Applied Mechanics and Materials
ISSN: 1662-7482, Vols. 522-524, pp 445-449
doi:10.4028/www.scientific.net/AMM.522-524.445

Online: 2014-02-06

Isolation and identification of main microorganisms in different water systems in northern Hangzhou, China

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Keywords: Hangzhou; River; Microorganism; Canonical Correlation Analysis(CCA)

Abstract. Microorganisms in aquatic ecosystems play an important role in the processes like water purification, nutrition regeneration and biochemical circulation. Some microorganisms are also related to human diseases. To analyze the main microorganisms in the water systems in northern Hangzhou, water was sampled from three different rivers for isolation and identification of microorganisms. The highest diversity and quantity of bacterium was found in the rivers in Banshan industrial district, with an average 127 clones in each plate. For the Beijing-Hangzhou Grand Canal and Longshan reservoir, the number was 20 and 7, respectively. Similar trends were also observed for actinomycetes and molds. CCA analysis showed that the microorganism abundance was mostly related to the NO_3-N, TP and TN values of water. Totally, 7 bacterium strains were identified, including *Bacillus* cereus, *Escherichia* coli., *Klebsiella* pneumoniae, and so on, which belong to 7 different genera. In an antibiotics sensitivity analysis, all of the 7 bacteria showed sensitivity to tetracycline.

Introduction

Microbial communities in water systems were important for water purification and ecological balance，because they play an important role in such processes as particulate carbon flux, nutrient regeneration and biochemical cycling[1-4]. Some microorganisms cause certain diseases in human. On the other hand, microorganisms in water could help us learning the instant environmental changing in the water systems, because microbial communities are generally sensitive to the environmental changing[5].Although some reports on investigation of microbial communities in some water systems are available now, only certain regions have been involved. The methods and techniques applied in these studies are also different[5-10]. Especially, researches on civil rivers inside cities are still not enough. Most cities in China experienced rapid economic development and city expansion in the past decades. While, it was rarely discussed how human activities affected the microbial community in the rivers inside downtown district.

Hangzhou located in the downstream of Qiantang River and belongs to the Yngtze River Delta region in China at the same time. Hangzhou was famous for its complex water systems. About 8% of the city area was covered by water including lake, river, canal, brook, west land and so on.

Northern Hangzhou is a special district, where located a lot of water systems, including Beijing-hangzhou canal, Shangtang river, reservoir, and so on. These colorful water sources provided convenient conditions for aquatic microbial community study. Analysis of water characteristics and microbial communities in these different water systems could help us learn the ecological changing and how human activities are affecting the microorganisms.

Materials and methods

Water samples collection. As shown in figure 1, water was sampled from three different rivers in northern Hangzhou during Junly, 2012. The northernmost site LS(N30°21.490′E120°11.738′) located in a artificial reservoir, Longshan reservoir. The middle site BS(N30°21.243′E120°10.022′) located in a river nearby steel plants and cement plants. The southmost site YH(N30°19.898′E120°08.309′) located in the famous Beijing-Hangzhou Grand Canal nearby residential area. Locations were determined using global positioning system (GPS). Water was sampled according to *Environmental quality standards for surface water of China* (GB3838-2002) [11].

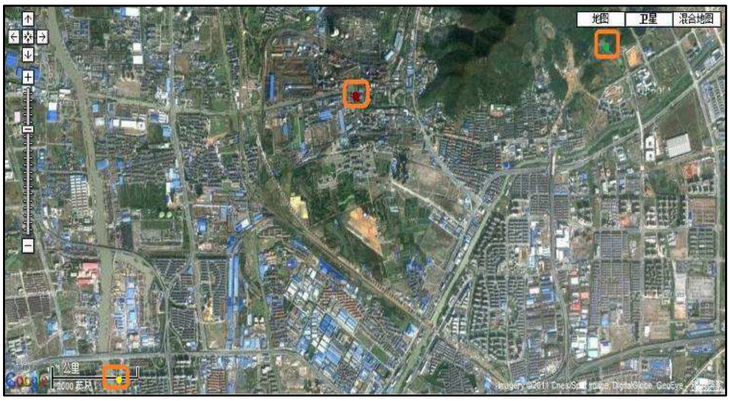

Fig. 1 Location of water sampling sites

Analysis of the physical and chemical characteristics of the water. Physical and chemical characteristics including temperature, pH, COD, NH3-N, Total nitrogen(TN), total phosphorus(TP), NO3-N, BOD, dissolved oxygen(DO), heavy metals was analyzed according Drinking water standard test method of China(GB/T5750) [12].

Isolation, culture and identification of microorganisms. The water sample was diluted serially(1, 10^{-1}, 10^{-2}) and inoculated into Beef extract peptone medium, Gauze's No1 medium, and czapek's medium respectively for culture of bacteria, actinomycete and filamentous fungi correspondingly. The culture of the microorganisms maintained for 3 to 6 days according to the different species. The microorganisms were determined by colony observation, microscopic observation, instrument determination. In colony observation, the colony morphology was recorded and counted. Finally the microorganisms were identified using microorganism identification system (BIOLOG Gen Ⅲ Microseation).

