

## RESEARCH ARTICLE

### Bioremediation

# Biodegradation of the cyanotoxin cylindrospermopsin by *Bacillus cereus*, *Micrococcus luteus* and *Alcaligenes faecalis*

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**Abstract:** Cylindrospermopsin (CYN) is a cyanotoxin found in natural waters, with potential risk to human health through the inhibition of protein synthesis. Despite the implementation of conventional water treatment procedures, complete removal of CYN remains a question due to its heat-stable nature. Hence, contamination of water sources with CYN is a challenge in providing safe drinking water throughout the world. The present study was conducted to test the ability to degrade CYN at 28°C and pH 7, of four bacterial strains: *Bacillus cereus*-Y, *Bacillus cereus*-S (*B. cereus*-S), *Micrococcus luteus*, and *Alcaligenes faecalis*, which were previously isolated from different water sources as different hydrocarbon degraders. The CYN degradation kinetics of each bacterial species were studied using High Performance Liquid Chromatography. The greatest CYN degradation ( $28.22 \pm 0.24\%$ ) was shown by the bacterium *B. cereus*-S in 5.0 mg/L CYN within 14 days. The CYN degradation by the other strains was lower than 10% under the same conditions. Further studies employing different initial concentrations of CYN revealed that *B. cereus*-S could degrade lower CYN concentrations at a higher percentage (1.0 mg/L, 2.5 mg/L, and 5.0 mg/L of CYN removal percentages were  $36.83 \pm 2.43\%$ ,  $32.25 \pm 1.25\%$ , and  $24.72 \pm 0.40\%$ , respectively, after 14 days of incubation at 28°C and pH 7). The maximum average degradation rates were recorded for 1.0 mg/L, 2.5 mg/L, and 5.0 mg/L CYN on the 6th ( $0.05 \pm 0.00$  mg/L/day), 8th ( $0.04 \pm 0.01$  mg/L/day), and 12th ( $0.02 \pm 0.01$  mg/L/day) days of incubation, respectively. The study showed the potentiality of the bacterium *B. cereus*-S on the application for degrading CYN among the tested bacteria species.

**Keywords:** *Bacillus cereus*-S, biodegradation, cyanotoxin, cylindrospermopsin, hepatotoxic.

## INTRODUCTION

Cyanobacteria are a group of prokaryotic microorganisms, which belong to the kingdom Bacteria. They produce cyanotoxins which are secondary metabolites, and these cyanotoxins are found in a range of chemical groups. Cylindrospermopsins (CYNs), a specific group of cyanotoxins, were initially isolated from the cyanobacterium, *Raphidiopsis raciborskii* (previously known as *Cylindrospermopsis raciborskii*). CYNs have since been detected in surface water bodies worldwide, with different cyanobacterial producers. CYN is an alkaloid with three distinct groups of tricyclic guanidine, uracil, and sulphate. It posse a molecular weight of 415.2 g/mol and is soluble in water and organic solvents (Adamski *et al.*, 2014). Furthermore, the toxin shows stability at room temperature ( $21 \pm 1^\circ\text{C}$ ) and in a wide pH range (3, 5, 7) (Adamski *et al.*, 2016).

CYNs enter the human body via different pathways, such as dermal contact during bathing, swimming, and other recreational activities and ingestion of water or food that are contaminated with CYN (Pichardo *et al.*, 2017). In most cases of CYN toxicity, the environmental concentrations of CYN have been recorded within the range of 1–10 µg/L, while exceptional values have been reported from aquaculture farms and farm dams in Australia (Pichardo *et al.*, 2017). CYN has been detected in mollusks, crustaceans, and fish with the maximum

