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Microbial Contamination in Drinking Water: Occurrence, Measurement and Control

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1. Microbial contamination in drinking water

Drinking water is essential for the health, everyday life and social development of human beings. Its importance is evident to all the people. Because China now is experiencing an unprecedented urbanization progress in the world, its demand for enough and clear drinking water is expanding every year. Unfortunately, many cities and towns in China are suffering from the pollution of source water, which results in the deterioration of drinking water quality. The so-called "micro-pollution in source water" has become a nationwide problem for the water utilities. This is even serious for the relatively developed areas such as the Yangtze River delta area and the Pearl River delta with Shanghai and Guangzhou as their central metropolises, respectively.

The contaminants in drinking water can be categorized into chemical ones and microbial ones. The former includes the trace level persistent organic pollutants, endocrine disrupting compounds and mutagenic disinfection byproducts (DBPs). The microbial contaminants in drinking water discussed in this mini-review include the following: (1) the emerging waterborne pathogens consisting of bacteria, protozoa and viruses, such as *E. coli*

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Dr. Yu has published over 70 peer-reviewed papers and served as a referee for over 10 international journals. He is also the inventor or co-inventor for over 10 Chinese patents on drinking water related technology. His research interest includes the following: (1) Detection of waterborne pathogens and antibiotic resistance genes using molecular tools; (2) Formation and control of the microbial aggregates such as biofilm and activated sludge; and (3) Biological and advanced water treatment processes.

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O157:H7, *Cryptosporidium parvum, Giardia lamblia*; (2) the antibiotic resistance genes (ARGs); (3) the microbial metabolic products during drinking water biological treatment process and the microbe leakage from the bioreactors like biofilters; and (4) the biofilms and other biofouling in pipeline systems.

The microbial contamination can cause both acute and chronic damages to human health. The pathogenic microbes can cause intestinal track disorders like vomiting and diarrhea, even fulminating infectious diseases such as cholera. The ARGs are the physical basis for the antibiotic resistance which can lead to the lack of anti-microbial drugs in clinics. The microbial products may be the precursors of the mutagenic DBPs. The leaked microbes from the biological treatment process increase the burden of the successive processes and also trigger the epidemiological risk of drinking water. And the biofilm can be a reservoir of pathogens besides corroding the pipeline material. Furthermore, this group of contamination usually does not change the physicochemical characteristics of the drinking water. For instance, the color and odor will keep unchanged if drinking water contains a waterborne pathogen with a level much higher than the water quality criteria. So the microbial contamination is even more dangerous since in many cases it can only be confirmed when the severe consequence such as death happens. For example, a massive outbreak of *C. parvum* oocysts occurred in Milwaukee, Wisconsin, U.S., about half of the population, around 0.4 million people, were infected and more than 100 died.

Household drinking water purifiers are popular in China for the emerging concern on drinking water safety. However, the bacterial quality of the effluent from the purifiers is not optimistic. Dr. Yu's investigation showed some ultra-membrane filter produced drinking water with even higher bacterial amounts than tap water.