Correlation analysis. Canonical correspondence analysis (CCA) (CANOCO 4.5; Biometris, Wageningen, the Netherlands) was performed on a statistical analysis of the microbial community abundance versus the physical and chemical characteristics of the water.

Sensitivity of the dominant microorganisms to antibiotics. Some microorganisms in river systems might cause infectious diseases in human. To determine whether the dominant bacteria keep sensitive to antibiotics, the sensitivity to tetracycline was evaluated using spectrophotometric method according the design reported before with a little changing[13]. Briefly, the dominant bacteria were cultured in beef extract peptone medium to OD_{600}=0.1, different concentrations of tetracycline（final concentration of 0mg/L, 0.5mg/L，1 mg/L，5 mg/L, respectively）were added respectively for culture at 37°C with 150rpm shaking. OD_{600} Value was checked at 2h intervals for evaluation.

Results

Physical and chemical characteristics of the water. The water characteristics of water were shown in Table 1. The water on BS site showed the highest value of temperature, pH, COD, BOD and the lowest DO value. The water on YH site showed a middle level of temperature, COD, BOD, DO value, and the lowest value of pH and NO3-N. The water on LS site showed the lowest value of temperature, COD, NH3-N, TN, TP, BOD, and the highest NO3-N value.

As to the metals, Al, Fe and Pb were found in all of the three sites, and the concentrations of them were obviously higher than other metals. Totally, the concentrations of most metals in LS site were lower than those in BS and YH sites. Based on a comparison between the three sites, the BS site showed high concentration of Ni, Fe, Li, Al, As and Mn. The YH site showed high concentration of Cu, Pb and Cd. The LS site showed high concentration of Cr.

Table 1 The physical and chemical parameters including metal content in the water

	BS site	YH site	LS site
Temperature(℃)	30.2(±0.19)	29.1(±0.15)	28.9(±0.13)
pH	7.99(±0.04)	7.12(±0.01)	7.72(±0.12)
COD(mg/L)	347.20(±10.67)	23.47(±1.12)	7.60(±0.95)
NH_3-N(mg/L)	2.27(±0.17)	1.43(±0.09)	0.38(±0.04)
TN(mg/L)	2.94(±0.03)	1.76(±0.03)	0.66(±0.03)
TP(mg/L)	0.06(±0.00)	0.03(±0.01)	0.03(±0.01)
NO_3-N(mg/L)	0.07(±0.03)	0.01(±0.01)	0.11(±0.03)
BOD(mg/L)	28.30(±0.90)	3.67(±0.12)	1.53(±0.06)
DO(mg/L)	1.51(±0.17)	2.87(±0.05)	6.51(±0.17)
Cu(mol/L)	0.021(±0.003)	0.044(±0.005)	0.010(±0.002)
Ni(mol/L)	0.003(±0.000)	0.001(±0.000)	0.000(±0.000)
Cr(mol/L)	0.004(±0.000)	0.008(±0.001)	0.023(±0.004)
Cd(mol/L)	0.002(±0.000)	0.004(±0.000)	0.000(±0.000)
Pb(mol/L)	0.108(±0.010)	0.128(±0.012)	0.123(±0.008)
Fe(mol/L)	0.716(±0.015)	0.315(±0.024)	0.355(±0.027)
Li(mol/L)	0.021(±0.007)	0.010(±0.002)	0.009(±0.001)
Al(mol/L)	1.386(±0.051)	0.536(±0.028)	0.366(±0.030)
As(mol/L)	0.022(±0.002)	0.003(±0.000)	0.004(±0.000)
Mn(mol/L)	0.021(±0.001)	0.013(±0.002)	0.010(±0.001)

Culture and identification of microorganisms.

Comparison of the microorganism quantity. After inoculation and culture, the quantity of microorganism colony in the plates was calculated. A general trend was observed that BS site showed highest quantity, followed by YH and LS site in order. For example, after inoculation using water sample without dilution, the BS, YH and LS sites caused 127, 20 and 7 bacterial colonies per plate(mean value), respectively. As to actinomycetes, after inoculation using water sample with a 10^{-2} dilution, the BS, YH and LS sites caused 10, 9 and 2 colonies per plate(mean value), respectively. As to mould, after inoculation using water sample with a 10^{-1} dilution, the BS, YH and LS site caused 12, 5 and 2 colonies per plate (mean value), respectively.

