

Comparison of two chemical extraction methods for proteins and polysaccharides of *Spirogyra fluviatilis* in extracellular polymeric substances

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Abstract. Under natural circumstances, *Spirogyra fluviatilis* excretes large amounts of extracellular polymeric substances (EPS) and forms a mucilage layer. This study used two algal EPS extraction methods such as NH₄OH and formaldehyde-NaOH to compare the extraction yields of EPS and the levels of its primary components of polysaccharides and proteins. The results indicated that the extraction yields of EPS using formaldehyde-NaOH and NH₄OH are 8.05 mg EPS/g-fresh algae and 5.26 mg EPS/g-fresh algae, respectively. The ratio of proteins to polysaccharides in EPS is around 0.24 to 0.34, which is not a significant difference between the two extraction methods. In other words, the level of polysaccharides is always higher than the level of proteins in EPS extraction with both two methods

1. Introduction

EPS are metabolites accumulated on the surface of microcells. They not only stabilize the structure of plasma membranes but also protect cells against external harsh environments. EPS are high molecular isomeric polymers, which primarily consist of polysaccharides, proteins, nucleic acids and fats [1]. Therefore, they can be transformed and become the source of carbon and energy during starvation [2].

EPS can be found in marine microorganisms and fungi [3, 4]. *Spirogyra* is a genus of large filamentous green algae commonly found in freshwater all over the world. The cell surface of *S. fluviatilis* can excrete large amounts of EPS and then form a unique mucilage layer. However, the extraction methods of *S. fluviatilis* and the features of EPS were not explored.

The application of microbial EPS in the industry has been valued highly [5]. The demands for natural polymers in various industrial applications have increased lately, and therefore, novel algae have been used to synthesize high-value products [6].

This study compared the EPS extraction yield of the large freshwater algae *S. fluviatilis* and the levels of its primary major components of polysaccharides and proteins with two different chemical extraction methods of NH₄OH and formaldehyde-NaOH. We expect to establish an EPS extraction-based biotechnology from *S. fluviatilis* and explore its potential in industrial development.



2. Materials and methods

2.1. Algae

The algae that were used in this study the species *S. fluviatilis* were collected from the campus of Kun-Shan University (N23°25', E120° 21') in Taiwan. The algae were washed twice with tap water, placed in a strainer at room temperature for 2 hrs to naturally drain the water, and then used for EPS extraction.

2.2. EPS extraction methods

In extraction methods, cationic exchange resin (CER) is not suitable for EPS extraction from large Filamentous algae. Therefore we used two chemical methods, formaldehyde-NaOH, and NH₄OH, to extract EPS.

(1) NH₄OH: 50g fresh algae+0.05%(w/w) NaCl solution to 100ml+50ml 25% NH₄OH at 4°C for 24h.

(2) Formaldehyde-NaOH: 50g fresh algae+0.05%(w/w) NaCl solution to 100ml+50ml 36.5% 1N Formaldehyde-NaOH at 4°C for 1h.

The treated suspension was centrifuged (10,000 × g) for 10 min with the suspension being collected. All supernatants were diluted by Mili-Q water to at the same level and then filtrate using 0.45µm cellulose nitrate membrane filter with filtrate collected.

The flowchart of extraction procedures are shown in Fig. 1.