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detection range of 0.00007–4.3 µg/g in Redclaw crayfish (*Cherax quadricarinatus*) in Australia. Certain studies suggest that CYN has a bioaccumulation factor (BAF) of 4–171, indicating the potential to bioaccumulate in aquatic species (Scarlett *et al.*, 2020). During chronic toxicity, it acts as a hepatotoxin, resulting in severe liver necrosis (Abeyasiri *et al.*, 2021b). Further, it affects kidneys (Abeyasiri *et al.*, 2021c) and has the potential to cause cytotoxicity (Poniedzialek *et al.*, 2014; Abeyasiri *et al.*, 2021a; Chichova *et al.*, 2021). It has been reported that CYN has the potential to inhibit the synthesis of proteins such as globin and antioxidants such as glutathione, leading to oxidative stress (López-Alonso *et al.*, 2013). Moreover, prolonged exposure to CYNs could result in genotoxicity, immunotoxicity, and tumour initiations (Pichardo *et al.*, 2017).

Numerous studies have confirmed the presence of CYN producing cyanobacteria, including *Raphidiopsis* sp., in water bodies of Sri Lanka. It is evident from these reports that *Raphidiopsis* sp. was not significantly dominant prior to the 20th century in Sri Lanka. The species has spread widely during 20<sup>th</sup> century, which might be due to increased human habitation that led to an increased number of point and non-point sources of aquatic pollution (Jayatissa *et al.*, 2006; Kulasooriya, 2017). Furthermore, *Raphidiopsis* sp. has been observed to be prevalent in a majority of water bodies located in the North Central Province of Sri Lanka, as compared to other regions of the country (Sethunge & Manage, 2010).

During the study of Abeyasiri *et al.* (2018b), a positive correlation ( $p < 0.05$ ) between the CYN-producing cyanobacterial cell density and the CYN concentrations was observed in the surface and groundwater sources of CKDu (chronic kidney disease of unknown aetiology) strike areas in Sri Lanka. Additionally, Abeyasiri *et al.* (2018a) recorded the presence of CYN in well water samples of CKDu endemic areas in the Anuradhapura district. Further, Arachchi & Liyanage (2012) have detected CYN in an average concentration of 0.137 ng/mL in the Kala Wewa, Nuwara Wewa, and Tissa Wewa of the Anuradhapura District, where CKDu is highly prevalent. In addition to the aforementioned facts, Piyathilaka *et al.* (2015) reported that CYN has the potential to cause severe damage to livers and kidneys due to chronic exposure. Consequently, the presence of CYN in drinking water sources in Sri Lanka is alarming.

Various studies have been carried out worldwide on physicochemical methods of removing toxic CYN through the drinking water treatment process. UV irradiation

catalyzed by titanium dioxide (TiO<sub>2</sub>) (Chen *et al.*, 2015; Camacho-Muñoz *et al.*, 2020), ozonation (Yan *et al.*, 2016), catalytic wet peroxide oxidation (Munoz *et al.*, 2019), non-thermal plasma approaches (Schneider *et al.*, 2020) and electrolysis approaches (Bakheet *et al.*, 2018) have been tested as effective in removing CYN. Although such physicochemical methods have proven to be effective in removing CYN, their high operational and maintenance costs have become a significant constraint in their application, specially in local drinking water treatment processes in developing countries.

Therefore, biodegradation is now accepted as the most efficient, cost-effective, and environmentally friendly method of removing cyanotoxins. Even so, there is limited information available on CYN biodegradation. Wormer *et al.* (2008) showed that biodegradation of CYN produced by *Aphanizomenon ovalisporum* is not achieved by the co-occurring natural bacterial community within 40 days, and concluded that previous exposure of bacteria to CYN has no effect on their ability to degrade the toxin. In contrast, several successful degradation studies have been reported lately. During the study of Ho *et al.* (2012a) the biodegradability of five cyanobacterial metabolites was tested and the ease of biodegradation was achieved in the order of MC-LR (Microcystin-LR) ≥ CYN > saxitoxins > geosmin ≥ 2-methylisoborneol. Maghsoudi *et al.* (2015) studied the simultaneous biodegradation of MC-LR, MC-YR, MC-LY, MC-LW, MC-LF, and CYN using particulate attached bacteria and phycocyanin in clarifier sludge of a drinking water treatment plant and a drinking water source in Canada. In this study, CYN was degraded with a half-life of 6.0 days in the sludge, but it was not degraded in the lake water samples. In contrast, Ho *et al.* (2012b) found that CYN was biodegradable in a tested source water in Australia, along with MC-LR, saxitoxins, and geosmin and the order of ease of biodegradability was reported as MC-LR > CYN > geosmin > saxitoxins. This study has supported the fact that the biodegradation of CYN occurs in waters with historical exposure to blooms of CYN producing cyanobacteria.

