Analysis of characteristics extracellular polymeric substances extracted from aerobic granular sludge by different methods

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Abstract: The characteristics of the extracellular polymeric substances (EPS) extracted with eight different extraction protocols from two different types of aerobic granular sludge were studied. The efficiency of four single methods including NaOH, sonication, heating and cationic exchange resin (CER) and four their combination methods was compared with each other. The EPS of the aerobic granular sludge investigated are predominantly composed of polysaccharides, proteins, and humic-like substances. The nucleic acid content of the EPS extracted show that the extraction whether induce abnormal cellular lysis or not. The EPS content in each biochemical compound varies depending on the sludge type and extraction technique used. Combination methods can provide more EPS than every single method, which suggests that there is a complementary and mutual promoting relationship between each method.

1. Introduction

In recent years, researchers have found that the main extraction methods of EPS are centrifugation, sonication, EDTA, heating, NaOH, CER, formaldehyde plus NaOH, chemical reagent plus electric field[1-5], etc. In order to achieve the optimal balance between high EPS extraction efficiency and low cell lysis, although many experimental studies have been carried out, the results are still controversial. Therefore, it is very necessary to determine an activated sludge EPS extraction method to maximize the extraction efficiency without destroying the cell wall[6,7]. It can not only reflect the relationship between activated sludge EPS and the properties of sludge more accurately, but also provide a theoretical basis for the operation of the process. In addition, different sludge EPS extraction methods have differences in the extraction efficiency of different activated sludge EPS, so determining different extraction methods for different sludge to achieve the highest extraction efficiency is of particular importance.

At present, there are few studies to combine multiple methods for extraction. This experiment aims to obtain a more effective extraction method. Four kinds of original single methods are combined. At the same time, a single method of control experiment is carried out to extract the same batch of sludge EPS, and component analysis is carried out, which laid the foundation for the establishment of sludge EPS extraction method.
2. Materials and methods

2.1. Origin of biomass
The aerobic granular sludge used was taken from two laboratory-scale sequencing batch reactors (SBR) with a working volume of 12L operated stably at 20°C for more than 4 months. SBRs were supplied with synthetic wastewater containing VFAs (400 mg/L as COD basis), One used acetic acid as carbon source (R1) and another used propanoic acid as carbon source (R2), NH₄Cl (40 mg/L as NH₄⁺–N basis), KH₂PO₄ (10 mg/L as PO₄³⁻–P basis), and trace element solution. The trace element solution consisted of the following compounds per liter: 0.1 mg ZnCl₂, 0.5 mg MgSO₄, 0.5 mg FeCl₃·6H₂O, 0.1 mg MnSO₄·H₂O, 0.1 mg NaCl, 0.1 mg CaCl₂, 0.1 mg H₂BO₄, 0.1 mg NiCl₂.

2.2. Chemical analysis
Proteins were quantitated by the modified Lowry method[8]. Carbohydrates were measured using the anthrone method[9]. DNA was measured by the diphenylamine colorimetric method[10]. The yield of EPS was measured as total organic carbon (TOC) by using a TOC analyzer (SHIMADZU, Japan). MLSS and volatile suspended solid (VSS) contents of sludge were analyzed according to the standard methods[11]. All chemical analyses were performed in duplicate using chemicals of analytical grade.

2.3. EPS extraction protocol
In order to study the effects of the extraction methods on the EPS characteristics, eight extraction procedures have been selected as shown in Fig.1.

3. Results and discussion
The two kinds of mature granular sludge both have a complete morphology, smooth surface, and dense structure, with an average particle size of 400μm and 800μm. Most of the sludge is white, and a small part is pale yellow, which is shown in Figure 2.
3.1. Comparison of single method extraction quantities

Figure 3 showed the amount of EPS extracted by eight methods in R1, including proteins, polysaccharides, and nucleic acids. It can be seen that from the perspective of the total amount of the three components, NaOH > CER > sonication > heating. NaOH method has the highest amount of polysaccharide and protein extraction. The results is similar to the tests of Zou et al. [12], Liu et al. [13]. Heating has the lowest extraction amount of polysaccharide and protein, the results of the test are similar to Huang et al. [14], Wang and so on [15]. Sonication has good extraction effect on EPS, the reason is that the power used in the experiment is high. On the other hand, from the point of view of cell destruction, NaOH and CER extract more nucleic acids, followed by heating, and sonication are the least. NaOH and CER are chemical methods, the damage to cells is greater, so the nucleic acid content is higher, Heating and sonication are physical methods, The conditions are relatively mild and the cell integrity can be preserved. It can be seen that the extraction amount of EPS is closely related to the extraction method. Figure 4 showed the amount of EPS extracted by eight methods in R2. It can be seen that from the perspective of the total amount of the three components, CER > NaOH > heating > sonication. In conclusion, the extraction amount obtained by the same method is also different, indicating that the extraction amount is related to the source of sludge and the means of analysis.

