figure 3). With the concentration of chitosan flocculant increased, the flocculation and decolorization efficiency increased. The maximum flocculation efficiency of 86.0% was obtained with 100 mg/L chitosan addition and a maximum decolorization of 85.9% was observed with 150 mg/L chitosan addition. Approximately 90% of the total sugars remained in extract as the chitosan concentrations were less than 100 mg/L. The optimal concentration of chitosan used to remove the debris from the extract in this study was higher than that used in harvesting algae.[38,39] For example, Lavoie and de la Noüe[38] reported 95% of algae could be flocculated at chitosan concentrations below 40 mg/L from an algal biomass at 100–150 mg dry weight/L. However, this concentration of algal biomass is much lower than the 3.73 g dry weight/L used in this study. Thus, we can conclude that higher biomass content requires higher chitosan concentration for optimal flocculation efficiency.

According to the results shown in Figure 3b the highest flocculation and decolorization efficiency was obtained at pH 7.0, which has also been reported as the optimal pH for harvesting intact and live algal cells by chitosan flocculation.[40] Chitosan is a polymer with high cationic charge density, and the overall charge of debris particle of the crude extract was negative at pH 7.0 (Figure 1b). Thus, the positively charged chitosan strongly adsorbs on the negatively charged debris particles resulting in most of the charged groups being

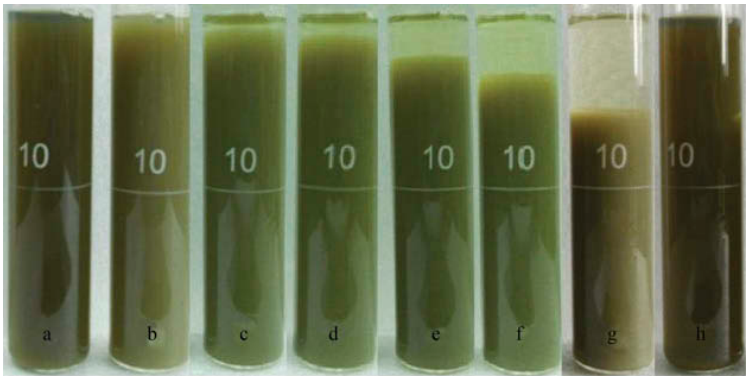

Figure 2. Photos of flocculation at different settling times (tube A: sample without chitosan addition. Tubes B–G show samples settled at 15 min, 1 h, 2 h, 3 h, 4 h and 20 h, respectively, after the addition of chitosan. Tube H shows sample without chitosan addition and settled for 20 h.

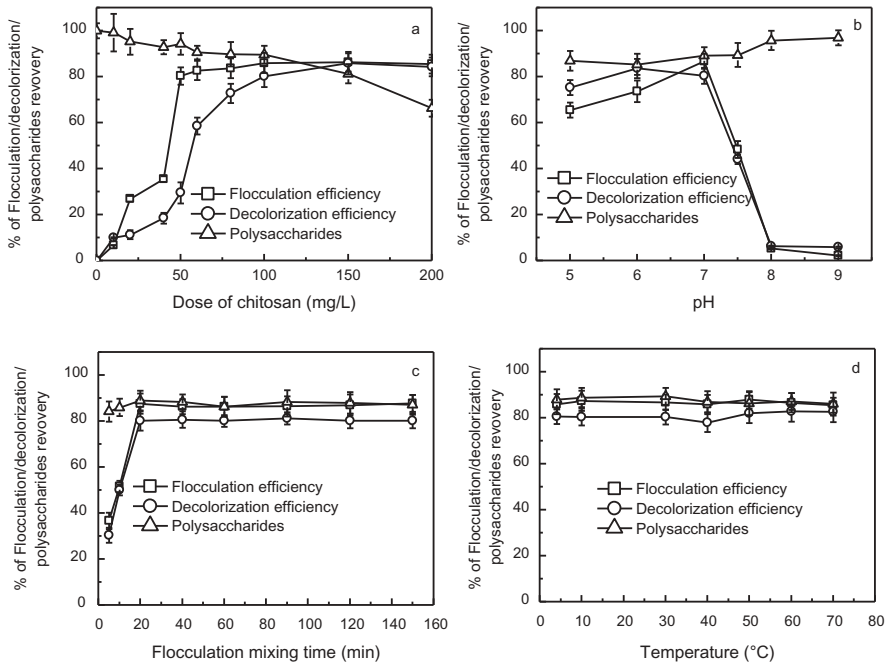

Figure 3. Effects of dose of chitosan concentration (Panel a), pH (Panel b), temperature (Panel c) and flocculation mixing time (Panel d) on the efficiency of flocculation, decolorization and polysaccharides recovery from crude extract from fresh *S. platensis*.