Besides, Mohamed & Alamri (2012) reported successful biodegradation of CYN using a *Bacillus* strain (AMRI-03) isolated from cyanobacterial blooms, which was previously reported as an MC degrader. Growth of the bacteria is observed in the presence of CYN, and the development has increased with an increasing initial concentration of CYN. Consequently, the researchers concluded that complete degradation of CYN depends on the initial toxin concentration. Complete degradation in this experiment occurred after six days at the highest

concentration (300 µg/L) compared to 7 and 8 days at lower concentrations (10 and 100 µg/L) and the highest rate of degradation (50 µg/L/day) was reported for the highest initial concentration. Moreover, Dziga *et al.* (2016) reported a 25% removal of CYN within 6 days of incubation with *Aeromonas* sp. isolated from Lake Rusalka, Poland. The effects of pH and temperature were studied too here, and CYN removal was reported as 47 and 49% at 20 and 30°C, respectively, and 48.9 and 41.5% at pH 6.5 and 8.0, respectively. Based on such literature, it is clear that the majority of the studies on CYN biodegradation have utilized microorganisms which previously showed the ability to degrade MCs and nodularin (Mohamed & Alamri, 2012). Nonetheless, the inadequate CYN degradation rates of these bacterial strains make them inappropriate for environmental applications. Therefore, it is important for researchers to focus more on CYN biodegradation, since this might become a huge environmental issue in the upcoming years without proper management.

The objective of the present study is to determine the efficacy of degrading CYN by four bacterial strains namely, *Bacillus cereus*-S (BC-S), *Bacillus cereus*-Y (BC-Y), *Micrococcus luteus* (ML-M), and *Alcaligenes faecalis* (AF-M) which were previously proven to degrade several other environmental pollutants including pesticides (Geed *et al.*, 2017), dyes (Thakur *et al.*, 2014; Ekanayake & Manage, 2017), polycyclic aromatic hydrocarbons (Liyanage & Manage, 2016; Dharmadasa *et al.*, 2017; Idroos & Manage, 2017) and antibiotics (Idroos & Manage, 2014). The BC-S strain employed in this study was isolated from the surface water of the Giradurukotte reservoir in Sri Lanka during a previous study and has been proven to degrade MC-LR completely within 8 days (Idroos & Manage, 2018). The other *Bacillus* strain used in this study, BC-Y, was previously isolated from crude oil contaminated surface water and identified as a hydrocarbon degrader (Liyanage & Manage, 2016). ML-M and AF-M, the two other bacteria employed in this study, were isolated from water and soil samples from textile wastewater in Sri Lanka (Ekanayake & Manage, 2020). *Micrococcus luteus* has been reported to have the ability to degrade environmental pollutants such as arsenic (Sher *et al.*, 2020) and biphenyl (Su *et al.*, 2015). In contrast, *Alcaligenes faecalis* has been studied for its biodegradation ability on plastics (Caruso, 2015) and has been used in the treatment of hospital wastewater (Rashid *et al.*, 2020).

