Biodegradation of microcystin [Dha7]MC-LR by a novel microcystin-degrading bacterium in an internal airlift loop bioreactor

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ABSTRACT

Cyanobacterial hepatotoxin microcystins are a health problem found in bodies of water in Thailand. Bacteria capable of biodegrading microcystin [Dha7]MC-LR were isolated from the Bueng Nong Khot reservoir in Khon Kaen, Thailand. The bacterium Novosphingobium isolate KKU25s was shown to degrade [Dha7]MC-LR at a concentration of 25 μg/l C0 1 at 30 °C within 24 h. Two intermediate by-products (linearized peptide and tetrapeptide) and a cluster gene (mlr A, mlr B, mlr C and mlr D) that encodes four putative enzymes involved in [Dha7]MC-LR degradation were detected in KKU25s. KKU25s was also shown to form strong biofilms in microtiter plate assays. These assays were carried out in preparation for use of the bacterium in a bioreactor for [Dha7]MC-LR degradation. In an internal airlift loop bioreactor, the biodegradation of [Dha7]MC-LR by the bacterium was established in batch and continuous flow experiments. In the batch experiment, KKU25s degraded [Dha7]MC-LR at a concentration of 25 μg/l C0 1 at 30 °C within 24 h, whereas in the continuous flow experiment, KKU25s degraded the toxin at the same concentration within 36 h. This study demonstrated that this bacterium could potentially be used to remove microcystins from water.

Key words | biodegradation, bioreactor, by-product, microcystin, mlr gene, Novosphingobium

INTRODUCTION

Microcystins (MCs) are a group of heptapeptide hepatotoxins synthesized by planktonic cyanobacteria (blue-green algae), which comprise a diverse range of species from the genera Microcystis, Anabaena, Planktothrix, Oscillatoria, and Nostoc (Carmichael 1992). Their occurrence has been thoroughly documented in eutrophic water throughout the world. These toxins result in the illness and death of wild and domestic animals worldwide and are known to be potential threats to human health (Zurawell et al. 2005). Chronic exposure can occur through the presence of microcystins in drinking water, and this is thought to be a contributing factor to primary liver cancer because of the known tumor-promoting activities of these compounds (Nishiwaki-Matsushima et al. 1992).

These toxins are stable and undergo hydrolysis in the presence of strong acids or high temperatures (Lawton & Robertson 1999). Microcystins can exist for months or years in distilled water in the absence of light. However, there have been reports that naturally occurring bacteria can decompose microcystins. Sphingomonas strain MJ-PV (ACM-3962), the first bacterial strain found to use MC-LR as carbon and nitrogen sources for its growth, was successfully isolated from the Murrumbidgee River, Australia (Jones et al. 1994).

Research on microcystin production has been carried out in Thailand. Microcystins were first reported by Mahakhant and co-authors in 1998. Five heptapeptide toxins, MC-RR, -LR, -YR, -LA, and -AR, were detected in two reservoirs and a pond in Thailand (Mahakhant et al. 1998). Peerapornpisal et al. (2002) investigated toxic cyanobacteria and cyanotoxins, particularly microcystins, in six raw water resources used for water supplies from April 2000 to March 2001. MC-RR was found at every site in higher amounts. MC-LR and -YR were also found in some reservoirs but in small amounts. Prommana et al. (2006) further reported the presence of microcystins in scum and water samples collected from a giant freshwater prawn farm in Chiang Rai Province. MC-LR and -YR were the dominant microcystin types. Based on the results of these studies, microcystin production was suggested to represent a risk of microcystin
accumulation in bodies of water in Thailand. The major objectives of this study were as follows: (1) isolate bacterial strains with microcystin-degrading abilities from natural water; (2) identify the bacterial strains based on morphological and biochemical characteristics as well as the 16S rRNA gene sequence; (3) examine the mechanisms of bacterial [Dha]$^7$MC-LR degradation by detecting intermediate byproducts and genes involved in [Dha]$^7$MC-LR degradation from the identified bacterial strains; and (4) investigate the biodegradation of the toxin in an internal airlift loop bioreactor under batch and continuous operations.