2. The research team on drinking quality control in IUE, CAS and its research focuses

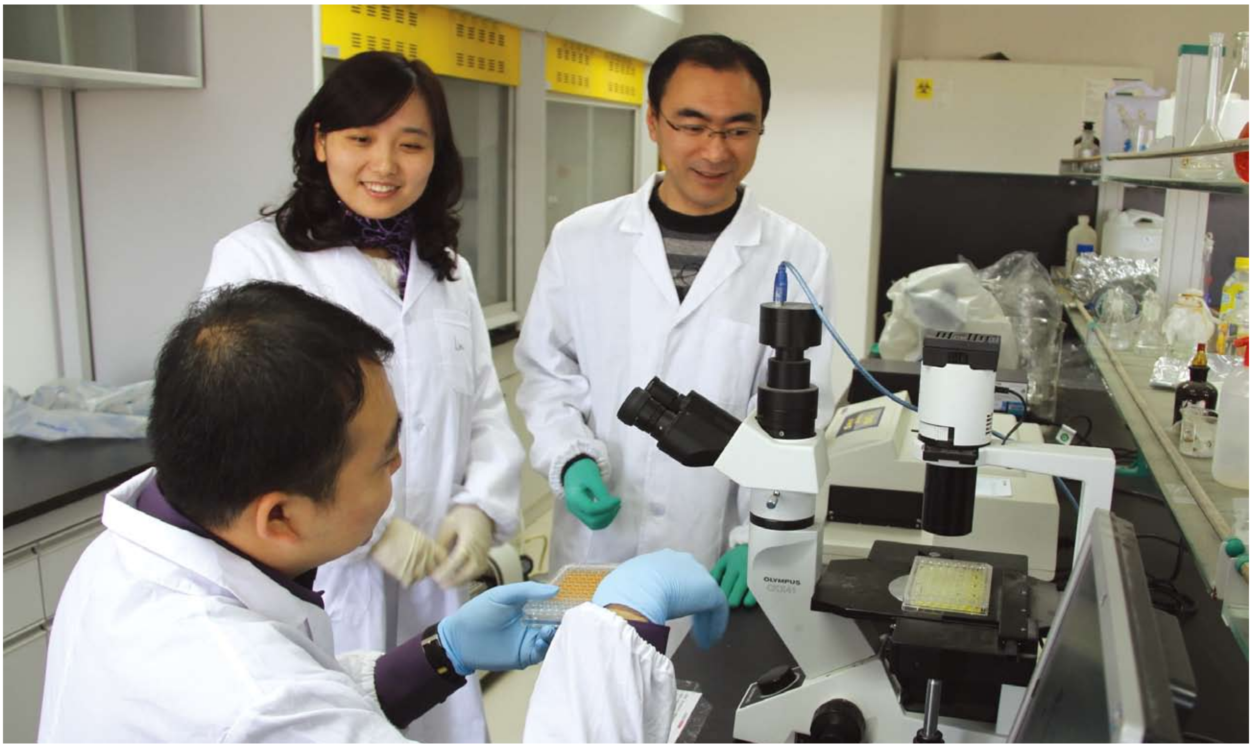
As the national research institution of China, Chinese Academy of Sciences (CAS) has several institutes aiming at improving the water environment quality with technical and engineering approaches. The Xiamen–based CAS Institute of Urban Environment (IUE) is just one of them. Established in 2006, IUE strives to find sustainable solutions to the environmental problems taking place during the rapid urbanization period in China. An IUE research team, led by Dr. YU Xin, focuses its efforts on the quality control and safety of drinking water. As is well known, drinking water quality and safety is a hot issue in environmental science and engineering. In order to distinguish itself from others, Dr. Yu' team zeros in on the following issues concerning the biological safety of drinking water and its control technologies.

2.1 Determination and survey of the background of emerging pathogens and ARGs in source and drinking water using molecular tools

A series of molecular tools such as PCR, real-time PCR and microarray was modified or developed to detect and/or quantify the pathogenic microbes and ARGs with respect to different throughput demands. The target gene numbers in typical water samples (from source water to tap water with clear or polluted source, and as the effluent of each treatment unit) were determined to provide the profile of these directly microbial contaminants in source and drinking water in China.

2.2 Characterization and control of microbial products and the leaked microbes during drinking water biological treatment

In order to remove trace level biodegradable organic matters and other bioavailable materials such as ammonia, biological treatments like biofiltration and O_3-BAC, are widely used in the drinking water plants in China. However, the microbial biomass will release metabolic products into the bulk water as they "eat" the bioavailable matters. Some of the released materials are direct or indirect threats to human health. Dissolved organic nitrogen (DON), a component of soluble microbial products (SMPs), is such a material since it can act as the precursor of nitrogenous



Dr. Yu and his group members isolated a bacterial strain from source water and found that it has the broad-spectrum biofilm inhibition ability. In this picture, they are observing and discussing the biofilm formation results using 96-well polystyrene plates.

DBPs. In addition, the leaked bacteria from biological reactors are also dangerous pathogenic sources for drinking water and drawing more and more attention nowadays. So this theme, *i.e*. the safety of the biological ones themselves, is becoming more important.

2.3 Biofilm formation and control technologies in biological reactors and pipeline systems

A biofilm is a preferred style for bacterial existence and growth other than planktonic growth. Because some bacteria are resistant to the disinfectant and can live in the distribution pipelines. They will utilize the very low level of organic carbons and other nutrients like N and P and form a biofilm attached to the pipe walls. The biofilm will corrode the wall material and reduce the water distribution efficiency, and release hazardous materials from the walls. Most importantly, the biofilm may be a reservoir of the pathogens. The biofilm formation is based on the cell-cell and cell-substratum interactions. The internal mechanisms and the ways to control its formation are now attracting many scientists' interest including those in IUE. They carried out studies from the view of quorum-sensing and cell-cell dependent interactions, promoting research in the field.