## MATERIALS AND METHODS

### Screening of CYN degradation using *Bacillus cereus*-Y, *Bacillus cereus*-S, *Micrococcus luteus*, and *Alcaligenes faecalis*

Four bacterial strains—two strains of *Bacillus cereus* named *B. cereus*-S (BC-S) and *B. cereus*-Y (BC-Y), *M. luteus* (ML-M), and *A. faecalis* (AF-M)—which have been isolated from previous studies (Liyanage & Manage, 2016; 2018; Idroos & Manage, 2018; Ekanayake & Manage, 2020) were tested to evaluate their ability to degrade CYN, following the method described by Manage *et al.*, (2016) and Manage *et al.*, (2009). A loop-full of each bacterial isolate was inoculated in 5 mL of sterile Luria Broth (LB) and incubated overnight at 28°C and at pH 7. The bacteria grown overnight were washed 03 times by adding equal volumes of 0.01 M phosphate-buffered saline (PBS), followed by centrifugation at 6000 rpm for 20 min. The resulting pellet was re-suspended in 0.01 M PBS and kept overnight to remove residual carbon, if any. The cell suspensions were centrifuged at 6000 rpm for 20 min, and the resulting pellet was washed three times using PBS (Manage, 2009). Then the optical densities of all bacterial suspensions were equalized ( $A_{590} = 0.35$ ) using the UV-Vis spectrophotometer. Approximately 0.5 mL of the equalized bacterial suspension (approximately  $10^5$  cells/mL) were inoculated into pure CYN purchased from Sigma Aldrich in sterile distilled water at a final concentration of 5 µg/mL. Triplicate samples were prepared for each bacterial strain and incubated at 28°C for 14 days. Sample aliquots (1000 µL) were removed at intervals of two days for 14 days, and the samples were frozen immediately at -20°C. For the analysis of CYN, the samples were freeze-dried, reconstituted in 1 mL of 80% (v/v) HPLC grade methanol followed by centrifugation at 6000 rpm for 20 min and the supernatant was removed, filtered through a 0.2 µm nylon syringe filter and 50 µL of the sample were subjected to PDA-HPLC analysis (Idroos & Manage, 2018).

### Detection of CYN using the high-performance liquid chromatography (HPLC) method

The remaining CYN in sub-samples were quantified using Agilent 1200 series High-Performance Liquid chromatography (HPLC) which was equipped with a diode array and fluorescence detector, following the method of Manage *et al.* (2018), with minor

modifications. The detection limit of the HPLC was 0.5 mg/L. The injected volume was 20  $\mu$ L, and chromatography was performed at 30°C. The mobile phase consisted of a mixture of HPLC grade methanol (Component A) and Water (Component B), 0:100 (v/v) was pumped at the beginning at a flow rate of 0.3 mL/min for CYN, followed by linear gradient elution of solvent B from 100% to 0%. The run time was set as 15 min, and the detector was set at the range of 200-300 nm to monitor the column effluent. The standard retention time for the CYN peak is 2 min, and the concentration

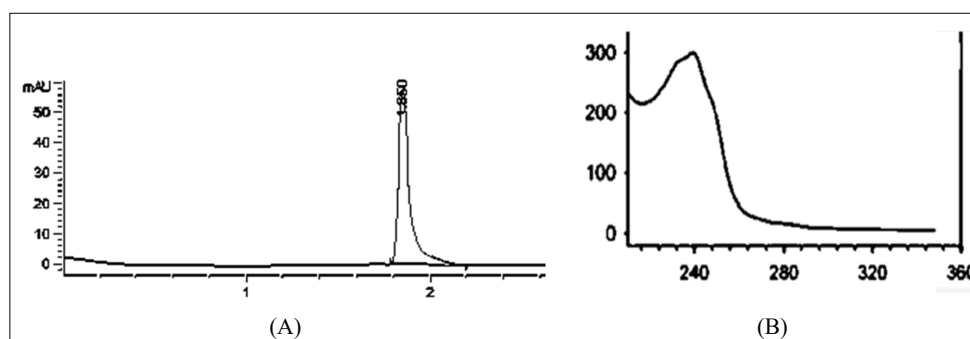
calculation was made using the equation given below.

$$Y = 43.433X + 11.945$$

where,

$Y$  = Peak area (mAU) and  $X$  = CYN concentration (mg/L)

The relevant HPLC standard CYN (5 mg/L) peak is given in Figure 1.



