



# Microbial Community and Urban Water Quality

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**Abstract** Urbanization of China is substantial and growing, and water resources are crucial for both economic and social sustainable development. Unfortunately, the frequency and intensity of water contamination events are increasing at an unprecedented rate and often accompanied by increased pollutant loading due to human activities such as irreversible industrialization and urbanization. The impacts of human pollution are most evident and of greatest concern at the microbial level. The research of the Aquatic Ecohealth Group, Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, has been focusing mainly on aquatic microorganisms in the urban environment, from drinking water and landscape water to waste water. Its projects fall into three categories: biomonitoring and bioassessment, microbial ecology and diversity, ecotoxicology and environmental microbiology. Its scientific topics include the aquatic ecological safety and microbial food web.

**Keywords** freshwater ecology, biomonitoring and bioassessment, microbial ecology and diversity, ecotoxicology and environmental microbiology



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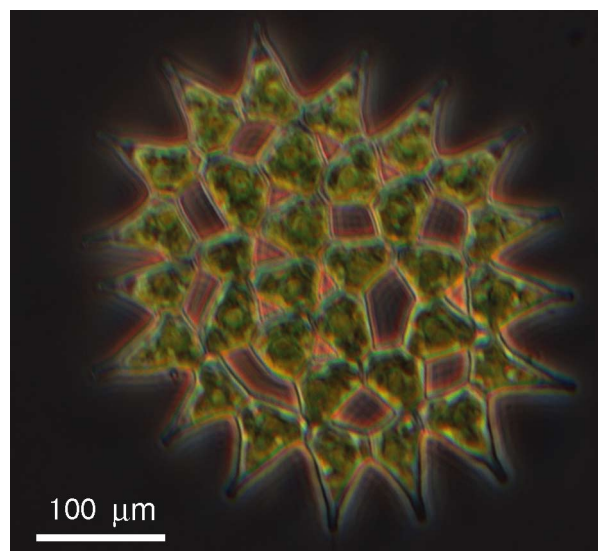
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Water resources are crucial for both economic and social sustainable development because they are used not only for agriculture, aquaculture, industry and recreation, but also for drinking water supply. Globally, safe and aesthetically acceptable water is a critical need in a modern city, and the eutrophication and pollution related water quality impairment has very substantial negative economic effects in developing and developed countries. Microorganisms, including bacteria, algae, protozoa and small metazoa, are ubiquitous and abundant components of aquatic ecosystems and play essential roles in the biogeochemical processes because they constitute the base of food webs and their changes in composition and structure can lead to profound changes at high trophic levels. As single-celled organisms with a short regeneration time, microorganisms respond more rapidly in terms of community structure and density to physical, chemical, and biological changes than larger animals and plants. Certainly, water quality is closely related to microbial community in the aquatic ecosystems.

Over the last 30 years China urbanization has experienced dramatic growth, and it has had not only positive environmental impacts but also created more environmental challenges. The degradation of water quality associated with excess levels of pollutants in aquatic ecosystems is one of the most serious and challenging environmental problems. Water contamination events can affect the urban water supply safety, threaten the aquatic

ecosystem sustainability, and endanger the long-term human health and wellbeing. The conflict between urban environment and sustainable development is becoming ever more prominent during the process of China's urbanization. The Aquatic Ecohealth Group strives to understand the relationship between microbial community and urban water quality, a multidisciplinary research topic spanning various fields, ranging from biomonitoring and bioassessment, to microbial ecology and diversity, and to ecotoxicology and environmental microbiology.



A light microscope photograph of *Pediastrum simplex* (a common green alga in freshwater lakes and reservoirs).

## 1. Algae community and eutrophication of subtropical reservoirs in Fujian

Currently, China is the greatest dam building country in the world and has more than 87,000 reservoirs with a total water storage capacity of 706 billion m<sup>3</sup>. Ideally, the reservoir water resources should be managed properly to fulfill the needs of human beings. Reservoirs in southeast China's Fujian Province are important water resources for urban sustainable development, although few have been studied so far. In recent years, growing population and increasing demands for water shifted the focus of many reservoirs from flood control and irrigation water to drinking water. However, cultural or anthropogenic eutrophication has become the primary water quality issue for many reservoirs due to high input of nitrogen (N) and phosphorus (P) from domestic sources, and extensive

agricultural and industrial activities. This eutrophication or algae related water quality impairment has very substantial negative economic effects, thus trophic state assessment has been rapidly gaining increased attention from the public and government globally. Understanding of typical characteristics of reservoir eutrophication in Fujian and potential factors that drive the community of reservoir algae is essential in developing effective water quality management approaches. Therefore comprehensive and regular monitoring programs of water quality for the reservoir's effective management are urgently needed.

