Research Progress of Biofouling Extracellular Polymeric Substances in Cooling Water System

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Abstract

Biofouling is one of major pollutants in circulating cooling water system, one important reason for formation of biofouling is the accumulation of extracellular polymeric substances, they are high polymers that are secreted to vitro by microorganisms, mainly bacteria. Bacteria formed deposits that can secrete mucus are the mainly bacteria causing dirt. This essay is to illustrate causes of biofouling formation, component of extracellular polymeric substances of which extracellular polysaccharide, extracellular protein extraction and identification methods and provide a theoretical basis for researching the control of extracellular polymers and developing green efficient detergent methods.

Keywords: Biofouling; cooling water system; extracellular polymeric substances; exopolysaccharide; extracellular protein

1. The formation of biological fouling in cooling water system

Water shortage is one of the resource crises that human are facing. Available fresh water resources are very meager and the waste of water resources is a very serious, coupled with consumption of industrial circulating cooling water system is huge. So increasing the utilization efficiency of circulating water system is one important ways of industrial water conservation. But the fouling in industrial water recycling system increases the difficulty for the reuse of circulating water. In particular, human still lack depth understanding of the formation mechanism of biofouling because of the very complex process. The formation mechanism was involved with many different academic subjects by reason of serious shortage of geodesic quantitative parameters and different development levels of academic subjects. There are multiple barriers in the respects of forecast in biofouling prediction parameters, and this situation is hard to change in a short time [1].

Suitable living environment and sufficient nutrient are propitious to the mass propagation of microorganisms together with producing biological slime that is including rubbery sediments, goo,
adhesive film and colored pastes [2]. The causes of biological slime formation are complex in the cooling water system. Zhang [3] studies circulating cooling water system using wastewater of oil refining. However, the wastewater of oil refining still contains a certain amount of suspended solids, sulfide, oil, ammonia nitrogen and so on. The presence of these substances has an influence on circulating cooling system. Suspended solids can form colloid easily and are hard to removal. Sulfide that influences the effect of fungicides can accelerate the corrosion of carbon steel and form sediments easily with zinc and other divalent ions. The suspended solids and sulfide provide a location for attachment of microorganism, and promote the formation of biological slime. Oil which is the organic giant molecules can be microbial nutrients, but it has a dispersive action and can decrease the surface tension of water and reduce the tendency of scaling. There is a dispersion, and it can reduce the scaling tendency to a certain degree. Ammonia nitrogen can affect the pH of water, promote microbial growth and consequently result in acceleration corrosion of pipes. Now Costerton and Lewandowski [4] have discovered that adhesion triggers the expression of a σ factor that derepresses a large number of genes so that biofilm cells are clearly phenotypically distinct from their planktonic counterpart. Each biofilm bacterium lives in a customized microniche in a complex microbial community that has metabolic cooperativity, primitive homeostasis and a circulatory system. Each of these sessile cells reacts to its special environment so that it differs fundamentally from a planktonic cell of the same species.

To sum up, the growth of microorganisms plays an important role for the formation of biological fouling, some bacteria can produce adhesive extracellular polymeric substances (EPS) that are also called the secretion of mucus, and they are the adhesion of biological fouling. The bacteria in cooling water system can secrete mucus are mainly bacteria formed deposits, they are saprophytic bacteria, Gram-positive, rod-shaped, no flagellum, short growth cycle, and can secrete a lot of mucus. The mucus surround and adsorb insoluble impurities in water. They form suspended substance that adhere to piping surfaces, and deposit in pipes and the dead angle of pipes, then form tuberculation corrosion products. These products affect heat transfer and contribute to the propagation of certain bacteria, such as iron bacteria, eventually lead to leakage of tube corrosion perforation [5]. The environments under tuberculation corrosion products provide anaerobic bacteria such as sulfate reducing bacteria (SRB) with good growing conditions. This case will accelerate the corrosion of pipes, result in foul smell and affect water quality.

2. The composition of EPS

Liu and Sponza consider that EPS which have complex composition are high molecular natural polymers. They are mostly composed of proteins and polyoses whose content are nearly 70-80%. There are also other constituents such as humic acid, uronic acid and nucleic acid and so on [6-7]. EPS are indicated into loosely bound (LB) and tightly bound (TB).

A. The composition of Exopolysaccharide

The composition of exopolysaccharide can be indicated into different degree of polymerization of repeated oligosaccharides units and a variety of non-sugar substituents. Nowadays, there are so many exopolysaccharides such as dextran, fructan, cellulose and other heterogeneous polysaccharide with different functional groups [8].