**Figure 1:** (A) UV chromatogram for 5 mg/L standard CYN toxin; (B) UV spectrum for 5 mg/L standard CYN toxin

### Determination of CYN removal percentage

The remaining CYN concentrations of subsamples were quantified, and the CYN removal percentages were calculated following the equation given below (Manage *et al.*, 2009).

$$\text{CYN removal percentage} = [(a-b)/a] \times 100$$

where,

$a$  = Initial CYN concentration

$b$  = CYN concentration on sampling day

The average degradation rates at which each bacterial strain degraded CYN for different time periods of incubation were calculated using the following equation.

$$h = [(C_0 - C_t)]/t$$

where,

$h$  = Average Rate of degradation (mg/L/day)

$C_0$  = Concentration of CYN at the beginning (mg/L)

$C_t$  = Concentration of CYN at the end (mg/L)

$t$  = Time interval

The bacterial strain which showed the highest degradation was selected and the procedure was repeated for the selected bacterial strain under three different concentrations of CYN (5.0 mg/L, 2.5 mg/L and 1.0 mg/L).

### Determination of adsorbance of CYN on the bacterial cells

The potential adsorbance of CYN onto the bacterial cells were determined at the end of the 14 days of the incubation period. The remaining samples were filtered using a 0.45  $\mu$ m filter to obtain the bacterial cells. Then the filter paper was cut into pieces and washed with distilled water to extract any cell bound CYN. The washing was repeated three times by periodic centrifugation and letting cell-bound CYN be dragged into it. The obtained samples were then subjected to Solid Phase Extraction (SPE). SPE was carried out using C18 ENV cartridge. The cartridges were attached to the vacuum manifold system and conditioned by passing 10 mL methanol and 10 mL water through each. Then the samples were passed through the cartridges and eluted with 3 mL of 80% methanol. The eluted samples were collected in sample tubes and analyzed for the presence of CYN, using the HPLC. The control samples of the three experimental



concentrations containing CYN without bacteria were also subjected to this procedure.

### Statistical analysis

One-way ANOVA was performed using Minitab 17 software, during the initial study to select the suitable bacterial strain which would efficiently degrade CYN and during the latter study to find if there is a significant relationship between the initial concentration and the rate of degradation for the selected bacterial strain.