Our study suggested that southeast Fujian reservoirs were diverse and complex with respect to algae assemblages. One hundred and twenty-nine taxa belonging to eight phyla (*i.e.*



Bacillariophyta, Chlorophyta, Chrysophyta, Cryptophyta, Cyanophyta, Euglenophyta, Pyrrophyta, Xanthophyta) were observed and the most diverse groups were Chlorophyta (52 taxa), Cyanophyta (20 taxa), Euglenophyta (17 taxa), Chrysophyta (14 taxa). The dominant groups were Chlorophyta (40.58%), Cyanophyta (22.91%), Bacillariophyta (21.61%), Chrysophyta (6.91%). Algae communities were structurally different between study reservoirs. The species richness, abundance, diversity, and evenness of algae varied significantly between reservoirs. These differences indicated a regional effect, which was related to trophic state, environmental factors, and human influences.

Unfortunately, neither oligotrophic nor mesotrophic reservoirs were found, and all investigated reservoirs were eutrophicated based on the TSIc values, thus our results provided an early warning of water degradation in Fujian reservoirs. Shallow reservoirs generally have higher values of trophic state index (TSI) and appear to be more susceptible to anthropogenic disturbance than deeper reservoirs. Trophic state is not the same thing as water quality, but trophic state determination certainly is an important aspect of water quality assessment. TSI results indicated that all 11 reservoirs were eutrophic, three of them were hypereutrophic, six were middle eutrophic, and two were light eutrophic. The comprehensive trophic state index (TSIc) was not correlated to taxa richness and abundance but significant correlations to Shannon-Wiener diversity and Pielou's evenness were found.

Our results also illustrated that temperature, transparency, conductivity, dissolved oxygen (DO), total carbon (TC),  $\text{NH}_4\text{-N}$ ,  $\text{NO}_x\text{-N}$ , total phosphorus (TP), and chlorophyll a were significant environmental variables affecting the distribution of algae communities. Furthermore, the trophic state plays an important role in shaping community structure and in determining species diversity of algae. Our TSIc was positively correlated with nitrogen, phosphorus and Chl a.

Freshwater eutrophication is likely to intensify in the coming decades due to rapid increase in human population, demand for more food, land conversion, and fertilizer use. For different purposes, therefore, the integrated monitoring and assessment methods of surface water quality for reservoirs are needed to support various decision making processes in local government agencies. Eutrophication information on the Fujian's water resources is critical to ensuring long-term availability of water that is safe for drinking and recreation. Algae species composition may be an important facet of water quality for the users and consumers because some species can impair the quality of drinking water. Further, the degradation of water quality associated with excess levels of nitrogen and phosphorus in Fujian reservoirs may be impacted by interactions among agriculture and urban factors. A watershed-based management strategy, especially phosphorus control, should be developed for drinking water source protection and sustainable reservoirs in the future.

The transparency and chlorophyll a were the strongest environmental factors in explaining the algae community data. The chlorophyll a itself was related to the abundance or frequency of Euglenophyta (hyper-eutrophication indicators). Accordingly, it might be recommended that regular biological monitoring should concentrate on the abundance, species richness and species diversity of Euglenophyta and Cyanophyta (bloom-forming species), rather than the whole algal flora; and that regular chemical monitoring should concentrate on TN, TP and Chl a. This limited number of variables seems to contain all the information necessary to establish the eutrophication status of these Fujian reservoirs. Therefore, long-term and regular monitoring of Euglenophyta, Cyanophyta, TN, TP and chlorophyll a in reservoirs is urgently needed to further understand the future trend of eutrophication and to develop a watershed-based strategy to manage the Cyanophyta bloom hazards.