**MATERIALS AND METHODS**

**Source of [Dha]$^7$MC-LR**

A large mass of *Microcystis aeruginosa* cell material was harvested from the Bueng Nong Khot reservoir, Khon Kaen, Thailand, between February and July 2010. The material was extracted and purified using the method as described by Somdee *et al.* (2014). Liquid chromatography mass spectrometry (LC-MS) analysis further identified the purified toxin, and high-performance liquid chromatography (HPLC) analysis determined the yields and purity.

**Analysis of the microcystin with HPLC and LC-MS**

Microcystin analysis employed an HPLC system with UV detection at a wavelength of 238 nm. The system contained a 600 pump controller, a 717 plus autosampler, a 2487 absorbance detector (Waters, USA) and a TSK-GEL ODS-80Ts column (150 × 4.6 mm) (Tosoh, Japan). The mobile phase consisted of acetonitrile:0.05 M phosphate buffer (pH 3.0) (30:70 v/v) and ran at a flow rate of 1 ml min$^{-1}$.

The LC-MS system consisted of a Waters Alliance 2695 liquid chromatograph with diode array detector (Waters, USA) and a Q-TOF 2 (quadrupole mass filter-time-of-flight) mass spectrometer with a Z-spray ES (electrospray) source (Micromass, UK). A 5 μm, 3.9 × 150 mm Symmetry C$_{18}$ column (Waters, USA) separated the toxins. The mobile phase consisted of acetonitrile:0.05 M phosphate buffer (pH 3.0) (50:70 v/v) and ran at a flow rate of 1 ml min$^{-1}$.

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The MS instrument without stream splitting.

**Isolation and identification of microcystin-degrading bacteria**

The sources of microcystin-degrading bacteria were water samples from the Bueng Nong Khot reservoir, Khon Kaen, Thailand, during a bloom of *Microcystis aeruginosa* in June 2012. The isolation process was carried out according to Phujomjai (2014). Bacterial isolate KKU25s was selected for further study.

Standard procedures according to Chan *et al.* (1993) characterized the morphological and biochemical properties of KKU25s. Additionally, scanning electron microscopy (SEM) was used to examine additional cell morphology. The samples for SEM were prepared according to Somdee *et al.* (2013).

Sequencing of 16S rRNA was also completed. Genomic DNA from bacterial isolate KKU25s was extracted according to Doyle & Doyle (1990). PCR (polymerase chain reaction) amplified the bacterial gene encoding 16S rRNA using the specific oligonucleotide primer set consisting of 16F27 (5’-AGA GTT TGA TCM TGG CTC AG-3’) and 16R1541 (5’-AAG GAG GTG ATC CAG CCG CA-3’). PCR was performed in a PTC-200 Peltier thermal cycler (MJ Research, USA) using 50 μl reactions containing 1× GoTaq® Green Master Mix (Promega, USA), 1 mM MgCl$_2$ (Promega, USA), 0.5 μM of each primer (Invitrogen, USA) and 20 ng of genomic DNA. The PCR amplification protocol was as follows: 94°C for 5 min, 35 cycles at (1) 94°C for 30 s, (2) 55°C for 30 s, (3) 72°C for 45 s, and a final extension step at 72°C for 5 min. PCR products were analyzed via 1% agarose gel electrophoresis. The MegaBACE 1,000 sequencing system (Amersham Biosciences, USA) performed the automated sequencing of the partial 16S rDNA. The BLAST network service (blastn) from the NCBI GenBank database conducted sequence similarity searches. A phylogenetic tree was constructed using the neighbor-joining algorithm method with 1,000 bootstrap replicates (MEGA 5.05 software).

