Microcystin-LR degradation by enriched bacteria isolated from different units of Drinking Water Treatment Plant

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INTRODUCTION

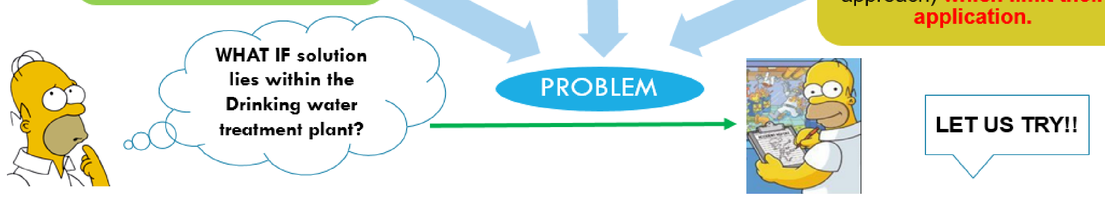
- The occurrence of cyanobacterial harmful algal bloom (CHABs) producing cyanotoxins affect fresh and marine systems as well as public health. Among all the cyanotoxins, microcystin variant MC-LR is the most toxic (due to their cyclic and persistent nature) and commonly found cyanotoxin in the aquatic ecosystem.
- When these cyanotoxin reaches the DWTPs, conventional treatment operations become inefficient in cyanotoxin removal (especially dissolved MCs) and produce toxic byproducts which are often difficult to characterize [1].
- The biological approach has not only shown promise in fully degrading cyanotoxins but it is also known to produce less toxic end products (up to 160-fold less) [2].
- Many studies involving degradation of MC-LR by isolating indigenous bacterial species have been explored including some in-situ bacterial species as well [3,4]. Here, the ability of different bacterial communities present in different operational units of the DWTP (i.e. influent water to the DWTP, sedimentation unit and the filtration unit) have been explored to develop a potentially sustainable solution for this global drinking water issue.

PROBLEM

Microcystin-LR (MC-LR) produced by harmful algal bloom pose **serious threat to the ecosystem** including public health (WHO guidelines in drinking water is **1 µg/L**) by entering the drinking water treatment facility/plants (DWTPs).

Conventional operations employed in drinking water treatment plant (DWTPs) such as ozonation, chlorination, algicides and advance processes such as photocatalysis, RO membrane, etc. are **energy intensive**, **requires supervision skills**, produces **toxic by-products** and are **uneconomical.**

Bacteria isolated from various sources such as rivers, lakes, etc. have already shown successful degradation of cyanotoxins. However, culturing them in DWTP **demands** employment of **new bioreactor** (suspended growth approach) **which limit their application.**



OBJECTIVE/METHODOLOGY

OBJECTIVES	METHOD	INSTRUMENT USED
To isolate and study growth curve of the bacterial community from different units of the DWTPs (Ste-Foy, Canada)	Cells isolated based on dominancy and color morphology	Basic molecular biology
To test their toxicity tolerance level in presence of microcystin-LR	Cell viability count in presence of 10 µg/L and 100 µg/L MC-LR	Statistical analysis (t-test/one-way ANOVA; p-value analysis)
Comparing degradation rate and overall removal efficiency of microcystin-LR before and after the acclimatization process	Equal cell concentration ($6x10^6$ cells/mL) of bacterial community isolated from different units of DWTPs were spiked in flask with mineral salt media (60 mL) containing 200 µg/L	High performance liquid chromatography (HPLC)
Identification of the formed by-products and their toxicity level	Relative abundancy of the by-products based on their m/z value during mass spectra analysis	Mass spectrometry (MS), Bio-indicator organism (*Rhizobium Melitoli*)