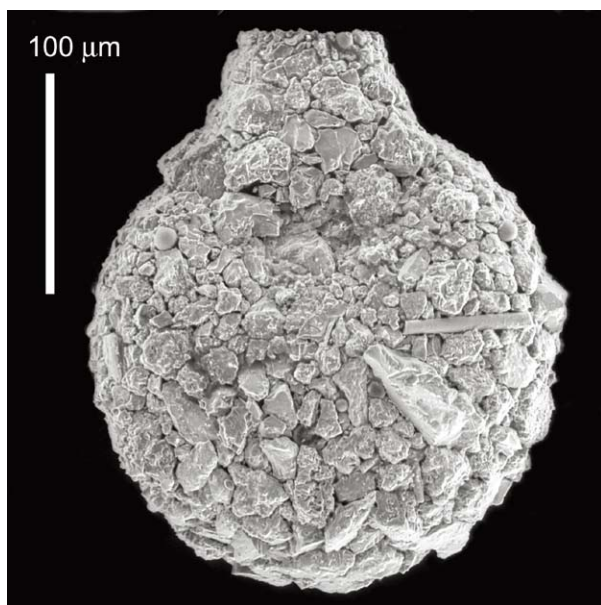
## 2. Microbial ecology and diversity in a subtropical river (Jiulong River)

It is important to investigate changes in microbial community for assessing aquatic ecosystem health. In the past few decades, we have developed a good knowledge of the temporal and spatial patterns of microbial abundance and production in some coastal regions, oceans and

temperate lakes. On the other hand, studies focused on patterns of microbial community composition in large subtropical river (hundreds of kilometers) are far less common. In this study, we investigated microbial planktonic communities by using denaturing gradient gel

electrophoresis (DGGE) analysis from a subtropical river (Jiulong River, southeast China), and their relationships with local environmental factors were studied. The Jiulong River, the second largest river in Fujian Province, southeast China, is an important water source for drinking, agricultural and industrial use. Its watershed is home to about nine millions people and characterized by intense agricultural activities. Unfortunately, the water quality problems, caused by agricultural pollution (the excess input of nitrogen and phosphorus), have deteriorated dramatically and rapidly in the upper Jiulong River over the past decades. Especially, in the dry season (*e.g.* winter), most nutrients were concentrated due to the negative balance between precipitation and evaporation, which had a detrimental impact on the water quality. In 2010, we investigated the genetic diversity pattern of microbial communities in the Jiulong River, determined the impact of environmental factors, particularly agricultural pollution and saltwater intrusion, on microbial communities and provided existing information for biomonitoring activities based on community-level molecular fingerprinting techniques.

The Betaproteobacteria (26%) were the most dominant taxa in prokaryotic clones derived from DGGE bands. The high detection frequency of these Betaproteobacterial phylotypes suggested that they were an important component of the prokaryotic community



A scanning electron microscope photograph of *Zivkovicia compressa* (a common testate amoeba in freshwater habitats).

and widely distributed in the Jiulong River. Seven eukaryotic 18S rDNA phylotypes (26%) identified as belonging to the Dinophyceae were detected frequently in the Jiulong River. In recent years harmful algal blooms have increased globally, and most of the known toxic or harmful algal species are dinoflagellates. A record dinoflagellate bloom lasted for one and half months from late January to March in 2009 in the upper middle Jiulong River. The bloom event led to a strong reduction in water quality and threatened the water supply for both Xiamen and Zhangzhou cities. Notably, one of our dinoflagellate sequences obtained from estuarine sites showed the highest similarity to that of dinoflagellate *Heterocapsa triquetra*. *H. triquetra* is non-toxic, but it significantly contributes to the total phytoplankton biomass and may be harmful to the ecosystems. Bloom of *H. triquetra* associated with *A. tamarensis* frequently occurred in the eastern coast of USA and Hong Kong waters. Therefore, it is urgent to improve the water management and to prevent the potential harmful algal blooms in the Jiulong River. The concentrations of all nitrogen and phosphorus in the upper stream sites were significant higher than those of middle-lower stream sites and estuarine sites due to agricultural pollution. Notably, the concentrations of TN at all sites and TP at six sites exceeded  $1.0 \text{ mg L}^{-1}$  and  $0.2 \text{ mg L}^{-1}$ , respectively, which are the standard limit for safe drinking water (Environmental Quality Standards for Surface Water, China GB3838-2002). PCA of 13 environmental parameters provides a clear distinction between three groups of sites. Group I was characterized by microbial communities from highly agricultural pollution sites. Group II was characterized by communities from less polluted sites. Group III consisted of 3 estuarine sites with high levels of conductivity and salinity. Further, the variation of microbial community closely related with the environmental factors because both cluster and ordination analyses of prokaryotic and eukaryotic DGGE fingerprinting resulted in the same three groups from the 15 sites. Group I was composed of 3 sites in the upper Jiulong River, Group II included 9 sites in the middle-lower Jiulong River, and Group III consisted of 3 estuarine sites. It appears that agricultural pollution (phosphorus and nitrogen) and saltwater intrusion (conductivity and salinity) maybe account for this pattern in genetic structure of both prokaryotic and eukaryotic communities. To distinguish the relative