**Detection of genes involved in [Dha]$^7$MC-LR degradation**

PCR primers previously used by Saito *et al.* (2003) amplified the $mlr$A gene, and the primers for $mlr$B, $mlr$C, and $mlr$D genes are listed in Ho *et al.* (2007). The PCR reactions consisted of 1× GoTaq® Green Master Mix (Promega, USA), 1 mM MgCl$_2$ (Promega, USA), 0.5 μM of each primer (Invitrogen, USA) and 20 ng of genomic DNA for a final volume
of 50 μl. A programmable thermal cycler (a PTC-200 Peltier Thermal Cycler; MJ Research, USA) amplified the reactions, with temperatures and times as previously described by Saito et al. (2003) and Ho et al. (2007). The MegaBACE 1,000 sequencing system (Amersham Biosciences, USA) completed the automated sequencing of the mtr genes. Cimarron 3.12 software analyzed the sequence data, and the sequences were compared in multiple alignments using ClustalW2 version 2.1 software. Blastn available at NCBI GenBank (www.ncbi.nlm.nih.gov/blast) obtained the reference strain data.

Detection of [Dha7]MC-LR-degrading by-products

The bacteria cultured in peptone yeast extract medium (PYEM) broth in a shaking incubator at 180 rpm and 30 °C for 48 h were centrifuged at 8,000 g for 15 min. The supernatants were decanted, and then the precipitates were added to sterile distilled water followed by centrifugation at 8,000 g for 15 min. This process of washing was repeated three times. Finally, the precipitate was added to sterile MSM broth with [Dha7]MC-LR for a concentration of 25 μg l⁻¹. The sample sat in the dark in a shaking incubator at 30 °C and 180 rpm for 24 h. Samples were taken every 6 h for 24 h. LC-MS analyzed the intermediate by-products as described in a previous section.

Degradation of [Dha7]MC-LR in an internal airlift loop bioreactor

[Dha7]MC-LR degradation of isolate KKU25s employed an internal airlift loop reactor. Previously, this type of bioreactor successfully removed a wide range of water pollutants, including 2,4-dichlorophenol and phenol, high-carbohydrate printing ink, and quinoline from wastewater. The bioreactor consisted of a glass cylinder bioreactor, a 10 L synthetic wastewater reservoir (0.1 g of (NH₄)₂SO₄, 0.5 g of KH₂PO₄, 0.5 g of Na₂HPO₄, 0.5 g of MgSO₄·7H₂O, and 0.02 g of yeast extract per litre; pH 7.2) plus [Dha7]MC-LR at the concentration of 25 μg l⁻¹, an air pump and a 0.22 μm air filter (Figure 1(a)). The center of the bioreactor unit contained a plastic medium (Figure 1(c)) with a diameter of 75 mm and a height of 180 mm; the bacterial cells were immobilized on this medium. A 0.22 μm sterile filter continuously fed clean air into the apparatus, and a diffuser placed inside the bioreactor at the bottom introduced the clean air at a flow rate of 5 L min⁻¹. Air circulation caused the ‘synthetic wastewater’ to flow upward in a loop over the reactor. Additionally, a recipient flask and a peristaltic pump were used when the bioreactor was operated in the continuous mode experiment.

Cell immobilization on a plastic medium

The bacterial isolate KKU25s grew in 50 ml of PYEM broth in a shaking incubator at 180 rpm and 30 °C for 48 h. The 48 h culture inoculated 1,500 ml (2% v/v) of fresh PYEM broth and was incubated in a shaking incubator at 30 °C and 180 rpm for 24 h. After 24 h of incubation, the bacterial culture was poured directly into the plastic medium so that the plastic medium immobilized the cells. The bioreactor, complete with the plastic medium unit containing immobilized cells, was then incubated at 30 °C for 24 h on a shaking incubator at 90 rpm. The entire bacterial culture therefore incubated for a total of 48 h (which corresponds to a late exponential growth phase for KKU25s and promoted sufficient biofilm formation on the plastic medium).

Batch experiment for [Dha7]MC-LR degradation

After the plastic medium immobilized the cells for 48 h, the plastic medium was washed with 10 L of sterile synthetic

![Figure 1](http://iwaponline.com/wst/article-pdf/73/2/267/464804/wst073020267.pdf)
wastewater to remove excess culture medium. The plastic medium was packed into the center of the sterile bioreactor (Figure 1(a)), which contained fresh synthetic wastewater plus [Dha\(^7\)]MC-LR at the concentration of 25 \(\mu\)g/C\(0\)\(^1\). It was shown in a previous study that this is the optimum concentration for microcystin degradation of KKU25s (Phujomjai 2014). The bioreactor was incubated in a water bath at 30 °C for 48 h. The samples (1 ml) were withdrawn from the bioreactor after 0, 6, 12, 18, 24, 30, 36, 42 and 48 h. HPLC analyzed the remaining [Dha\(^7\)]MC-LR. The controls consisted of sterile synthetic wastewater in the absence of bacteria on the plastic medium with the toxin at the concentration of 25 \(\mu\)g l\(^1\).