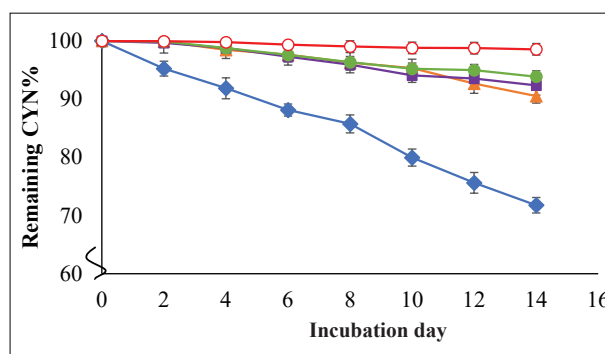
## RESULTS AND DISCUSSION

### Selection of potential bacterial strain which degrades CYN

Starting with a value of 5.0 mg/mL, bacterial strains had different CYN degradation percentages. After 14 days, the percentage degradations for *B. cereus*-S (BC-S), *M. luteus* (ML-M), *A. faecalis* (AF-M), and *B. cereus*-Y (BC-Y) were reported to be  $29.67 \pm 0.24$ ,  $10.94 \pm 0.28$ ,  $9.09 \pm 0.16$ , and  $7.58 \pm 0.14\%$ , respectively. The control sample, which did not contain bacteria, only revealed a  $1.45 \pm 0.05\%$  decrease in CYN, which could be attributed to natural degradation of the toxin due to the temperature. Considering this fact, the exact bacterial degradation was calculated by deducting natural degradation from the values obtained for each sample set. Accordingly, the real bacterial degradation values are 28.22, 9.49, 7.64 and 6.13% for BC-S, ML-M, AF-M and BC-Y respectively. The bacterial strains AF-M, ML-M and BC-Y showed significantly lower degradations compared to the degradation of BC-S. This lack of CYN degradation by rest of the bacteria could be due to the inhibition of their growth by CYN, or because they might be possessing enzymes with low reaction kinetics. According to the results of one-way ANOVA, there are significant differences ( $p < 0.05$ ) between the percentage degradations of all the bacterial strains and the highest mean value of percentage degradation was obtained for BC-S. As a result, BC-S was chosen as a potential bacterial candidate for degrading CYN. Figure 2 illustrates the reduction of the toxin by the four bacterial strains throughout 14 days of degradation study.

### Degradation of different CYN concentrations by the bacterium *B. cereus*-S (BC-S)

Under three different initial concentrations of CYN, the degradation kinetics of the selected bacterial strain, BC-S, were studied (5.0, 2.5, and 1.0 mg/L). For a 14-day incubation period, the degradation kinetics

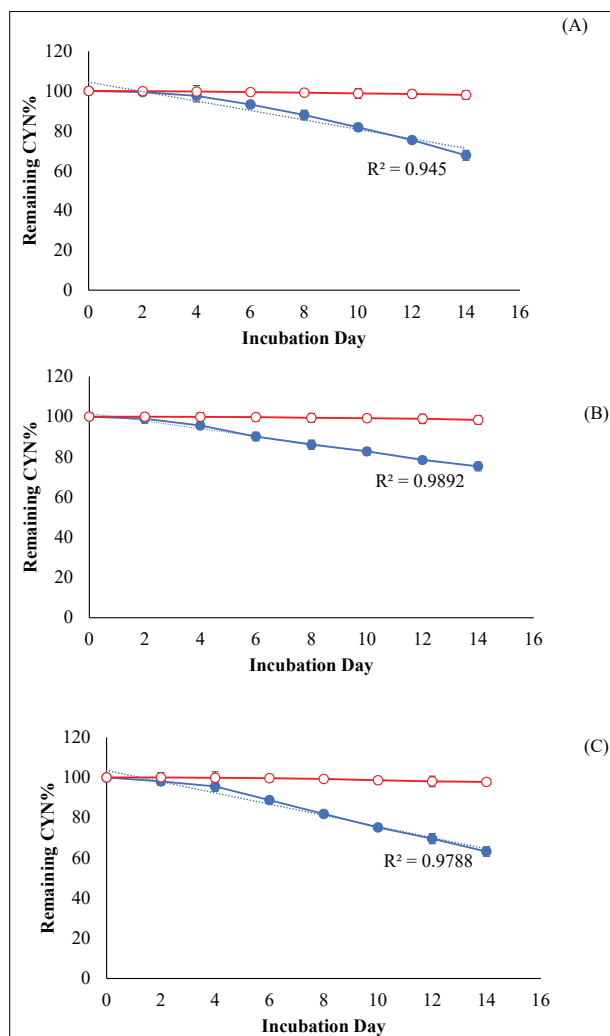


**Figure 2:** Remaining CYN after incubating the four bacterial strains under 28°C for 14 days with 5.0 mg/L CYN. When error bars are not shown, the standard deviation is less than the width of the symbol. (Closed diamond; *Bacillus cereus*-S, closed circle; *Bacillus cereus*-Y, closed triangle; *Micrococcus luteus*, closed square; *Alcaligenes faecalis* and open circle; Sterile control).