Some scholars have researched different ingredients of many EPS of bacterial cells. The practice of Cong [9] approves that the essential exopolysaccharide of Pseudomonas aeruginosa is alginate that is constituted by D-mannuronic acid and L-guluronic acid. More hydroxide radical of polymer can promote the aggregation between a bacterial from others. Some exopolysaccharides are not peculiar to one bacterial. Sutherland [10] indicates that Agrobacterium sp., Rhizobium sp. and Alcaligenes sp. all can produce succinoglycans which are the more characteristic octasaccharide repeating units(four main-
chain+four side-chain sugars) and highly charged macromolecule in which there are two main-chain D-glucuronosyl residues, as well as non-stoichiometric acetylation of both D-galactosyl and D-glucosyl residues. The configurations of exopolysaccharide are various. Liu [11] uses nuclear magnetic resonance spectroscopy to analyze the EPS configuration of Klebsiella oxytoca XCH-1. In the main-chain rhamnose has α and β configurations, glucose is mainly β configuration and galactose is mainly α configuration. Galactose on the side-chain links the main-chain by 1-2 bond which is also β configuration. This study is the first time to investigate the EPS of Klebsiella oxytoca XCH-1 in domestic, and it is endowed with promised future. The adhesive extracellular polymers result in the formation of bacterial biofilm and they are also the gather of EPS. Sun [12] denudes polysaccharide whose Mw is 4.8×10⁶ from membrane bioreactor, and it is a heteropolysaccharide that is made up of glucose, rhamnose, xylose, mannose and so on. The glycosidic bond type is 1-3 and 1-6 configurations.

B. The composition of Extracellular protein

Pan[13] utilizes Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry to identify eight extracellular proteins of Aspergillus oryza 3.951. They include Glucoamylase B, Take-amylase A precursor, Chitinase, Acid Amylase A, Assume protease AN0443.2, Alkaline protease, Xylanase F1, A-chain neutral protease, Alkaline protease precursor and so on. These and protein profiling of Aspergillus oryzae 3.951 that have been finded in 2009 provide integrated extracellular protein profiling. Liu [14] collects the extracellular proteins in the cultural supernatants of virulent and avirulent Flavobacterium columnare during the late logarithmic growth phase, then isolates and identifies 3 proteins. They are Sliding protein K, gland anhydride methionine synthase and a possible membrane protein. The result provides another powerful basis for the study of Flavobacterium columnare virulence factors. The discussion of extracellular protease is beginning. Chi [15] has found that the extracellular protease and serine proteinase of Cryptococcus neoformans are not variant at 30 or 37℃, the activity of Clinical isolates is stronger than Environmental isolates and Capsule deficient strain. There is no significant difference between different serotype strains. Serine protease inhibitor can inhibit the extracellular protease and serine proteinase of Cryptococcus neoformans.

3. Extracting method of EPS

At the present time, Extracting methods of EPS are summarized in physical, chemical methods, and combined technique which has higher output capacity of EPS and a lower level of damage is more effective than single method. This is an example of technology updates and progress. Specific methods following in TABLE 1:

4. Extraction and identification of Exopolysaccharide

Extractions of exopolysaccharide are multitudinal. The process can be generally divided into extraction of crude polysaccharides, removal of protein, identification and so on. Yu [27] determines an optimal route of extraction in studies of Cordyceps exopolysaccharide. After getting the crude polysaccharides of Cordyceps, she analyzes them, and futher examination shows that there are D-mannose, D-glucose, D-galactose, D-arabinose, D-xylose in EPS. This process is complex, and workload is heavy. It needs a lot of precise instruments. DEAE-DE52 chromatographic column has some insufficiencies, but this experiment is subject to different methods and correlation of biological activity detection to determine extracting technic. It avoids the case that high yield of polysaccharide is inconsistent with their biological activity, makes results more precise, and laids a good foundation for exploitation of Cordyceps exopolysaccharide. Wang [28] gets the crude polysaccharides from fermentation liquor of Clitocybe sp. AS 5.112 strain, and
removes the protein by Sevag method. Further examination shows that the fraction of polysaccharide is homogenous, and the monomer is D-glucose molecule whose molecular weight is about 67,638. It is β-glycoside bond type, no α-glycoside. The main chain is β-(1-4) Pyran glycoside bond type, and point of branching is β-(1-6). The unique about this experiment is the usage of Sevag method and analysis of the yield of polysaccharide using Design ExPert software. The operation is complex but results are accurate and carry so much conviction. For another example, Liu [29] extracts the exopolysaccharide and mycelia polysaccharides of Cantharellescibarius, his method is easier, and the flow is: Mycelium ground, hot water extraction, ethanol precipitation, polysaccharides, re-dissolution. In this study he gets the optimal experimental conditions by using the orthogonal experiment. Results show that effecting factors of extracting Cantharellescibarius polysaccharide are extraction temperature, ethanol concentration, extraction time according to priority. This has greatly improved the technology of Cantharellescibarius polysaccharide extraction. Luo [30] extracts the crude Armillariella tabescens exopolysaccharide by boiling water extraction and then measures polysaccharide by phenol-H2SO4 colorimetry. Further purification and detection get single polysaccharide IPS2B2 which is α-2D-(1-6)-2 pyranose glucan, and considered that it has a good ability of inhibition by a mice in vivo experiment.