**Continuous experiment for [Dha\(^7\)]MC-LR degradation**

These experiments were carried out using a similar setup to that used for the batch experiments with the exception that the bioreactor was changed to flow-through mode with the addition of a peristaltic pump with which the synthetic wastewater, containing [Dha\(^7\)]MC-LR at the concentration of 25 \(\mu\)g/C\(0\)\(^1\), continuously flowed from an adjacent ‘reservoir’ through the bioreactor to accumulate in a recipient flask. Figure 1(b) shows a schematic diagram of the continuous experiment. A water bath covered with aluminum foil was used to incubate the continuous bioreactor at 30 °C for 48 h in the dark. The influent and effluent flowed at a rate of 2 ml min\(^1\), and the hydraulic retention time was 8 h. Every 6 h for 48 h, an aliquot of solution was withdrawn from the effluent tube. HPLC analyzed the remaining [Dha\(^7\)] MC-LR. The control was sterile synthetic wastewater in the absence of bacteria on the plastic medium with the toxin at the concentration of 25 \(\mu\)g l\(^1\).

**RESULTS**

**Isolation and identification of microcystin-degrading bacteria**

To obtain bacteria that were capable of degrading [Dha\(^7\)]MC-LR, water samples obtained from the Bueng Nong Khot reservoir, Khon Kaen Province, Thailand, were incubated in MSM broth with [Dha\(^7\)]MC-LR as the sole carbon and nitrogen source. A total of 18 bacterial strains were isolated; only one isolate, KKU25s, was able to degrade [Dha\(^7\)]MC-LR at the concentration of 25 \(\mu\)g l\(^1\) at 30 °C within 24 h. Therefore, the bacterial isolate KKU25s was used for further study.

The bacterial isolate KKU25s possesses [Dha\(^7\)]MC-LR degradation ability and is an aerobic, Gram-negative, slow-growing bacterium that formed pale-yellow colonies on PYEM agar. The colonies were particularly adhesive to the agar medium and could not be easily removed. A SEM found that the morphology of the bacterial cells was rod-shaped, 1.2–1.5 \(\mu\)m in length, and 0.3–0.5 \(\mu\)m in width.

The isolate KKU25s gave negative results in all tests for conventional biochemical methods, with the exception of the oxidase and catalase reactions (data not shown). The isolate could not utilize glucose, xylose, mannitol, lactose, sucrose or maltose as a carbon source. It grew on TSA (trypticase soy agar) but not on MacConkey agar. The temperature range permissive for growth was 10–37 °C, and the optimum temperature for growth was 30 °C. There was slow growth at 37 °C, and there was no growth at 45 °C.

The 16S rRNA sequence of KKU25s was determined and compared with those in the GenBank, EMBL, and DJB prokaryote databases. The databases revealed that the 16S rRNA sequence of the bacterium resembled that from the *Novosphingobium* strain MG37 (accession number AJ746094) with 98% sequence homology (Figure 2). *Novosphingobium* sp. MG37, a bacterium isolated from cultured bacteria present in hemodialysis water and fluid found at the Hospital Son Llatzer (Palma de Mallorca, Spain) (Gomila et al. 2005), is classified as a *Novosphingobium* sp. and exhibits 97% homology with the 16S rRNA sequence of *Novosphingobium stygium*. Therefore, at this stage, the isolate KKU25s was tentatively classified as *Novosphingobium* sp. KKU25s.