RESULTS AND DISCUSSION

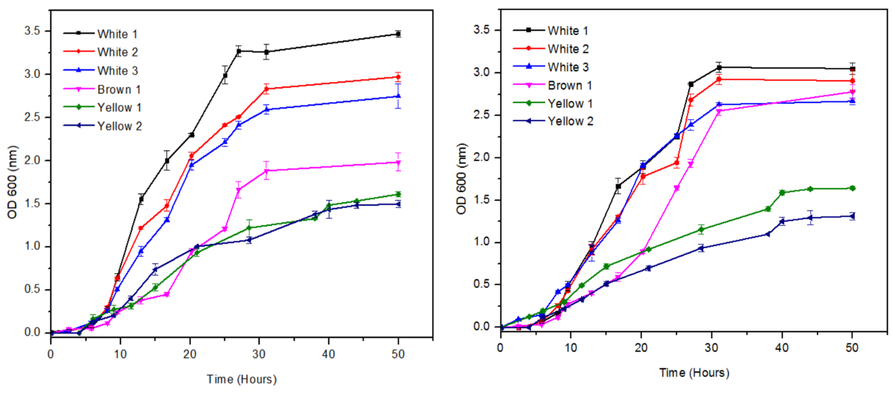


Figure 1: Growth curve for all six isolated bacteria when tested with 10 µg/L (left) and 100 µg/L (right) MC-LR

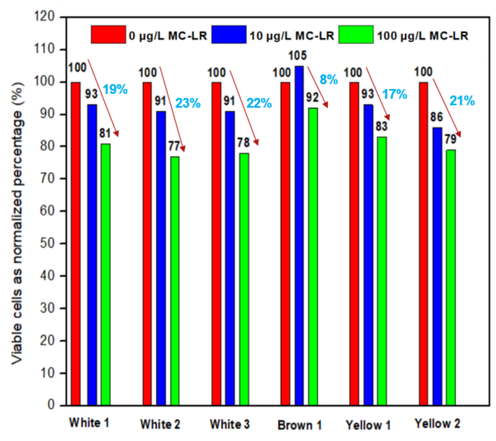
Table 1: Statistical analysis for analyzing significant difference between the data sets of two curves shown in figure 1

Bacteria	P-value
White 1 (POU)	0.60
White 2 (ESSU)	0.81
White 3 (TSFU)	0.99
Brown 1 (TSFU)	0.61
Yellow 1(ESSU)	0.90
Yellow 2 (TSFU)	0.59

Formulated hypothesis for growth curve:

Null Hypothesis (H_0): No significant difference between the two sets of observation
Alternate Hypothesis (H_1): Significant difference between two data sets.
No significant difference (p-value > 0.05) was observed between the growth rate of all six bacteria **when spiked with 10 µg/L and 100 µg/L MC-LR** (based on p-values).

Bacterial population decreased only by **19%, 23%, 22%, 8%, 17%, 21%** when tested with **100 µg/L MC-LR** for White 1, White 2, White 3, Brown 1, Yellow 1 and Yellow 2 bacteria respectively **as compared to zero MC-LR case**.



RESULTS AND DISCUSSION

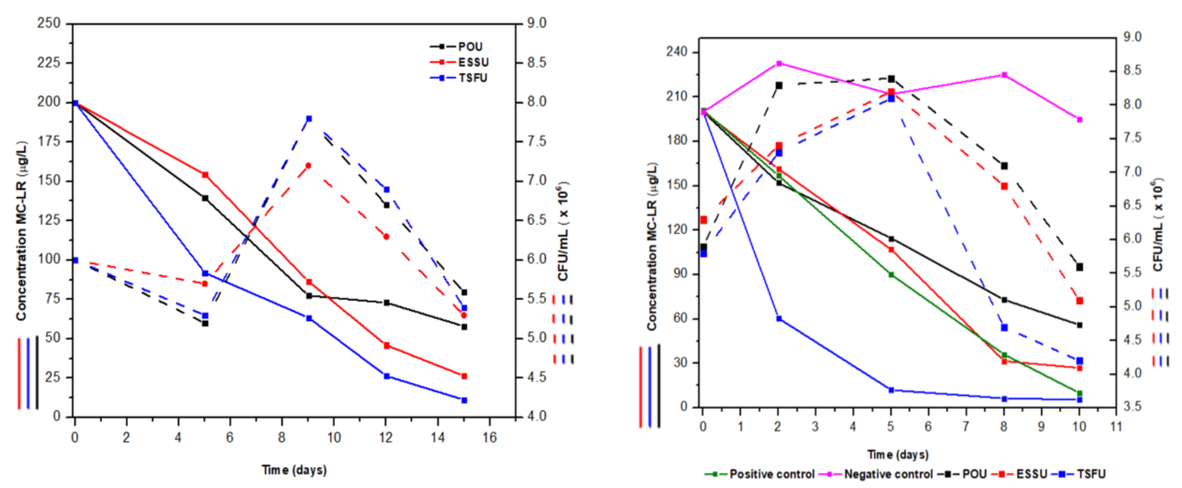


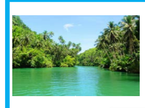
Figure 2: Draw-down curve for acclimatization phase (left) and post acclimatization (right) phase