2.4 Treatment technologies for a higher biological stability and lower genotoxicity in drinking water

It is important to develop and modify high efficient treatment technologies to remove various contaminants and improve the drinking water quality. In IUE, these technologies include biological pretreatment, enhanced conventional treatment and O_3-BAC, membrane filtration. The novel treatment process, the combination between them and the conventional ones were tested to remove the complicated pollutants cocktails in drinking water.

Because of the rapid increase of the R&D investment of the Chinese government in environmental studies, many scientists in this area have obtained mass financial support from both government and industry. This is also the case for Dr. Yu's group. Over the recent 3 years, for instance, they have won nearly 10 grants from the National Natural Science Foundation of China (NSFC), Fujian Provincial Department of Science and Technology, and Xiamen Municipal Bureau of Science and Technology. The highest grant amounts to 3 million yuan. Besides, they have also conducted research projects with support of water plants. With these funds, the group could afford advanced facilities to do their research. The following is some of its study cases.

3. Research cases

In the recent 3 years, Dr. Yu's group has published nearly 40 peer-reviewed papers in international and Chinese journals, presented their work in about 20 different academic conferences, symposium and workshops. Herein its representative studies are summarized.

3.1 Molecular detection of targeted microbial species and ARGs in source and drinking water

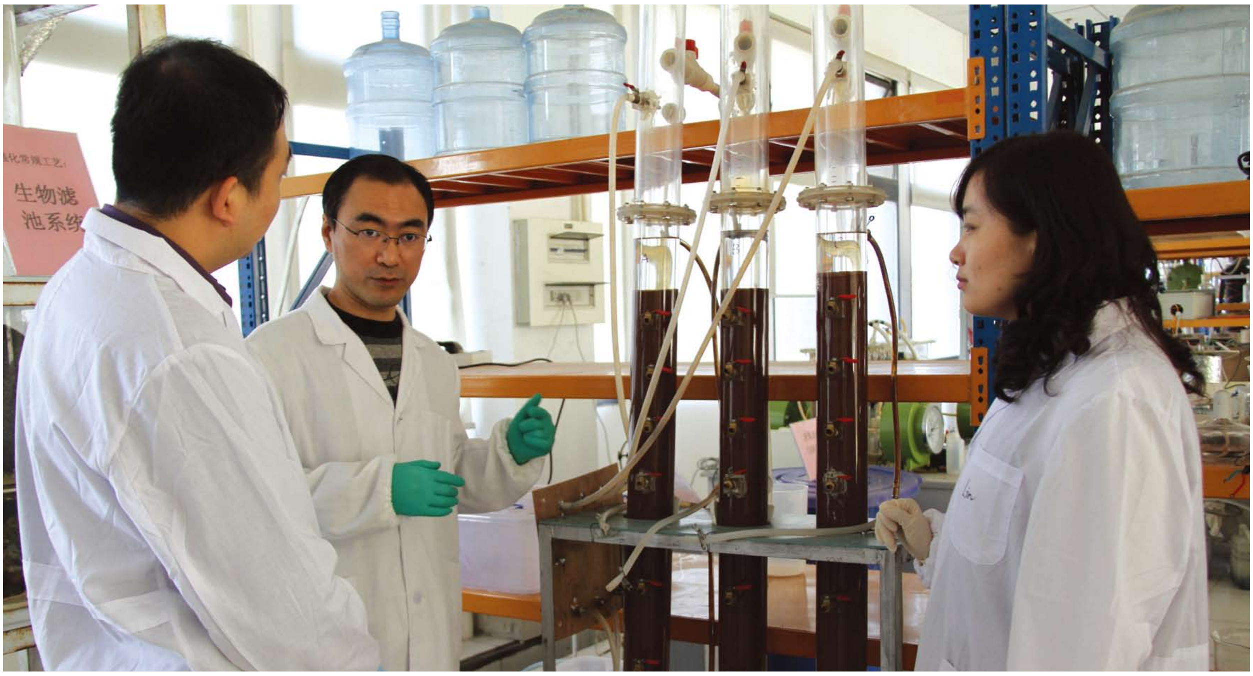
The first step to determine or quantify the targeted microbes is to get enough nucleic acids from the samples. There are various inhibiters in water for DNA or RNA extraction. Here we introduce a novel protocol on DNA extraction from activated granule samples. The BAC (biological activated carbon) process is used widely in water treatments, because activated carbon granules were suitable and affordable microbial carriers. This treatment is efficient in removing organic contaminants including natural organic matters, synthesized organic compounds, and other bio-available materials. For most soluble bio-available materials, bio-degradation acts as the essential mechanism in the contaminant removal by BAC filters, rather than physical filtration. Therefore, it is important to reveal the microbial community structure and function of the attached biofilm in order to optimize the reactor performance. However, the genomic DNA of the biofilm attached to carbon granules is hard to obtain because of the absorbing ability of activated carbon. This is an obstacle to the further molecular analysis. Dr. Yu's group has tested five pretreatments to find a solution, and adding skim milk followed by ultrasonic vibration proved to be the optimal choice. This method was further tested using the BAC granule samples from the full-scale biofilter of Pinghu Water Plant. All results suggested that the optimal protocol could produce qualified genomic DNA as a template from the BAC filter for downstream molecular biology researches (Zhang , *et al*., 2010).