3.2. Comparison of the proportion of each component of the single method

The extraction method not only determines the amount of extraction, but also has a significant impact on the content of each component. Figure 5 and Figure 6 shows the distribution of the proportions of the three components in the EPS obtained by the eight methods in R1 and R2. It can be seen that polysaccharides are the primary component of EPS, accounting for 65% to 90% of the total extraction, followed by proteins accounting for 4% to 31%, and nucleic acids accounting for 1% to 4%. Due to the different primary components obtained by different methods, it can not be inferred from Figure that the main component of the sludge EPS is polysaccharide or protein. For polysaccharides, either method can account for most of the total amount, indicating that the polysaccharide component is the most easily extracted part. For proteins, sonication and heating methods account for a very limited proportion, while NaOH methods can account for more than 30%, indicating that the conditions for protein extraction are more stringent. In conclusion, CER method is an ideal extraction method based on the synthesis of cell integrity and total extraction.
3.3 Comparison the extraction quantities of different combined methods

In order to further improve the extraction amount of EPS, a more effective EPS extraction method was obtained. Four single methods were combined to test and EPS was extracted from sludge in order of priority. Figure 3 and Figure 4 are the extraction effect of each component by using four combination methods in R1 and R2. It can be seen from the figure that the sonication + CER was used to obtain the highest amount of extraction, followed by sonication + NaOH + heating and sonication + NaOH, and finally NaOH + heating in R1. Each step plays a very important role in the extraction of polysaccharides, and each method can extract a part of polysaccharides. However, for proteins, in the method of adding NaOH, the extraction effect is quite similar, further indicating that NaOH is the key to extracting proteins. When sonication is treated alone in R2, the extraction amount is very limited, but it is used as a pre-treatment of other methods to disperse the sludge flocculation, which can play a role in enhancing the effect of polysaccharides extraction. From Figure 4, it can be seen that after adding sonication pretreatment, the extraction of polysaccharides will increase by 11.7% to 18.6%, while the extraction of proteins will remain. On the other hand, the degree of cell destruction is also an important indicator of the quality of extraction methods. All four combination methods are a combination of physical and chemical methods. They are processed by 2 to 3 single methods. Therefore, the nucleic acid content of these combination methods is higher than that of a single method. This is unavoidable defects. It shows that sonication as a pretreatment, the damage degree of cells will be further increased, the more cumbersome the extraction process, the more difficult to guarantee the integrity.

3.4 Comparison of the components proportion of different combined methods

Similarly, the proportional distribution of the components of the four combinatorial methods can be obtained, as shown in Figure 5 and Figure 6. Polysaccharides account for 60% to 90% of the total extraction, proteins account for 8% to 36%, and nucleic acids account for 2% to 5%. After the addition of NaOH, the proportion of proteins generally increases. Comparing the single methods and the combination methods, it is speculated that the polysaccharide in the sludge EPS may be the highest content component. Combined with the total extraction amount and nucleic acid content, sonication is a good pre-treatment method. The combination with any method can enhance the extraction effect. Using ultrasound + CER method, while the proportion of nucleic acids is comparable to that of other methods, it can maximize the extraction of proteins and polysaccharides, which is the most ideal extraction method for the test.
4. Conclusion

- When the extraction is done by a single method, CER method is an ideal extraction method based on the synthesis of cell integrity and total extraction.
- The polysaccharide components of the sludge sample in this test are easier to extract, and the protein needs to be extracted more fully under alkaline conditions. According to the extraction results of eight methods, it is assumed that polysaccharide is the most important component in the sludge EPS.
- As a combination of pretreatment and other extraction methods, sonication can play a role in enhancing the extraction effect of polysaccharides. The extraction amount of the combined methods is generally higher than the single method extraction amount.
- The total extraction amount and the effectiveness of the extracted components are combined to determine that sonication + CER is an ideal extraction method.

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