importance of agricultural pollution (TN, NH<sub>4</sub>-N, NO<sub>x</sub>-N, TP and PO<sub>4</sub>-P) and saltwater intrusion (conductivity and salinity) in determining community structure of prokaryotes or eukaryotes, variation partitioning was performed. The results of variation partitioning revealed that agricultural pollution (phosphorus and nitrogen) and saltwater intrusion (conductivity and salinity) were the main factors impacting microbial community composition, by explaining more than two-thirds of the total variation in both prokaryotic (67.0%) and eukaryotic (70.5%) communities. The relative contribution of agricultural pollution factors was considerably larger than that of saltwater intrusion factors in both communities. For prokaryotic communities, the pure variance explained by agricultural pollution (39.0%) was higher than that explained by pure saltwater intrusion (19.0%). Similarly, for eukaryotic communities the pure variance explained by agricultural pollution (44.3%) was substantially higher than that explained by pure saltwater intrusion (14.6%).

In conclusion, our data indicate that great variations in environmental conditions of the Jiulong River throughout space resulted in different microbial communities. The agricultural activities in the upper Jiulong River Watershed are the major sources of nitrogen and phosphorus due to heavy chemical fertilizer application and intensive livestock production. The genetic structure of both prokaryotic and eukaryotic microbial communities changed significantly from the upper Jiulong River sites to the estuarine sites. These changes were closely related to the agricultural pollution and saltwater intrusion. Somewhat surprising was the finding that the genetic diversity patterns were similar between prokaryotic and eukaryotic communities. Moreover, the robust and quantifiable relationship between DGGE results and environmental variables indicated that the community-level molecular fingerprinting techniques could support the physicochemical assessment of riverine water quality and ecosystem health.

### 3. Microbial ecotoxicology: Arsenate toxicity in a freshwater protozoan

Nowadays, water pollution is a part of our life which heavily destroys the aquatic ecosystem and threatens our health. Toxicity tests are an important component for assessing the impact of chemicals on aquatic ecosystems. Groups of selected biological organisms are employed to determine the potential adverse effects of different kinds of pollutants, such as heavy metals, persistent organic pollutants (POPs) and microcystins, *etc.* Meanwhile, biological remediation is also considered as one of the most potential effective approaches for the recovery of polluted aquatic environment. Currently, one of our research interests is studying the arsenic (As) toxicity and metabolisms in a freshwater protozoan, *Tetrahymena pyriformis*. Arsenic (As) is a ubiquitous and potentially toxic element in the environment. Chronic exposure to arsenic through contaminated drinking water occurs worldwide, and is associated with a variety of diseases, including cancer, diabetes and developmental disorders. In natural waters, As exists predominantly as the pentavalent state, As (V). Its mobility and bioavailability is influenced not only by abiotic factors, but also by the activities of aquatic plants, animals and microbes. Recently, bioremediation to clean up arsenic-contaminated environments has been widely

hailed as environmentally friendly and cost effective, as compared to the physico-chemical methods. In addition to the popular phyto-remediation method, aquatic microorganisms are considered to be reliable candidates for arsenic bioremediation in waters.

Understanding the responses of varied microorganisms to arsenic will be an important knowledge base for developing efficient and selective bioremediation approaches. Arsenic metabolism in microbes, such as archaea, bacteria, fungi and algae, has been extensively studied in the past. Microbes respond to arsenic in a variety of different ways, such as chelation, compartmentalization, exclusion, and immobilization. Most of them can methylate arsenic giving rise to monomethyl, dimethyl, and/or trimethyl derivatives, which are volatile and are rapidly released to the atmosphere. However, it is a pity that one of the dominant players in global aquatic ecosystems, protozoa, was neglected in the past. So far, little is known about arsenic metabolism in aquatic protozoan cells.

The unicellular protozoan is one of the largest and most complex of microorganisms in aquatic ecosystems, following bacteria and all other smaller microbes. As dominant bacterial grazers in microbial food webs, they

influence to a great extent the bacterial community size and population structure, and play important roles in the biogeochemical cycles of elements, such as carbon, nitrogen, and sulfur, *etc.*. Moreover, as unicellular organisms with a short regeneration time, protozoa respond rapidly with great sensitivity to the presence of pollutants in nature. This has resulted in them being used as test systems for assessing ecological risk.

