Form 2

Dissertation Abstract

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Dissertation title	EFFECTS OF HIGH PRESSURE ON GROWTH, SECONDARY METABOLITES FORMATION AND BUOYANCY REGULATION OF FRESHWATER CYANOBACTERIA (淡水シアノバクテリアに対する圧力増加および長期暗条件の影響について)		

"Cyanobacteria" previously known as "Blue green algae" are photosynthetic prokaryotes which are considered as world oldest species. Cyanobacteria are important fresh water phytoplankton in the view point of fresh water ecology. Cellular and filamentous forms of cyanobacteria are primary production in the natural water bodies and responsible for most of the transfer of Carbon dioxide (CO2) and Nitrogen (N2) from the atmosphere to the water. However due to the environmental pollution and climate change, growth of cyanobacteria in oceans and inland waters has become an uncontrollable problem recently. Formation of eutrophic lakes by over nutrient flow from the catchment areas and thermal stratification in summer season, are the main reasons for excessive cyanobacterial blooms occurrence in lakes and water reservoirs. There are plenty of physical, chemical and biological treatments methods are applying on site level, yet any of method could not achieve to the level of ecologically optimum level of the control of cyanobacteria. Cyanobacteria growth is governing by many factors in natural waters and the responses of cyanobacteria for the different environmental conditions are complex bio-chemical phenomena. Not like other phytoplankton, cyanobacteria have unique morphological and physiological features such as accessory pigments and gas vesicles to endure severe environmental conditions. Application of controlling measures with less of concern about cyanobacterial ecology could be realize as the main reason for the failure of most controlling mechanisms. Hence developing better understanding about effects of different environmental factors on cyanobacteria is necessary in this field. Light irradiance level, nutrient conditions and temperature were main governing factors of most cyanobacterial growth, yet effects of environmental factors such as ultra violet radiation, water turbulence, anoxic condition and hydrostatic pressure condition on cyanobacteria growth are still remaining uncertain. Among them effects of hydrostatic pressure occurred in deep water conditions, on growth, photosynthesis pigments, cell morphology and biosynthesis of secondary metabolites of fresh water cyanobacteria were studied during recent study. And trapping of cyanobacteria in hydrostatic pressure and prolonged dark conditions occurred in deep waters were simulated at lab scale to study its effects of cyanobacterial growth and photosynthetic pigment concentrations. Fresh water cyanobacteria species Pseudanabaena galeata and Microcystis aeruginosa were tested. Observed results showed significant decrease of gas vacuoles in cyanobacteria cells and significant increase of settling velocity of cells with increase of external applied pressure. The prolonged exposure of high pressure feather reduced the quantity of gas vacuoles, cell concentration; photosynthesis pigments (chlorophyll-a, allophycocyanin, phycocyanine and phycoerythrin) concentrations and secondary metabolite (2- methylisoborneol) formation of tested species. Furthermore the pressurization treatment was compared with naturally available ultra violet intensity levels and low dosages of ultra-sonication treatments in the point of gas vacuole collapse and settling velocity increment. Pressurization treatment increased the cell settling velocity effectively than UV-A and ultra-sonication treatments. Effects of high pressure on cyanobacteria can dived to two parts as acute and prolonged effects. Fresh water filamentous cyanobacterium Pseudanabaena galeata which was grown under prolonged hydrostatic pressure more than 35 days shows clear reduction of cell growth rates, even with sufficient irradiance level. Prolonged darkness combined with high pressure made considerable adverse effects on cell growth and pigmentation of cyanobacteria. However at the early stage of high pressure application, main photo energy harvesting pigment chlorophyll-a (chl-a) and accessory pigments including

carotenoids and phycobilisomes concentrations of Pseudanabaena galeata slightly increased in lower pressure values (0.2 MPa) as an acute response. This pigmentation provided high survival capacity to pressure treated cyanobacterial cell, hence even with very high and prolonged pressure condition they can propagate. In contrast to that with dark condition growth of cyanobacterial cells was very low. Yet when cyanobacteria are facing to a high pressure and darkness in cyclic pattern, percentage of survival cells from initial cell amount is gradually increased. Cyanobacteria are reducing during the high pressure and dark period and cells were able to re-grow during the light exposure period with higher increasing rate than control. These results suggest that Pseudanabaena galeata have an adequate tolerance to moderate pressure and fluctuating conditions of irradiance.

2-Methyliobornel (2-MIB) is a major cause of musty odor in water resources contaminated with Pseudanabaena galeata. The effect of hydrostatic pressure and darkness on variation of growth, pigmentation and 2-MIB synthesis in P. galeata were reduced significantly ($f= 98.911$, $p= 0$) while all the pigments concentrations inside the cell have increased significantly (Chl-a $f= 6.52$ $p=0.015$; Car $f= 24.433$ $p=0$; PC $f= 11.864$ $p=0.003$; PE $f= 9.38$ $p=0.005$). The decrease of 2-MIB per cell was caused by inhibition of 2-MIB synthesis in side cells by hydrostatic pressure. Further observation was obtained by Q-PCR analyses of genes related with 2-MIB synthesis were supported to this 2-MIB reduction. Relative gene expression has also being affected both light and pressure. Movement of cyanobacteria Pseudanabaena galeata to deep water could limit the production of 2-MIB significantly.

The effects of sudden (acute) high pressure on the morphology of gas vacuole and settling velocity (Vs) of Pseudanabaena galeata, and Microcystis aeruginosa were statistically significant. Observed Vs of acute pressurized cells, under several pressure values from 0.05 to 0.5 MP were increased gradually with increasing applied external pressure. Application of pressure altered the buoyancy state of the cells to average Vs of up to 0.05 mm/s (maximum Vs were observed at 0.5 MPa). Increase of pressure caused deformations and collapses of cyanobacterial gas vesicles. An apparent relationship between Vs and gas vacuole volume change could be observerd in this study. Moreover, the effects of prolonged pressure on the protein and polysaccharide contents were decreased, which reduce the cell ballast weight and Vs. This study, tested to obtain the effective sedimentation of cyanobacteria cells. Comparison of settling velocities of cells grown under two naturally available treatments (external pressurization relevance with hydrostatic pressure in deep water; ultra violet-A (UVA)) and one artificial treatment (ultra-sonication) were significantly varied with treatment method. Increasing external pressure ($f=136$, $p<0.05$), ultra-sonication ($f=127$, $p<0.05$) and UVA ($f= 133$, $p<0.05$) have significant negative effect on Vs. UVA treatment has minimum effect on settling while ultra-sonication has much higher effect. Yet the destruction of cell integrity by sonication raises the turbidity of water. However the pressurization treatment shows rapid sedimentation of cells compared to other treatments. Hydrostatic pressure is naturally occurred while the water depth increases therefore application of high pressure on cyanobacterial cells using pressurization vessel or pumping of upper cyanobacterial scum to deep water layer can be improved farther as a controlling technique of Microcystis aeruginosa.

Hydrostatic pressure is naturally occurred while the water depth increases therefore application of high pressure artificially on cyanobacterial cells using pressurization vessel or pumping of upper cyanobacterial scum to deep water layer to subject them to natural hydrostatic pressure are suggested for further researches as a controlling technique of fresh water cyanobacteria in lakes.