close to the surface of the cell debris.[41] Neutralization promotes flocculation by reducing the electrostatic repulsion between the debris particles.[33] Thereafter, chitosan can bind and bridge debris to produce flocs, causing the suspended debris to settle. When the pH was increased from 7.0 to 8.0, a sharp reduction in the flocculation efficiency was observed. One reason for this result may be the poor solubility of chitosan under alkaline conditions, reducing the amount of flocculent available for flocculating the debris particles.[37] Another reason may be that the increased concentration amount of OH^- ions can compete with the negatively charged debris particles in attracting positively charged chitosan, weakening the ability of chitosan to adsorb on and capture solid particles.

The effect of mixing time on the flocculation efficiency was investigated at 200 rpm and the results are shown in Figure 3c. The flocculation efficiency achieved the maximal value in 20 min. Negligible variations in total sugar recovery were observed with mixing time. Maximum flocculation efficiency within 20 min was similar to that reported by Ahmad *et al.*[41] for the flocculation of microalgae cells by chitosan. Variation in temperature had no influence on the chitosan and decolorization flocculation efficiency and polysaccharide recovery (Figure 3d).

Crude polysaccharides extract from fresh S. platensis

In our study on optimizing the conditions for the water extraction of polysaccharides from fresh *S. platensis*, we found that the highest recovery of total sugar (56.2 ± 2.0%) was obtained when 32.5 g/L fresh *S. platensis* was extracted with mechanical stirring at 80°C and neutral pH for 4 h. Some polysaccharides and sugars were discarded together with the debris of cell because the cell wall of *Spirulina* is made of complex sugars and protein.[22] Since the total sugar content of the polysaccharide extract was ~67%, the yield of polysaccharides from fresh *S. platensis* extraction was 9.1% dried sample weight.[2] This yield is higher than that of 8.3% reported by Chaiklahan *et al.*[4] for extraction of polysaccharides from dried *Spirulina* but lower than that of 13.6% obtained using the ultrasound-assisted extraction.[7]

Purification of polysaccharides solution with macroporous resins in the static adsorption operation

Spectrophotometric analysis of the crude polysaccharides solution displayed strong absorption peaks in the range of 200–290 nm (Figure 4). In this spectrum range

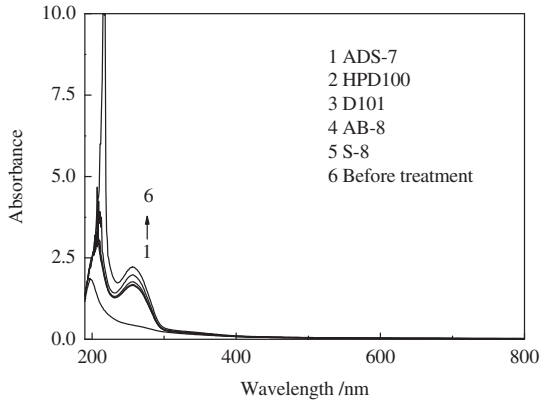

Figure 4. The UV-vis spectra of the crude polysaccharides solution after treatment by different resins.

of absorption, the peaks can be ascribed to nucleic acids, peptides, proteins and pigments released from the broken cells of *S. platensis*. Macroporous resins usually demonstrate a high capacity for adsorbing nucleic acids, protein and other pigments.[18,42–44] Therefore, macroporous resins were next evaluated for their ability to remove the UV-absorbing substances from the crude polysaccharides solution obtained from fresh *S. platensis*. Based on the results of our previous studies, macroporous resins AB-8, ADS-7, D101, HPD100 and S-8 were selected and tested for their ability of removing the UV-absorbing impurities from the crude polysaccharides solution of fresh *S. platensis*. The spectra of the crude polysaccharides solution treated by different resins in the static adsorption operation are displayed in Figure 4. ADS-7 resin showed the highest capacity of removing UV-absorbing substances from the crude polysaccharides solution because the absorption peak at 260 nm disappeared completely after processing. The purity of polysaccharides was increased from 86.5% to 94.62% (total sugar/total solid) with the treatment of ADS-7 resin (Figure 5). The total sugar recovery ratio was 92.7% in the case of ADS-7 treatment.