were calculated every two days. At the end of the 14-day incubation period, different amounts of CYN were detected in each sample (5.0, 2.5, and 1.0 mg/L). BC-S degraded  $39.06 \pm 2.43\%$  of 1.0 mg/L CYN after 14 days of incubation at 28°C, while  $34.22 \pm 1.25\%$  and  $26.35 \pm 0.40\%$  of CYN were degraded in the 2.5 mg/L and 5.0 mg/L samples, respectively. In all three samples, CYN concentrations were gradually declining at different rates. The removal patterns of CYN by BC-S at different concentrations suggest that high initial concentrations of CYN will result in comparatively low degradation rates. During the incubation period, there was no significant loss of CYN in the sterile controls, and only  $2.23 \pm 0.15$ ,  $1.97 \pm 0.15$ , and  $1.63 \pm 0.08\%$  decreases were found in the 1.0, 2.5, and 5.0 mg/L control samples, respectively. Accordingly, the natural degradations were deducted from the obtained values in order to determine the exact bacterial degradation. After the reduction the obtained values were 24.72, 32.25 and 36.83% for 5.0, 2.5 and 1.0 mg/L samples respectively. The remaining CYN percentages of the three samples, 5.0, 2.5, and 1.0 mg/L, and the relevant control samples, are displayed against the incubation day in Figure 3. The graphs show a clearly declining CYN trend as the incubation day advances.

The average degradation rates were also calculated every two days, and it was found that the average rates changed throughout the incubation period. This variation in rates of degradation could be attributed to fluctuations in enzyme activity during different phases of bacterial growth. The highest average rates were recorded for the 5.0 mg/L ( $0.02 \pm 0.01$  mg/L/day), 2.5 mg/L ( $0.04 \pm 0.01$  mg/L/day), and 1.0 mg/L ( $0.05 \pm 0.00$  mg/L/day) samples

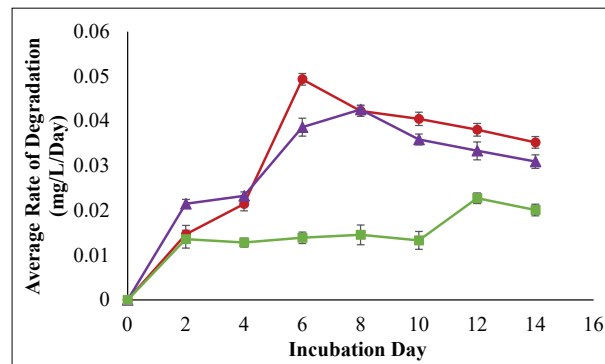
on the 12th, 8th, and 6th days of incubation, respectively. The average degradation rates determined for the three CYN concentrations are illustrated in Figure 4.



**Figure 3:** Remaining CYN percentages after degradation by the bacterial strain *Bacillus cereus*-S under (A) 5.0 mg/L, (B) 2.5 mg/L, and (C) 1.0 mg/L initial CYN concentrations after incubating at 28°C for 14 days. When error bars are not shown, the standard deviation is less than the width of the symbol. (closed circle -sample treated with bacteria, open circle – control sample).

Based on the results of the one-way ANOVA analysis of the initial CYN concentrations (1.0, 2.5, and 5.0 mg/L) (all the P values were less than 0.05) there is a relationship between the initial CYN concentrations (1.0, 2.5, and 5.0 mg/L) and the average rates of degradation. According to the results, the highest mean degradation

rate (0.05 mg/L/day) was obtained for a 1.0 mg/L initial concentration, whereas the lowest (0.02 mg/L/day) was obtained for a 5.0 mg/L initial concentration. As a result, the degradation rate at lower initial CYN concentrations has become significant.