Antibiotic resistance is one of the emerging challenges to drinking water safety, and this pollution spreads via antibiotic resistance genes (ARGs) which encoding the resistance. Hundreds of ARGs observed in water environments could be classified to five classes according to the different antibiotics they resist to. The five classes are: (1) tetracycline resistance genes; (2) aminoglycoside resistance genes; (3) macrolide, chloramphenicol, and vancomycin resistance genes; (4) sulphonamide and trimethoprim resistance genes; and (5) β-Lactam and penicillin resistance genes.

The Jiulong River is an important water source for the southern part of Fujian Province. The group monitored eleven ARGs in the River. In the study, it was found that many factors, including urban and agricultural activities, as well as water environment changes, could significantly impact the occurrence and variation of antibiotic resistance genes. The urban activities including sewage discharge and the swine industry can release ARBs and change the water parameters to influence the growth conditions of microorganisms, resulting in changes of the microbial community structure. They can significantly enhance the levels and relative abundances of ARGs. Then the increased salinity may decrease the relative abundances of ARGs in the river, it was speculated that the bacteria carrying ARGs were unable to adapt to the high level of salinity, then gradually lost the advantages in the microbial consortiums. Another finding in this study was that in high-flow period, the lack of NH_4^+ and PO_4^{3-} can attenuate the growth of ARBs, resulting in a positive relation between the abundance of ARGs and nutrient levels. However, in low-flow period, the inhibition disappeared.

In the investigation of ARGs in a full-scale drinking water treatment plant, the group has monitored two treatment processes, an advanced one and a routine one, in the Yangtze Delta Area. Among the eleven ARGs we had tested, nine ARG families (*tet*A, *tet*G, *str*A, *erm*B,

Molecular tools are essential for current research in the biological safety of drinking water. Dr. Yu's group members are using PCR for the qualitative determination of emerging waterborne pathogenic microbes.



Dissolved organic nitrogen (DON) is the derivative contaminant from biological filtration which was regarded as a safe technology for drinking water treatment. The occurrence of DON suggests the safety of biological processes should be re-considered. Dr Yu and his group members are discussing this issue in front of a set of biofilters simulating the formation and control of DON.

*cml*A5, *dfr*A1, *sul*II, *bla*TEM-1 and *blaoxa*-1) were found in most treated water inside the plant. The copy numbers of ARGs were obviously decreased along the treatments, but the reduction of the relative abundances of ARGs related to biomass was not as significantly as expected. Among the treatments, coagulation/ sedimentation, sand filtration and two-stage O_3-BAC can reduce the levels of ARGs, but ARGs carrying bacteria may regrow in the distribution system. However, to decrease the relative abundances of ARGs is difficult, only sand filtration and two-stage O_3-BAC filter may play a role. In contrast, coagulation/ sedimentation and disinfection may cause an increase in the abundances of ARGs, as well as the distribution system. As a result, it seems that the abundance was not significantly decrease eventually. Some water parameters may influence the removal efficiency. During a low temperature period, the lack of carbon and vial bacteria can limit the increase of ARGs, and during a high temperature period, DO (dissolved oxygen) becomes the limiting factor. We also found that during a low temperature period, levels of 16s rRNA gene and vial bacteria (HPC) have impacts on the abundances of ARGs, and then during high temperature period, the impacts disappeared.