Temperature, pH and ionic strength are considered as important factors affecting the adsorption properties of macroporous resins.[12,45,46] However, changing temperature from 25°C to 35°C and the buffer concentration from 0.025 to 0.35 mol/L demonstrated no impact on the recovery ratio and purity of *S. platensis* polysaccharides purified with ADS-7 in the static adsorption operation (data not shown). The influence of the variation in pH on the purification is demonstrated in Figure 6a. The recovery of total sugar increased from 81.6% (pH 4.0) to 95.5% (pH 8.0) when the pH of the solution increased from 4.0 to 9.0. This indicates a loss of polysaccharide by adsorption to ADS-7 resin with the rising pH. A similar effect of pH on recovery of polysaccharide by the macroporous resin S-8 was reported by Liu *et al.*[19] The ability of ADS-7 to remove UV-absorbing substances increased with the rise of pH (Figure 6b). This may be due to the ionic interaction between UV-absorbing compounds and the amino groups on ADS-7 resin.[12] The results are in agreement with Liu *et al.* showing the impurity removal ratio of S-8 resin decreasing with an increase of pH values.[19]

Purification of polysaccharides in the packed bed column of ADS-7

Dynamic adsorption operation for removing impurities from the polysaccharides solution of *S. platensis* was evaluated in a column (10 mm × 20 mm) wet-packed with ADS-7 resin. The polysaccharide solution containing 5.0 mg/mL of total sugars and 0.8 mg/mL impurities was applied to the column. The pH of operation was maintained at 8.0 and the solution was fed from the top of the column at a speed of two bed volumes per hour. The impurities were almost completely absorbed

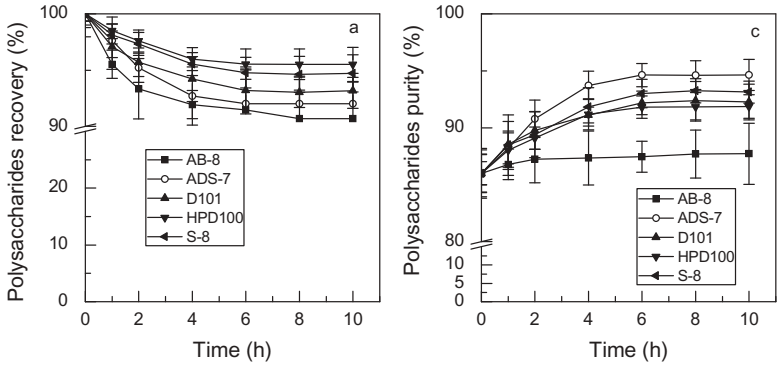

Figure 5. Kinetics of total sugar recovery (a) and purity of polysaccharides (b) with different resins treatments.

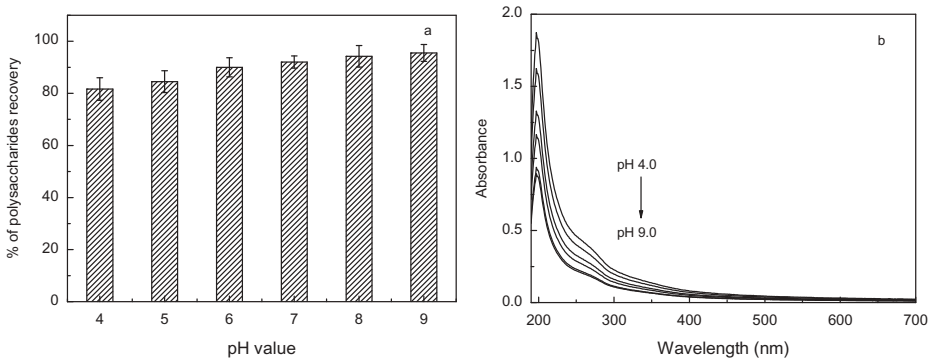

Figure 6. Effects of pH on total sugar recovery (a) and the UV spectra (b) of crude polysaccharides solution using ADS-7 resin in batch operation.

by ADS-7 resin within two bed volumes. This capacity of ADS-7 was similar to that of S-8 resin used in the removing impurity from crude polysaccharide solution from pumpkin residues when the cumulative volume applied to the column was no more than two bed volumes.[18]

The influence of the amount of solution applied to the column on the impurity removal efficiency of the packed column was tested and the results are displayed in Figure 7. The impurity removal was more than 80% while the recovery of total sugar was about 100% at 16 bed volumes of polysaccharide solution. When the cumulative volume of polysaccharide solution added to the column was greater than 16 bed volumes, the content of impurities in the effluent increased sharply. The removal ratio of impurity decreased to 31.5% at 30 bed volumes of solution added to the column due to the reduction in the number of active sites on the resin available for adsorption.