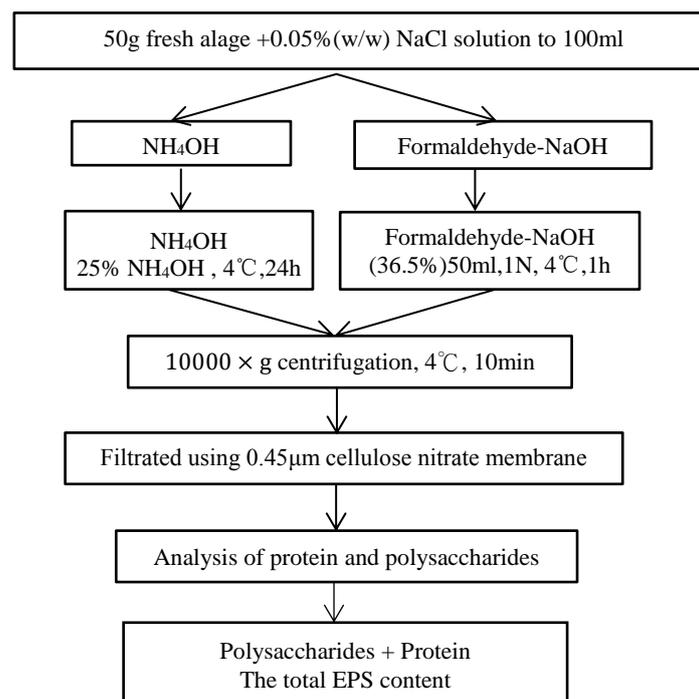


Figure 1. Procedures for the 2 extraction methods of *S. fluviatilis* EPS.

2.3. Analysis method

All chemicals used in this work were of analytical grade. Polysaccharide content was determined by the phenol-sulfuric acid [7] using glucose as a standard. Protein content was determined according to Bradford (1976) [8] with bovine serum albumin BSA, Sigma A2153 100mg/ml) [9]. The total EPS content was measured as the sum of these two substances.

3. Results and discussion

3.1. Comparison of different chemical extraction methods for assessing the levels of proteins and polysaccharides of EPS

The protein levels from different chemical extraction methods were measured using common testing kits. The results were shown in Fig. 2. The results revealed that the protein level with the formaldehyde-NaOH method was significantly higher than the protein level with the NH_4OH method. Similarly, the polysaccharides levels from different chemical extraction methods and are shown Fig. 3. The data reflect the average results from 6 repeated measurements, which showed that the average polysaccharides weight of EPS extraction with the formaldehyde-NaOH method was 6.07 mg-polysaccharides/g-fresh algae, w/w, but the average weight of polysaccharides with NH_4OH was only 4.08 mg-polysaccharides/g-fresh algae, w/w. Based on the aforementioned data, we can conclude that formaldehyde-NaOH also has a greater extraction yield of polysaccharides than NH_4OH .

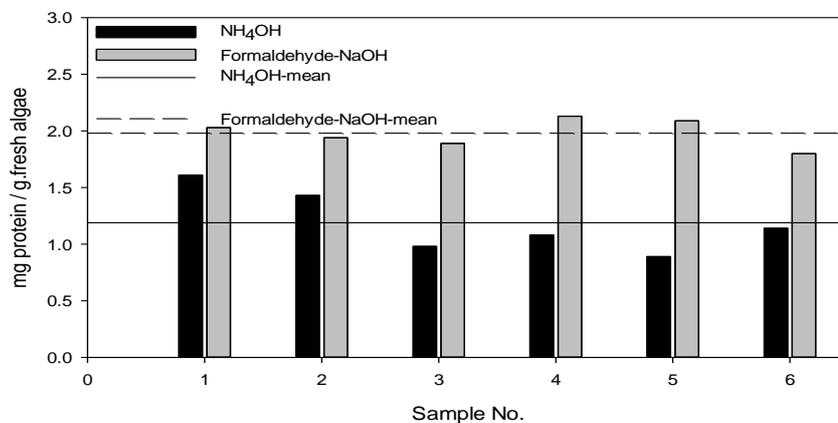


Figure 2. Protein level for formaldehyde-NaOH and NH_4OH EPS extraction method.