- **Acclimatization phase**: Bacterial cell concentration isolated from ESSU, POU, TSFU decreased to $5.2x10^6$, $5.7x10^6$ and $5.3x10^6$ CFU/mL respectively at the end of 5 days but increased to 7.8 $x10^6$, 7.2 $x10^6$, 7.8 $x10^6$ CFU/mL respectively after 9 days.
- **Post-acclimatization phase:** Bacterial cell concentration isolated from ESSU, POU, TSFU increased to $8.4x10^6$, $8.2x10^6$ and $8.1x10^6$ CFU/mL respectively at the end of 5 days gave sign of positively getting acclimatize to the MC-LR environment.
- Mass spectra revealed several accompanied ions at m/z 155.99, 213.14, 268.24, 332.93, 553.29, 571.27, 599.34 and 862.48. By-product with m/z of 862.5 forms due to hydrolysis of mother ion (m/z 995) followed by the further loss of Adda portion and amino groups (**microcystinase enzyme encoded by *mlrA* gene is responsible**) [5].
- These hydrolyzed linear by-products formation is also linked to the **reduced toxicity levels (up to 160-fold).**

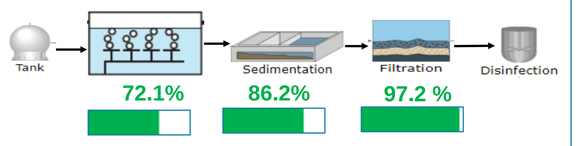
Table 2: Degradation rate and removal efficiency of MC-LR by bacterial community isolated from three different units of DWTP

Treatment Unit	Degradation rate (before acc.) acc.= Acclimatization	Removal efficiency (before acc.)	Degradation rate (after acc.)	Removal efficiency (after acc.)	Increase in degradation rate
Pre-ozonation Raw water (POU)	9.46 µg/L/day	71.1 ± 7.37 %	14.46 µg/L/day	72.1 ± 6.4 %	52.8 %
Sedimentation unit (ESSU)	11.56 µg/L/day	86.7 ± 3.19 %	17.32 µg/L/day	86.2 ± 7.3 %	49.8 %
Filtration unit (TSFU)	12.58 µg/L/day	94.35 ± 10.63 %	19.45 µg/L/day	97.2 ± 8.7 %	54.6 %

FUTURE SCOPE



In-situ bacterial biofilm can find application in future for cyanotoxin removal within DWTPs!!



Change in color of MTT dye (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide: **Yellow** to **Blue** ppt) indicating cell viability in degraded MC-LR samples when tested for toxicity.

- Highest removal efficiency of MC-LR (97%) is possible with enriched bacterial community present in filtration unit of DWTPs.
- Also, the toxicity analysis as observed by bioindicator organism (*Rhizobium Melitoli)* showed significant amount of living bacteria present (based on absorbance value due to change in color) for final degraded samples as compared to standard microcystin-LR (300 µg/L).
- In future, suitable biofilm formation over filtration media (sand, anthracite) can serve the purpose of total cyanotoxin biodegradation. In this way, no additional treatment unit will be required.

CONCLUSION

- Indigenous bacterial community isolated from different units of DWTPs (water sample from pre-ozonation unit (POU), effluent-sludge mixture from sedimentation unit (ESSU) and top-sand layer water sample from filtration unit (TSFU)) showing potential in degrading MC-LR.
- **Maximum degradation** of MC-LR was reported for **enriched bacterial community** isolated from TSFU achieving 97.2 ± 8.7% followed by 86.2 ± 7.3 % and 72.1 ± 6.4 % for enriched bacterial culture isolated from ESSU and POU, respectively.
- The ultimate degradation efficiency achieved was almost similar to non-enriched bacterial degradation but the **biodegradation rate increased** by 52.8% (9.46 µg/L/day to 14.46 µg/L/day), 49.8% (11.56 µg/L/day to 17.32 µg/L/day) and 54.6% (12.58 µg/L/day to 19.45 µg/L/day) for POU, ESSU and TSFU **enriched bacterial community** respectively.
- Mass spectra analysis depicts linearization involving hydrolysis of MC-LR structure (which is linked to reduced toxicity level) confirming *mlrA* gene presence from previous studies.

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