![Figure 2](http://iwaponline.com/wst/article-pdf/73/2/267/464804/wst073020267.pdf)

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Detection of genes involved in [Dha\textsuperscript{7}]MC-LR degradation

The bacterium KKU25s was screened for the \textit{mlr}A, \textit{mlr}B, \textit{mlr}C and \textit{mlr}D genes. The PCR products were visualized using gel electrophoresis (Figure 3). It was found that KKU25s contained the listed genes. The sequences of \textit{mlr}A, \textit{mlr}B, \textit{mlr}C and \textit{mlr}D from \textit{Novosphingobium} sp. KKU25s were examined using blastn. The \textit{mlr}A sequence of the isolate KKU25s exhibited close similarity to \textit{mlr}Ao of \textit{Sphingomonas} sp. MD-1 (accession number AB114202) with 99% identity, whereas the sequences of \textit{mlr}B, \textit{mlr}C and \textit{mlr}D from the isolate KKU25s were 99% similar to the homologous genes from \textit{Novosphingobium} sp. THN1 (HQ664118).

Detection of microcystin-degrading by-products

The biodegradation of [Dha\textsuperscript{7}]MC-LR by KKU25s was induced using [Dha\textsuperscript{7}]MC-LR. The [Dha\textsuperscript{7}]MC-LR concentration began to decrease, and HPLC detected two peaks, referred to as intermediate by-products ‘A’ and ‘B’ (chromatogram not shown). The peaks consistent with the by-products increased slowly, whereas the peak consistent with the parent toxin decreased. The intermediate by-products were further investigated using LC-MS. The [M + H]\textsuperscript{+} observed at \textit{m/z} 981.5745 confirmed that [Dha\textsuperscript{7}]MC-LR was present in the samples (Figure 4(a)). The weaker \textit{m/z} 999.6807 was a fragment of the linearized peptides of [Dha\textsuperscript{7}]MC-LR (by-product A), which is 14 mass units less than the linearized peptides of MC-LR (\textit{m/z} 1013), as reported by Bourne et al. (1996). Biodegradation by-product B exhibited a base peak at \textit{m/z} 601.5466 (Figure 4(b)). By-product B also exhibited fragment ion peaks at \textit{m/z} 584.5497 ([M + H]-NH\textsubscript{3}) and \textit{m/z} 450.8043 ([M + H]-PhCH\textsubscript{2}CHOMe-NH\textsubscript{2}) (Figure 4(b)), related to the tetrapeptide.

Degradation of [Dha\textsuperscript{7}]MC-LR in the bioreactor

Figure 5 shows the degradation by KKU25s of [Dha\textsuperscript{7}]MC-LR at the concentration of 25 \textmu g l\textsuperscript{-1} in batch and continuous experiments. In the batch experiment, the toxin was completely degraded within 24 h (Figure 5(a)). The degradation rate was calculated to be approximately 1.04 \textmu g l\textsuperscript{-1} h\textsuperscript{-1}. However, in the continuous experiment, [Dha\textsuperscript{7}]MC-LR was degraded by approximately 75\% after 24 h incubation and completely degraded within 36 h (0.7 \textmu g l\textsuperscript{-1} h\textsuperscript{-1}) (Figure 5(b)).
The presence of microcystins in freshwater is an increasing problem and poses a threat to human and animal health worldwide. These toxins are well recognized as stable and persistent compounds; however, reports have shown that the toxins are vulnerable to breakdown by indigenous bacteria (Jones et al. 1994). The bacterium *Sphingomonas* sp. strain ACM-3962 (Mj-PV) was one of the first microcystin-degrading bacteria to be identified (Jones et al. 1994). Since then, the number of known microcystin-degrading bacteria has increased. Thus far, these bacteria have been identified as belonging to a number of genera, including *Arthrobacter*, *Brevibacterium*, *Bordetella*, *Burkholderia*, *Novosphingobium*, *Paucibacter*, *Pseudomonas*, *Rhodococcus*, *Sphingomonas*, *Sphingopyxis*, *Sphingosinicella*, and *Stenotrophomonas* (Dziga et al. 2013). In this study, a microcystin-degrading bacterium was isolated from the Bueng Nong Khot reservoir, Khon Kaen Province, Thailand. Based on a morphological, biochemical, and phylogenetic analysis, KKU25s most likely belongs to the *Novosphingobium* sp.