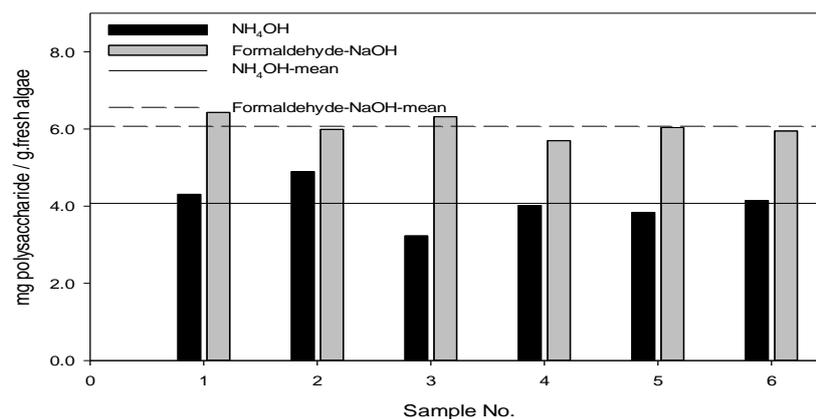


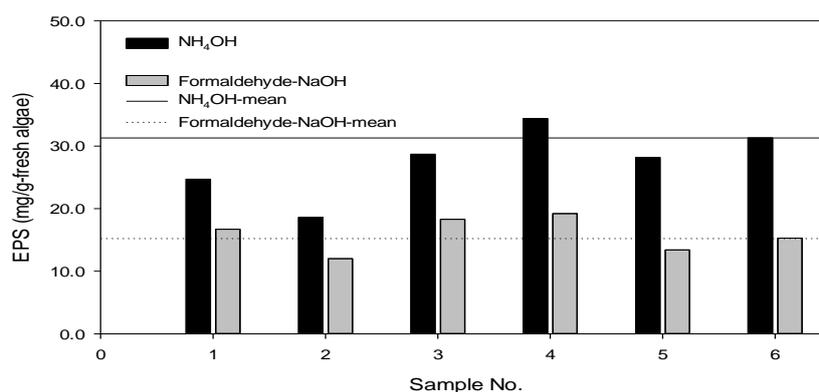
Figure 3. Polysaccharides level for formaldehyde-NaOH and NH_4OH EPS extraction method.

3.2. Comparison of formaldehyde-NaOH and NH_4OH extraction methods for the extraction yield of EPS

The results are shown in Table 1 and Fig. 4. The results demonstrated that formaldehyde-NaOH has a greater extraction yield of EPS than NH_4OH .

Table 1. Amounts and compositions of EPS extracted by different methods

| Extracted method | Organic matter contents (mg g ⁻¹ fresh algae) | | | |
|--------------------|--|----------------|--------------------------------|--------------------------|
| | proteins | polysaccharide | EPS(proteins+ polysaccharides) | proteins/polysaccharides |
| Formaldehyde-NaOH | 1.98±0.13 | 6.07±0.26 | 8.05±0.27 | 0.327±0.028 |
| NH ₄ OH | 1.19±0.28 | 4.08±0.55 | 5.26±0.77 | 0.291±0.047 |

**Figure 4.** EPS for Formaldehyde-NaOH and NH₄OH EPS extraction method.

This study further compared the primary components of EPS (polysaccharides and proteins) with different extraction methods. The results showed that although different extraction methods can lead to different extraction yields of EPS, the ratio of proteins to polysaccharides in EPS also significant differences.

4. Conclusion

To compare the extraction yields of EPS from large *S. fluviatilis* and the changes in its primary major components of polysaccharides and proteins with different chemical extraction methods, the study results showed that: (1) Formaldehyde-NaOH has a greater extraction yield of EPS than NH₄OH method. (2) The ratio of proteins to polysaccharides in EPS is around 1.05 to 1.38, which is not a significant difference with the two extraction methods. In other words, the level of polysaccharides is always higher than the level of proteins in EPS extraction with the two methods.

Genus of *Spirogyra* is a biomaterial with great potentials. In its natural habitat, *S. fluviatilis* can produce large amounts of EPS, therefore it has great potential in EPS extraction. These preliminary study results helped us understand the extraction methods of EPS applicable to large algae, and the influences on its primary components. We conclude that NH₄OH and Formaldehyde-NaOH are both suitable for EPS extraction methods for large algae.

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