3.2 Formation mechanism and remove of dissolved organic nitrogen

Dissolved organic nitrogen (DON) is drawing more and more attention in drinking water treatment currently for its potential threat to human health as the precursors of carcinogenic disinfection byproducts (DBPs). DON can also react with disinfectants to form nitrogenous disinfection by-products (N-DBPs), which present higher genotoxicity than the conventional characterized carbonaceous carcinogenic DBPs.

Existing in soluble microbial products (SMPs), extracellular organic material (EOM) of algae, forest litter and agricultural fertilizers, DON contains a range of nitrogen-containing functional groups that belong to various NH classes and amino categories, including nitriles, purine, pyrimidine, and nitro compounds. Currently, studies about DON are focusing on its analytical measurement, structural composition, occurrence and potential role in N-DBPs formation. However, no standard for the DON concentration in drinking water has been set to date in the whole world. In our recent study, DON was found increasing during biological processes in full-scale drinking water treatment plants. Thus, the results challenged the viewpoint that biological processes were genotoxicologically safe.

Dr. Yu's group has paid great concern on the formation mechanism and remove of DON. A research project was conducted in a plant with a production capability of $2.50\times10^4 m^3$ drinking water per day in Pinghu city, Zhejiang Province, PR China. Except the conventional drinking water treatment processes including coagulation, sedimentation, biofiltration and disinfection, advanced processes including biological fluidized bed pretreatment, coagulation, sedimentation, sand filtration, the first stage O_3-BAC, the second stage O_3-BAC, and disinfection was used because the source water was severely polluted.

The DON concentration and other related parameters along the media depth of the biofilter were investigated. DON concentration in biofilter presents a rapidly decrease at the surface media (0-10cm) of the biofilter, and a slow increase at the deeper media (10-100cm). At the initial stage of the biofiltration, DON was utilized much faster than SMPs generation, leading to a dramatic reduction of DON concentration. However, with the decrease of the level and biodegradability of the substrates, the SMPs began to accumulate along the deeper media depth, as well as the BAPs became predominant in SMPs, resulting in the finally substantial increase of DON concentration. EEM results confirmed the nitrogen-enriched aromatic protein-like fractions of SMPs contributed to the increase of DON concentration in biofilter.

A comprehensive investigation was made in this study on the variation of DON during a whole backwashing cycle of the biofiltration for drinking water treatment. In such a cycle, the normalized DON concentration ($C_{effluent}/C_{influent}$) was decreased from 0.98 to 0.90 in the first 1.5 h, and then gradually increased to about 1.5 in the following 8 h. Finally, it remained stable until the end of this hour cycle. This clearly 3-stage profile of DON could be explained by three aspects as follows: (1) the impact of the backwashing on the biomass and the microbial activity; (2) the release of soluble microbial products (SMPs) during the biofiltration; (3) the competition between heterotrophic bacteria and nitrifying bacteria. All the facts supported that more DON was generated during later part of the backwashing cycle. The significance of the conclusion is that the shorter backwashing intervals between backwashing for the drinking water biofilter should further decrease the DON concentration in effluent of biofilter (Liu, *et al*., 2012).

The effect of pre-oxidation and biological pretreatment in downstream coagulation on DON removal was investigated under different process conditions. DON removal in coagulation was augmented by the pre-oxidation. Cationic polymer could effectively promote DON removal and its dosing amount should be appropriate. Pre-oxidation could convert aromatic nitrogen to aliphatic nitrogen, with the Zeta potential absolute value of particles decreased and high-molecular-weight fraction increased degrading nitrogen enriched compounds in water. DON removal in coagulation was reduced by the biological pretreatment. It was found that biological fluidization pretreatment could change the composition of the compounds and generate more nitrogen-enriched compounds. It might be the reason of the lower DON removal of biological pretreated water in downstream coagulation. As a countermeasure, adding cationic polymer could effectively promote removal of DON in coagulation, and particularly the promotion of biologically pretreated water was more obvious (Zhang, *et al*., 2011, 2012).