The residence time of solutes in packed column determined how long the solutes can interact with resins to accomplish the process of adsorption. Thus, it was important to investigate the influences of different residence time of solutes in the packed column by changing the flow rate of polysaccharide solution. According to the results shown in Figure 8a and 8b, the removal of impurity decreased with the increased flow rate. The lower flow velocity results in longer residence time for impurities to contact the resin in column, facilitating the increase in removal of impurities. The highest capacity for removing impurities was obtained in the case with 2 bed volumes/h of flow rate. Liu *et al.*[19] also found 2 bed volumes/h to be the optimized flow rate for removing impurities from the crude levan extract from *Paenibacillus polymyxa* EJS-3. It may be easier for the solute molecules to reach and to adsorb on the resin surface and inside the resin at a lower flow rate. With increase of flow rate, the removal of impurity decreased while the recovery ratio of polysaccharides increased. Thus, the shorter residence time of solutes in the ADS-7 packed bed at the higher flow rates reduced the adsorption loss of polysaccharides on the resin.

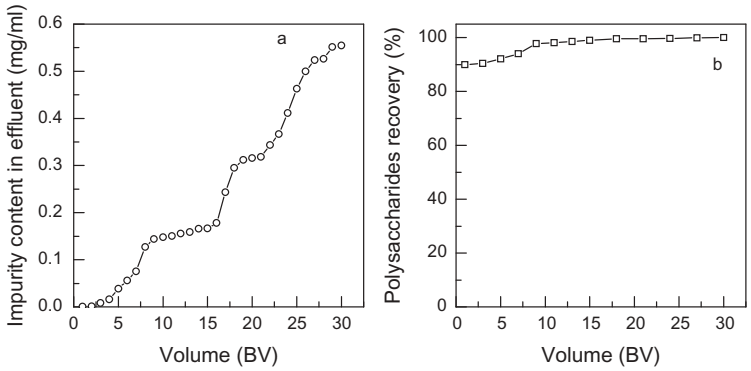

Figure 7. Impurity removal efficiency of polysaccharides solution from *S. platensis* in the packed column of ADS-7 (The influent contained 5.0 mg/mL of total sugars and 0.8 mg/mL of impurity at pH 8.0). BV is bed volumes.

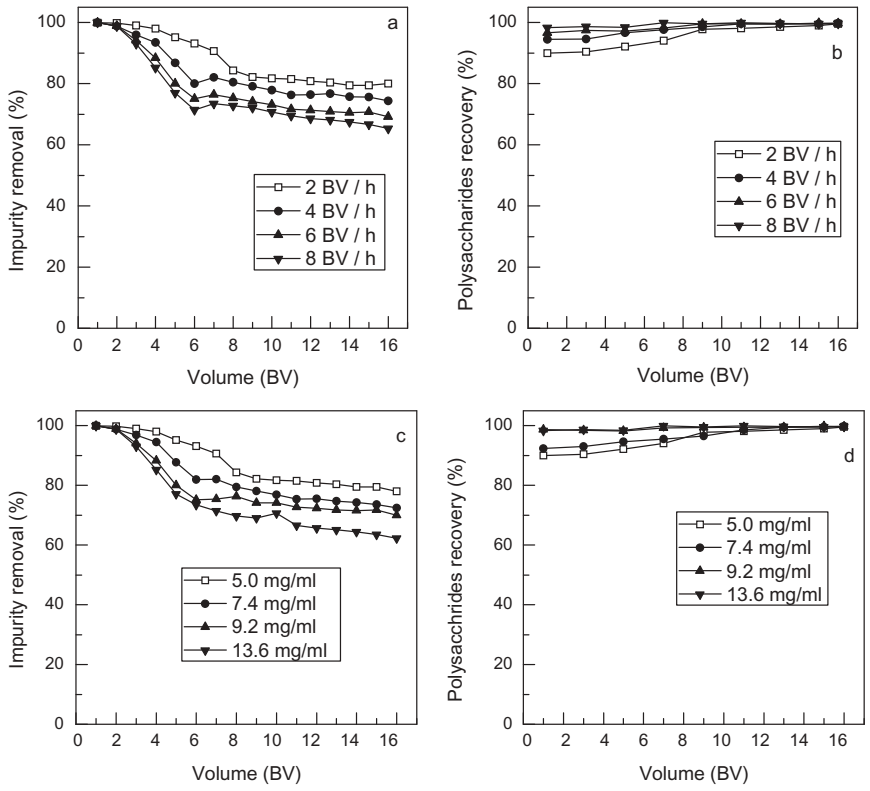

Figure 8. Effects of various flow rates and different concentrations of influent on the impurity removal ability of ADS-7 resin at pH 8.0 (the total sugar content of influent was 5.1 mg/mL). BV is bed volumes.