In natural waters, the widespread presence of arsenic has forced most organisms, from bacteria to mammals, to develop various strategies to counter-act arsenic toxicity or to utilize arsenic as an electron donor/receptor in energy production. Considering the important ecological status of protozoan in aquatic system, protozoan may have also developed certain approaches to respond to the widespread existence of arsenic. On this basis, a ciliated model protozoan *Tetrahymena pyriformis* was employed as one case to study the arsenic metabolism. Our aim is to evaluate the toxic effects of the As (V) to *T. pyriformis*, and explore the potential arsenic detoxification mechanisms in *T. pyriformis* culture, which may have hopes to be applied in technology for bioremediation of arsenic-contaminated localities.

The most easily recognized symptom of arsenate toxicity was the growth inhibition of *T. pyriformis*. The 18-hour EC50 arsenate concentration, causing a 50% decrease in growth rate of *T. pyriformis*, was calculated to be *ca.* 40  $\mu\text{M}$ . Under the treatment with 40  $\mu\text{M}$  arsenate, the cell mobility of *T. pyriformis* was slowed down significantly. Meanwhile, at above 18 h of exposure, significant morphological changes were found in most of the treated cells. The cell body became a little shorter and stouter, and the cell surface became riddled with a number of depressions ranging in diameter from 0.3-0.5  $\mu\text{m}$ . However, interestingly we found that *T. pyriformis* could still grow reasonably well in a medium containing up to 30  $\mu\text{M}$  of arsenate, revealing this protozoan has some capacity to mitigate against arsenic toxicity. Arsenic speciation analysis by using HPLC ICP-MS showed that

most intracellular As (V) of *T. pyriformis* was transformed to As (III), and the methylated derivatives of MMA (V) and DMA (V). The transformation rate reached as high as 94% and 98% in the cells under treatments with 0.67 and 40  $\mu\text{M}$  of As (V), respectively. Among them, the most abundant species were DMA (V) and MMA (V). As the toxicity of As (V) is much higher than DMA (V) and MMA (V), we consider that oxidation/reduction and methylation is likely one of the major detoxification pathways in *T. pyriformis*. Moreover, we found that both As (III) and DMA (V) could be continuously excreted from the cell, effectively reducing the arsenic cytotoxicity. Finally, comparative proteomics was employed to investigate the stress responses of *T. pyriformis* to arsenate exposure, and unveiled significant changes in the expression of multiple proteins involved in anti-oxidation, sugar and energy metabolism, proteolysis, and signal transduction.

It revealed multiple pathways of arsenate detoxification in *T. pyriformis*. Arsenic oxidation/reduction and biomethylation is the major detoxification pathway. Also, the effective efflux for As (III) and DMA can reduce the arsenic cytotoxicity. In addition, the following pathways were considered as the potential detoxification approaches, such as: 1) enhancing the scavenging activity of free radicals to prevent oxidative damages; 2) employing the ubiquitin-dependent proteolysis to avoid accumulation of misfolded or damaged polypeptides that are toxic to cells; 3) raising the sugar metabolic efficiency to supply more energy for arsenic extrusion.

Because protozoan is abundant and widespread in global aquatic environment, if the arsenic detoxifying process of *T. pyriformis* is not just a special case, but commonly exists among other protozoan, the protozoan-mediated transformation is proposed to have an important impact on the global arsenic cycle. Also, the high efficient biotransformation of arsenic by *T. pyriformis* provides baseline information for further explorations regarding the exploitation of protozoa for arsenic removal in contaminated water.

#### 4. Environmental microbiology: Nitrogen and phosphorous removal by microalgae

Human activities, particularly urbanization, have increased the entry of chemical and biological contaminants into the water systems. It is easy to cause

eutrophication when the high concentrations of nitrogen and phosphorus released into water system. So these effluents should be treated properly to reduce contaminants