DON removal by using Al-pillared bentonite adsorption was also studied. The method based on pillared bentonite adsorption is proved to be effective in removing DON and related substances. The maximum adsorption capacities of the bentonites vary from 7.11 to 7.61 mg/g. Kinetic studies show that the adsorption of DON on the bentonites can be described by the pseudo-second-order kinetic model, and the intra-particle diffusion controls the limiting rate. PH is found to be the key parameter affecting DON removal. Acidic conditions are favored while alkaline conditions are impeded. Furthermore, it is found that the adsorption capacity of the pillared bentonites depends on the ratio of OH^-/Al^{3+} during the pillaring process. Three-dimensional excitation–emission matrix (EEM) is also applied to assess the effect of adsorption on the removal of different DON fractions, and the results show that the tyrosine-like fractions are easier to be adsorbed (Gu, *et al*., 2010, 2011).

3.3 Formation mechanism and control of biofilms in drinking water pipelines

A biofilm is an assemblage of microbial cells that usually accumulate at a solid-liquid interface and are encased in a matrix of highly hydrated extracellular polymeric substances (EPS). Biofilm-dwelling cells have a high tolerance for stresses, biocides and host defenses, and are attributable to an enhanced risk of health threats. Bacterial biofilms also cause a multitude of industrial problems associated with corrosion and biofouling.

Drinking water distribution systems (DWDS) often face the problem of microbial contamination mainly due to biofilm formation. The presence of biofilms in DWDS can deteriorate the water quality of the distributed water, develop the bio-corrosion of the pipe as well as contribute to offensive odors. In order to control these biofilms, it is necessary to better understand the mechanisms for biofilm formation in DWDS. Quorum sensing (QS) systems have been proved to play a prominent role in the regulation of microbial biofilm formation, and inhibition of QS systems may be a promising approach in controlling biofilms in DWDS.

In its study, isolates from drinking water environments, including source water, biofilters, and finished water were taken into account by Dr. Yu's group. To address the hypothesis that AHLs play a role in mediating biofilms for the drinking water environment bacteria, each isolate was incubated in the presence and absence of four types of AHLs with final concentrations from 0.1 to 100 nM during the biofilm formation period and then the biofilm biomass amount was measured. As the results showed, the biofilm biomass did not appear to increase with increasing AHLs in some cases. However, for some strains, the supplementation of AHLs had promoted biofilm formation, and there were clear dose-effect correlations between AHLs levels and biomass in certain ranges. About 1/4 of the total strains had this positive response to the addition of AHLs, suggesting that the biofilm development of a significant number of strains in the drinking water environments was regulated by QS. For strains that had QS signals and were affected by the additional AHLs, it gave the direct evidence that AHL have impacts on the biofilm biomass, and it implied that AHL-mediated QS regulate biofilm formation for these drinking water environmental bacteria. For stains that did not have QS signals but were also affected by the additional AHLs, a possible mechanism may be that these bacteria encoded a putative AHL receptor, but not an AHL synthase, and with the addition of external synthetic AHLs, AHL-mediated biofilm formation was triggered.

In view of the role of QS in biofilm formation, the disruption of the QS systems of microbes seems to be a promising method for controlling biofilm formation. The isolates were taken into account to test the biofilm inhibition of MX and 2(5H)-furanone. MX, a series of chlorination disinfection byproducts which naturally occurs in drinking water pipelines, is a furanone compound, and its structure is similar to the AHLs. 2(5H)-furanone has already been proved to be a competitor for AHLs in some studies. Results showed that there were no significant differences for biomass with addition of 100 nM of MX and 2(5H)-furanone, respectively. The discrepancy of QS inhibitory effect of 2(5H)-furanone may be attributed to the differences in species considered and the inhibition reported previously may be species-specific. The fact of MX may be explained that biofilms in drinking water environments are not inhibited by the chlorination disinfection byproducts, though it is structure analogues of furanone.

In conclusion, AHLs play a certain role in biofilm formation for some of the drinking water environmental bacteria. It also indicates that QS may have important effects on biofilm formation in DWDS and should therefore help in developing strategies based on QS inhibition principle. Nevertheless, potential QS inhibitors, MX and 2(5H)-furanone, influenced biofilm formation negligibly in this study. For further work, more QS inhibitors and more complex multiple-species system should be applied for more drinking water bacteria (Zhang, *et al*., 2011).