Fluctuation of the concentration of substances in the sample loaded on the column is another important factor influencing the efficiency of the packed bed resin column, especially in industrial applications. Polysaccharide solutions containing 5.0, 7.4, 9.2 and 13.6 mg/mL of the total sugars with a corresponding impurity content of 0.8, 1.2, 1.3 and 2.2 mg/mL were investigated to determine the influence of different total sugar contents and impurity content on the efficiency of ADS-7, the packed bed column. The lower the impurity content loaded, the higher the amount of impurity removed based on the results shown in Figure 8c and 8d. When the total sugar content and the impurity content in solution were 5.0 and 0.8 mg/mL, respectively, the impurity removal was more than 80% using a cumulative volume of no more than 16 bed volumes (Figure 6). The recovery of total sugar increased with the rise of the total sugar content in the sample loaded on the column (Figure 8d).

Finally, the optimized conditions, for the ADS-7 resin packed bed purification, were a pH of 8.0 and a flow rate of 2 bed volumes/h. The maximum purity of polysaccharides could reach 96.14% when 2 bed volumes of the polysaccharide solution were fed into the column under optimal conditions. Hence, the dynamic adsorption operation consumed shorter time than the static adsorption operation (Figure 4) and resulted in the highest value of purity of polysaccharides.

Molecular weight and sugar component analysis of the polysaccharides

By means of high-performance gel permeation chromatography, the molecular weight distribution of the purified polysaccharides from *S. platensis* was determined (data not shown). Three components of polysaccharides were found with average molecular weights of about 1400 (A), 420 (B) and 2 kDa (C), respectively. The weight ratios of the components A, B and C were 44.5%, 29.6% and 25.9% (w/w), respectively. The analysis of sulfate residues and uronic acid content of the polysaccharides were 3.7% and 0%. Polysaccharide with a molecular weight more than 1000 kDa is considered as the "immulina" from *S. platensis*,[9] as it can increase mRNA levels of interleukin-1α and tumor necrosis factor-α and shows clinical potential for cancer immunotherapy.

Many researchers have suggested that the polysaccharides from *S. platensis* are comprised of various

monosaccharides and carboxylic acids, such as rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid and galacturonic acid.[3,8,11,47] The polysaccharides extracted from the fresh *S. platensis* with water were subjected to sugar composition analyses. After hydrolysis to monosaccharides, analysis by HPAEC-PAD showed that the polysaccharide consisted of nine sugars. The series of retention times identified the monosaccharides as L-fucose, D-glucosamine, L-rhamnose, L-arabinose, D-galactosamine, D-galactose, D-glucose, D-xylose and D-mannose with a relative molar concentration ratio of 1.16:0.078:2.63:0.865:0.136:2.56:96.75:1.18:1.79. The major component was glucose, accounting for around 89.13% of the total sugars. Mannose, rhamnose and galactose accounted for around 2.04%, 2.42% and 2.91%, respectively. The remaining sugars accounted for less than 2% of the total sugars. The polysaccharide of *S. platensis* from a different producer showed a great disparity in the ratio among monosaccharide components. Madhavi Shekharam *et al.* reported that the polysaccharide of *S. platensis* prepared in India was comprised principally of glucose (54.4%) and included rhamnose and mannose.[48,49] Whereas, Majdoub *et al.*[50] found that rhamnose was the main component (49.7%) of the polysaccharide extracts from *Spirulina* of Tunisia. The polysaccharide of *S. platensis* from Taiwan contained mainly galactose (46.4%) as the major component.[47] Thus, further research should be conducted to examine the genetic differences underlining these differences in the monosaccharide composition of the polysaccharide of *S. platensis* from different sources.

Conclusions

The water extract of polysaccharides from the fresh *S. platensis* can be carried out at a higher biomass (32.5 g/L) than polysaccharide extraction from the dried *S. platensis* powder. The combination of Brownian force, high viscosity of fluid and electrostatic repulsion between particles prevented the coagulation and precipitation of debris particles in the crude extract. Therefore, flocculation with chitosan was investigated to facilitate the separation of the cell debris resulting in 86.0% flocculation and 85.9% decolorization. The purity of polysaccharides could be further increased from 86.5% to 96.2% (total sugar/total solid) with the treatment of ADS-7 resin. Although the difference between the purity of polysaccharides obtained in the static adsorption operation (94.6%) and that in the dynamic adsorption operation (96.2%) was small, the dynamic adsorption operation took shorter time and resulted in higher polysaccharide purity. The purification process developed in this study is simple and easy for scale-up, showing a practical potential of industrial application for separating water-soluble polysaccharide or other bioactive substances from blue-green algae.

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