### TABLE 1. Comparison On Extraction Methods Of EPS

| Methods          | Operation methods and features                                                                                                                                                                                                 | refs |
|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Physical method  |                                                                                                           |      |
| Ultrasonication  | Samples are ultrasonicated then centrifuged. The amount of cell disruption is comparatively small and the extracellular polymer yield is relatively high, no chemical reagent contamination.                                      | 16   |
| Heating          | Samples are in phosphate buffer conical flask. Heating at 80°C and then centrifugate to remove cells. Cells are easy to damage, the income of protein and polysaccharide is not high.                                     | 17   |
| Steaming         | Samples are steamed in an autoclave and then centrifuged while still hot. During centrifugation the temperature is reduced In order to reduce the disruptive effects on the cells of boiling or autoclaving.                               | 19   |
| Centrifugation   | Samples are ultrasonicated. Accelerate particle settling velocity, under mild conditions, extraction efficiency is low, damage is small.                                                                                               | 18   |
| Mixing           | Samples are ultrasonicated, add deionized water to the original sample volume, stir it at high-speed supernatant and then centrifugating. Extraction efficiency is low, the level of cell disruption is low.                              | 16   |
| Chemical methods |                                                                                                           |      |
| NaOH             | Centrifuge and then discard the supernatant, add NaOH to the original sample volume, centrifuge and discard the pellet. Extraction efficiency and he level of cell disruption is high. Operation period is long.                           | 17, 19 |
| H2SO4            | H2SO4 is added to samples, after twice centrifugation, filtering the supernatant, getting the supernatant after dialysis. The solubility of EPS is large, the level of cell disruption is high.                                                                 | 20   |
| Formaldehyde     | Sodium chloride solution of formaldehyde is added to samples, get the supernatant after centrifugation. It is propitious to fixe cells, but it destroys structure of protein.                                                      | 21   |
| Formaldehyde-NaOH| Add formaldehyde and mix, and then add NaOH and mix. Centrifuge it twice and filter supernatant, obtain extract after dialyzing. High extraction efficiency, formaldehyde influences on analysis.                                                      | 6    |
| EDTA             | Add EDTA into sample, and keep 3h. Then centrifuged, filter the supernatant. High extraction efficiency and serious damage of cells.                                                                                             | 16   |
| PBS              | Add quantitative PBS into sample and mix 2-4h in water bath. Low extraction efficiency.                                                                            | 22   |
5. Extraction and identification of Exopolysaccharide

Ni [31] gathers the germs cultures of aeromonas hydrophila J-1 strains during logarithmic phase, then purifies and gets the extracellular protein. After analysis and identify, the result is obtained with the PCR test: 10 immunogenic identified proteins are mostly toxins and enzymes which are secreted by bacteria, include aerolysin, hemolysin and other proteins. Extracellular protein generally has medicinal value. This method is a simple extraction procedures. In particular, this experiment establishes PCR detection which is specific. Scientific and accurate method increases the reliability of experimental results. In another example, Wang [32] and others extracts crude extract of extracellular protein from Curvularia CLE bacteria strains. They determinate the protein content with Bradford. The extracellular proteins can induce the activity of antioxidant enzymes, and inhibit the rice sheath blight. Studies about the signal transduction protein are not deep enough, but the extraction and analysis methods are simple. Zhang [33] and others extrac the extracellular proteins of Streptococcus suis type 2 virulent strain and avirulent strain and then analysis dimensional electrophoresis spectrums by Image Master 6.01 software. They find 180±10 checkpoint proteins, and there are 50 peculiar checkpoint proteins in avirulent strains but none in virulent, 52 peculiar checkpoint proteins in virulent strains but none in avirulent, these results are contribute to aspects of virus infection mechanism about two strains. In recent years, there are big breakthroughs in protein analysis methods. He [34] establishes the method of two-dimensional gels electrophoresis to separate the extracellular and membrane proteins in mycobacterium tuberculosis, analyze the gel image. This method could be helpful to reveal the mechanism of M. TB cellular immunity and intracellular survival secrets. This is the first choice to detect proteins. Moreover, Shen [35] measures proteins in extracellular polymeric substances from activated sludge by a spectral probing stain method, and the microsurface adsorption-spectral correction (MSASC) technique that uses Congo Red probing is applied to determine the content of proteins in extracellular polymeric substances of activated sludge. The measurement results are consistent with those of the Folin method which is commonly used for protein measurement. It is simple, rapid, economical, accurate and a new quantitative analysis method of proteins.

6. Extraction and identification of Extracellular protein

The existence of biofouling in circulating cooling water system brings a lot of damage to industry, life and environment and it also poses a severe challenge to the energy-saving and environmental protectors [1]. Bacteria forming microbial slime are mostly the bacteria formed deposits which produce sticking EPS. The secretion of mucus is the main reason for microbial fouling which is easy to form and difficult to removal. The study about EPS of bacteria formed deposits can be viewed as the key solution, and the extraction of EPS has become a top priority. Our group has started studying the conditions and process forming dirt of bacteria formed deposits, and the extraction of EPS has been regard as the focus of our mission. It is possible to make secretion mechanism of EPS clear and provide a good prospect of
environmental protection and efficient removal methods.

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