Identification of dominant microorganisms. For BS site, based on the quantity, the dominant bacterium was *Bacillus* cereus, *Escherichia* coli., *Klebsiella* pneumoniae, *Sphaerotilus* natans, *Crenothrix* polyspora and Serratiamarcescens in order. For YH site, the dominant bacterium was *Klebsiella* pneumoniae, *Bacillus* cereus, *Escherichia* coli. and Serratiamarcescens in order. For LS site, the dominant bacterium was *Escherichia* coli.,*Bacillus* cereus and *Pseudomonas* fluorescens in order. These bacteria fall into 7 different genus. *Escherichia* coli. and *Bacillus* cereus were found in all of the 3 sites. *Klebsiella* pneumoniae were found in BS and YH sites. Also, *Klebsiella* pneumoniae, *Escherichia* coli. and *Bacillus* cereus were thought to be pathogenic to human. In addition, 3 kinds of moulds (*Rhizopus* nigricans, *Aspergillus* terreus and *Aspergillus* clavatus) were also identified.

Correlation analysis between microorganism abundance and water characteristics. The correlation between microbial diversity and 8 water indexes(Temperature, pH, COD, BOD, NH_3-N, TN, TP and NO_3-N) were analyzed using CAA mehod. The results were shown in Fig. 2. The microbial diversity in the rivers in northern Hangzhou showed to be mostly related to NO_3-N, TP and

TN value. Among these factors, TN and TP are positively correlated to the first axle, indicating the microbial abundance is in proportion to these factors. NO_3-N is negatively correlated to the microbial abundance, indicating the microbial abundance is inversely proportional to NO_3-N value. The microbial abundance is less connected to temperature and pH of the water.

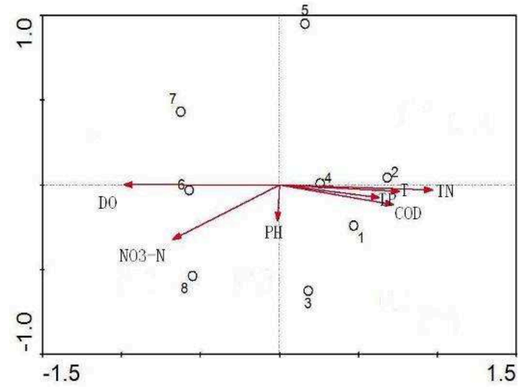

Fig. 2 Canonical Correlation Analysis between microorganism abundance and environmental factors

The sensitivity of dominant bacteria to tetracycline. The OD_{600} value after 5h of culture was shown in Table 2. The 7 bacterium strains selected here all showed growth inhibition by tetracycline. The OD_{600} value of the treated groups is lower than the control group($p<0.05$). And a positive correlation between the dose and the inhibition was also observed.

Discussion

Aquatic microorganisms are important members in the aquatic ecology. The abundance of microorganisms is closely related to the water characteristics. In the biogeochemical cycling process, microorganisms take part in degradation of organic matter (including biological body) into inorganic salts (phosphate, nitrate), CO_2 and so on, which could be used by plants. Some bacteria work as the most important contributors in the water purification process and in most wastewater biochemical treatment process. On the other hand, excessive proliferation of certain bacteria might cause water pollution and disease outbreak in human.

A variety of new methods for microbial community analysis have been established now, for example, PCR-DGGE, PCR-SSCP and T-RFLP. Most of these methods are based on molecular identification technology, which could identify microorganisms at DNA level[10,14]. But it is difficult for these technologies to decide the living or death of the microorganisms or the migration capacity of them. The traditional microorganism isolation and culture technology could indicate the living capacity of microorganisms and could provide living material for further research.

The results in this study indicated that the diversity and quantity of microorganisms are abundant in the rivers in northern Hangzhou. More microorganisms were found in the rivers nearby industrial district and human living district. The distribution of microorganisms is closely related to the fertility of water. Totally 7 species of dominant bacteria were identified, at least 3 of them had been confirmed to cause diseases in human. For example, *Escherichia* coli. in some cases can cause peritonitis and diarrhea. *Klebsiella* pneumoniae could enter the upper respiratory tract and intestinal tract of human, and cause acute pneumonia. *Bacillus* cereus exist widely in the environment and in some cases can cause food poisoning[15]. *Serratia* marcescens exist widely in the environment, which could also be regarded as conditional pathogenic bacteria. The other bacteria found in this study mostly are important for water purification and nutrient recycling in the aquatic ecology. *Sphaerotilus* natans could use many simple organic compounds(alcohols, organic acids and carbohydrates) as carbon or energy source. It could transform the soluble Fe^{2+} into insoluble Fe^{3+}, which could accumulate in outer sheath of cells. *Pseudomonas* fluorescens could degrade various synthetic compounds, so it is widely used for environment protection.

Acknowledgements

This work was financially supported by Zhejiang province-famous university cooperation project (2012E80002), Zhejiang Provincial Natural Science Foundation of China (Y5110135, Y5110130 and LY12C03009)and Zhejiang Shuren University foundations (2013A21005 and SH2013S05).

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