**Figure 4:** Average rates of CYN degradation under different concentrations of by the bacterial strain *Bacillus cereus*-S after incubating at 28°C for 14 days. When error bars are not shown, the standard deviation is less than the width of the symbol. (closed circle; 1.0 mg/L CYN, closed triangle; 2.5 mg/L CYN, closed square; 5.0 mg/L CYN)

The reason for this result can be attributed to several external and internal factors. The availability of the substrate is a main factor for any enzymatic bacterial degradation. The enzyme-substrate reaction might be limited due to the availability of limited number of active sites in the enzyme. It can be hypothesized that once the enzyme is saturated with the substrate, the rate of reaction might reduce. It is important to note that the specific mechanisms underlying the degradation of CYN by *Bacillus cereus* may involve factors such as the presence of co-metabolites, genetic variations among bacterial strains, and environmental conditions. Further research is necessary to gain a more comprehensive understanding of the exact reasons for the observed pattern in degradation rates.

During the study of Mohamed & Alamri (2012) using *Bacillus* strain (AMRI-03) for degrading three different CYN concentrations (300, 100, and 10 µg/L), it was found that the average rate of degradation increases with increasing initial concentration. In contrast, the present study indicates an increase in average degradation rate with decreasing initial concentration. Both studies conclude that the average degradation rate depends on the toxin's initial concentration, although the observations between the two studies are contradictory. Furthermore,

both Mohamed & Alamri (2012), and the present study also used *Bacillus* sp. and reported significant CYN degradation rates. This reveals an important fact: *Bacillus* sp. can be exploited as a promising CYN degrader.

Furthermore, in the present study, the maximum average degradation rate employing BC– S was 47.9 µg/L/day for 1000 µg/L (1 mg/L) CYN concentration. Yet, Mohamed & Alamri (2012), found the highest average rate of CYN degradation by *Bacillus* sp. to be 50 µg/L/day for 300 µg/L CYN concentration. The present study achieved a noticeably higher average degradation rate of CYN toxin, even under a higher CYN concentration compared to the previous studies. This finding offers valuable insight into developing an effective means of degrading CYN toxin through method optimization.

Although the HPLC analysis reported a reduction of the toxin during 14 days of incubation, it was uncertain whether this reduction was actually a degradation or due to the toxin's adsorbance on the bacterial cells. Therefore, a further study was conducted to determine the potential adsorbance of CYN on bacterial cells. No adsorbance was reported here, and it can be concluded that the toxin reduction was solely due to a degradation process occurring within the bacterial cells.

## CONCLUSION AND RECOMMENDATIONS

The study aimed to develop a viable method for degrading the CYN toxin by employing native bacteria. It was found that *Bacillus cereus*-S is an excellent degrader of CYN, with the greatest degradation rate of  $0.05 \pm 0.00$  mg/L/day on the 6th day when CYN was introduced in 1.0 mg/L concentration, per the biodegradation study. The degradation rates reported in the present study are greater than previously documented values, despite the high initial CYN concentrations used. Therefore, it is concluded that *Bacillus cereus*-S is a promising candidate for the degradation of CYN toxin in water sources during the water treatment process, ensuring that consumers receive safe drinking water.

The reported bacterial degradation could be improved by optimizing environmental parameters, particularly those that affect the growth and activity of bacteria, such as temperature, pH, light, and nutrients. Additionally, providing a suitable substrate for the bacteria to attach to, such as biochar, would impact its degradation efficiency. According to literature, such bacterial degradations are enzyme-driven mechanisms, with enzymes that

can function within or outside the cell. Hence, further research is necessary to identify the enzymatic activity of this bacteria, which will pave the way for enzymes to be isolated from the bacteria and applied in the field. This could minimize the adverse effects of introducing bacteria itself, as it would have other consequences, such as competition with native species. Furthermore, it is necessary to identify other factors which specially affect the rate of degradation of the toxin by the selected bacteria and their limitations. Moreover, the intermediate products of this biodegradation route must be researched, as the intermediate compounds can sometimes be beneficial or harmful.

## Conflict of interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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