to environment. Special attention has been focused on nitrogen and phosphorus removal from wastewater using biological, physical and chemical methods. Conventional treatment of wastewater goes through primary physical and chemical methods, secondary biological process, removing only a fraction of nutrient in the wastewater. Some harmful material are not effectively eliminated as the conventional technology of treatment used in wastewater treatment plants. More often, the effluent from the wastewater treatment plant fail to meet with the effluent standards set by the local government. Microalgae can be used for treatment of wastewater due to their capacity to assimilate nutrients including nitrogen and phosphorus. Moreover, these inorganic nutrients are suitable and cost-effective for microalgae cultivation. Algae are important bioremediation agents, and are already being used by many wastewater facilities. Microalgae treatment of wastewater does not generate additional pollution which can offer a more ecologically safer, cheaper and efficient means to remove nutrients than conventional methods. Moreover, microalgae cultures offer an interesting alternative for wastewater treatment because they provide a biotreatment coupled with the production of potentially valuable biomass and reduction of greenhouse gas emission. The major disadvantages associated with current wastewater treatment practices are: 1) Many wastewater treatment processes generate large amounts of biological waste sludge that must be sent off-site for disposal. Handling and disposal of this sludge is typically the largest single cost component in the operation of a wastewater treatment plant. 2) Most wastewater treatment processes cannot effectively respond to diurnal, seasonal, or long-term variations in the composition of wastewater. A treatment process that may be effective in treating wastewater during one time of the year may not be as effective at treating wastewater during another time of the year. 3) High energy requirements will make many wastewater treatment methods unsuitable for low per-capita energy consumption countries. 4) High operation and maintenance requirements, including production of large volumes of sludge, make them economically unviable for many regions.

To use microalgae for wastewater treatment is not a new idea, and many researchers have developed techniques for exploiting the fast growth and nutrient removal capacity. The term microalgae refers to all algae too small to be seen properly without microscope, and often

includes both eukaryotic microalgae and the prokaryotic cyanobacteria. The most important common feature of all eukaryotic microalgae and cyanobacteria is that they have oxygen-evolving photosynthesis and that they use inorganic nutrients and carbon. Microalgal biomass can be used for hydrogen gas production, bioenergy conversion and production of pharmaceutical substances or food. During the U.S. Department of Energy's Aquatic Species Programme (ASP), it was found that for the algae remediation of wastewater, energy outputs were twice the energy inputs, based on digester gas production and requirements for pumping the wastewater, mixing the ponds, etc. The overall economics were very favorable because of the wastewater treatment credits. In Virginia (USA), researchers at Old Dominion University have successfully piloted a project to produce biodiesel feedstock by growing algae at municipal wastewater treatment plants.

To construct algae based wastewater treatment system, it is essential to consider both wastewater treatment as well as algal cultivation. Cell retention time, nutrient addition rate, water depth, and degree of mixing are the common parameters consider for growth of algae. In addition to these parameters BOD (biochemical oxygen demand) reduction, TDS (total dissolved solids) reduction, pH, nitrogen removal rate and phosphorus removal rate are commonly considered for wastewater treatment. Hence the system should be designed accordingly to allow the growth of algae as well as wastewater treatment. Algae-based wastewater treatment technology is suited for tropical countries where the temperature is warmer and sunlight is optimum. Environmental factors play a major role in algae cultivation. Maintenance of optimum temperature and lighting in algae ponds are difficult. Apart from these environmental factors, there are a number of biological problems and operational problems can arise in the mass cultivation of microalgae using wastewater. These issues include contamination and grazing. Control measures for avoiding contamination by bacteria and other algal species are sterilization and ultra-filtration of the culture medium. Grazing by protozoans and diseases like fungi can eventually be treated chemically.

In our laboratory, a freshwater unicellular microalgae *Chlorella* sp. was used for removing nitrogen and phosphorus from influent and effluent municipal wastewater which were diluted to four different proportions, namely,

100%, 75%, 50% and 25%. The growth of *Chlorella* sp. in different proportions wastewaters was monitored over a period of 24 days. The level of wastewater proportions greatly influenced algal growth. Interestingly, *Chlorella* sp. grew fastest in 50% wastewater both in influent and effluent wastewater. *Chlorella* sp. showed a fairly high nutrient removal efficiency. The removal efficiencies of nitrogen (TN, NH<sub>4</sub>-N, and NO<sub>x</sub>-N) and phosphorus (TP and PO<sub>4</sub>-P) were

different in different proportions wastewaters. *Chlorella* sp. grew well after PO<sub>4</sub>-P was exhausted which indicated that PO<sub>4</sub>-P is not the limiting factor for growth of this alga. These results indicate good prospects for being able to cultivate *Chlorella* sp. in both influent and effluent wastewater to remove nutrients and produce microalgae biomass. In future, *Chlorella* sp., *Botryococcus braunii* and *Scenedesmus* sp. will be employed for wastewater treatment.

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