Furthermore, Dr. Yu and his colleagues addressed the effect of pipe materials, nutrients and temperature on biofilm formation in simulated drinking water distribution system using the annular reactors. When biofilm formation on four different pipe material coupons were compared, the biomass was in the sequence of casting iron > copper > PVC > aluminum, the microbial growth rate was as (casting iron, copper)> PVC > aluminum; PVC was the ideal pipe material for drinking water distribution system; A variety of nutrient elements influenced the biofilm formation, and phosphorus played the greatest role; Temperature between 20°C and 30°C was preferable for the growth of biofilm and higher or lower than this range could inhibit the biofilm growth; The impact of lower temperature seemed more substantial.

Microbial process is the key step in wastewater treatment, during which the bio-available or bio-adsorbable organic and inorganic pollutants are degraded or removed. Activated sludge process, where the aerobic biofloc (ABF) acts as the biocatalyst, is most widely applied for wastewater processing. So we also pay great attention on the variations of both bacterial community and extracellular polymers in aerobic granule sludge.

To investigate the inducements of increase of cell hydrophobicity from aerobic biofloc (ABF) and granular sludge (AGS), in this study, as the first time the hydrophilic and hydrophobic bacterial communities were analyzed independently. Meanwhile, the effect of extracellular polymers (EPS) on the cell hydrophobicity is also studied. Few Bacteroidetes were detected (1.35% in ABF and 3.84% in AGS) in hydrophilic bacteria, whereas they are abundant in the hydrophobic cells (47.8% and 43% for ABF and AGS, respectively). The main species of Bacteroidetes changed from class Sphingobacteria to Flavobacteria in AGS. On the other hand, EPS is directly responsible to cell hydrophobicity. For AGS, cell hydrophobicity was sharply decreased after EPS extraction. Both quantity and property of the extracellular protein are related to hydrophobicity. Our results showed the variation of cell hydrophobicity was resulted from variations of both bacterial population and EPS (Guo, *et al.*, 2011).

4. Challenges and perspectives

In past 30 years, China is keeping a rapid development and now it is the second largest economy in the world. However, this economic booming brings Chinese people not only the improvement of living conditions and social progress but also the problems of environmental pollution. The Chinese people now have an unprecedented requirement for a sustainable environment including clean air and water. So it is expectable that Chinese environmental scientists will continue to have a golden age for rather long period of time in the future.

In fact, the research themes of IUE now will still give high priority to the future challenges for the drinking water biological safety, e.g. the sensitive and high throughput detection of waterborne pathogens, the high efficiency and low cost technologies to remove the microbial pathogens and relative contaminants from drinking water, the prevention of the formation of genotoxic materials from microbial related contaminants, the effective approach to prevent the formation of biofilm in distribution system. Furthermore, emerging pollutants will be found continuously with the development of S&T instruments and their introduction into the aquatic environment from other environment. Novel problems will pose new challenges, too. The scientists in IUE will join with their colleagues in other CAS institutes to keep on their studies and endeavor to find more solutions.

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References

Gu, L., *et al*., 2010. Dissolved Organic Nitrogen (DON) in the processes of polluted source water treatment. *Chinese Sci. Bull*.: 59, 3098–3101.

Gu, L., *et al*., 2011. Dissolved Organic Nitrogen (DON) adsorption by using Al-pillared bentonite. *Desalination*: 269(1-3), 206–213.

Guo, F., *et al*., 2011.Variations of both bacterial community and extracellular polymers from aerobic biofloc to granule: The inducement of increase of cell hydrophobicity. *Bioresource Technol*.: 102, 6421–6428.

Liu, B., *et al*., 2012. DON profile during backwashing cycle of drinking water biofilter. *Sci. Total Environ*.:414, 508–514.

Zhang, H., *et al*., 2011. The effect of biological pretreatment on dissolved organic nitrogen removal in downstream coagulation and the countermeasure. *Fresen. Environ. Bull*.: 20(7), 1–7.

Zhang, H., *et al*., 2012. Pre-oxidation advances dissolved organic nitrogen removal in downstream coagulation. *Fresen. Environ. Bull*. (accepted).

Zhang, S., *et al*., 2011. Effect of interspecies quorum sensing on the formation of aerobic granular sludge. *Water Sci. Technol*.: 64(6), 1284–1290.

Zhang, S., *et al*., 2010. Development of DNA extraction protocol from biofilm attached to biological activated carbon (BAC) granules. *Front. Environ. Sci. Eng. China*: 4(4), 459-465.