Notably, most of the microcystin-degrading genera, including *Burkholderia*, *Paucibacter*, *Pseudomonas*, *Sphingomonas*, *Sphingosinicella*, *Sphingopyxis*, *Stenotrophomonas* and *Novosphingobium*, are Gram-negative, obligate aerobic rod-shaped bacteria. Saito et al. (2003) suggested that the ability to degrade microcystins is present only in specific strains of the genus *Sphingomonas* and related genera, including *Novosphingobium* sp. KKU25s isolated in this study. Therefore, the bacteria that belong to these genera may be derived from common ancestors that have the ability to break down microcystins. The genus *Novosphingobium* was previously part of the genus *Sphingomonas*. Based on 16S rRNA sequences and cellular lipid and fatty acid composition, the genus *Novosphingobium* was reclassified by Takeuchi and colleagues in 2001 as a new genus within the family *Sphingomonadaceae* on the basis of phylogenetic, chemotaxonomic and phenotypic studies (Takeuchi et al. 2001).

Studies on microcystin biodegradation have primarily focused on the degradation of the MC-LR analog because it is found worldwide and is highly toxic (Zurawell et al. 2003). The degradation of other microcystin analogs, such as MC-RR, MC-YR, MC-LW and MC-LF, has also been examined (Dziga et al. 2013). In this study, [Dha\(^7\)]MC-LR was used as a substrate to characterize the pathway by which KKU25s degrades these toxins. Based on the intermediate degradation by-products detected, at least three hydrolytic enzymes are involved in this pathway. The first enzyme catalyzes the aromatic ring of [Dha\(^7\)]MC-LR (cyclo(-D-Ala\(^1\)-Leu\(^2\)-D-MeAsp\(^3\)-Arg\(^4\)-Adda\(^5\)-D-Glu\(^6\)-Dha\(^7\)), MW = 981) at the Adda-arginine peptide bond to yield linearized peptides (NH\(_2\)-Adda-D-Glu-Dha-D-Ala-L-Leu-D-MeAsp-L-Arg-OH; MW = 999) (by-product A). The second enzyme cuts linearized [Dha\(^7\)]MC-LR at the alanine–leucine peptide bond, producing a tetrapeptide (NH\(_2\)-Adda-D-Glu-Dha-D-Ala-OH; MW = 601) (by-product B). Finally, the third enzyme presumably cleaves small peptides to produce undetectable or unidentified peptide fragments. This enzymatic pathway is identical to that previously identified by Bourne et al. (1996).

To further understand the mechanisms of microcystin degradation, Bourne et al. (2001) performed the cloning and molecular characterization of four genes from the *Sphingomonas* strain ACM-3962 to identify the microcystin-degrading gene cluster consisting of *mlr* \(A\), *mlr* \(B\), *mlr* \(C\) and *mlr* \(D\). The *mlr* \(A\), *mlr* \(B\), *mlr* \(C\) genes express three peptidases, whereas the *mlr* \(D\) gene is predicted to be a transport protein involved in microcystin degradation (Bourne et al. 2001). In this study, the *mlr* \(A\), *mlr* \(B\), *mlr* \(C\) and *mlr* \(D\) genes in the genomic DNA of KKU25s were detected, and the amplified nucleotide sequences of the *mlr* genes were identical,

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**DISCUSSION**

**Figure 5** Percentage of [Dha\(^7\)]MC-LR remaining in the bioreactor in (a) batch mode and (b) continuous flow experiments.
indicating that the bacterium has the ability to degrade microcystins in the same manner as *Sphingomonas* strain ACM-3962. Therefore, the key findings from by-products A and B and the detection of *mlrA*, *mlrB*, *mlrC* and *mlrD* genes indicate that the degradation of [Dha7]MC-LR by KKU25s occurs via a similar mechanism for the degradation of MC-LR as described by Bourne *et al.* (1996, 2001).

Biodegradation of microcystin was previously tested in a small scale bioreactor. Tsuji *et al.* (2006) revealed that MC-RR was decomposed in a bioreactor using immobilized cells of the bacterium *Sphingomonas* sp. (strain B-9) on a polyester resin. In this study, the internal airlift loop bioreactor was used to decompose microcystin. A bioreactor with plastic medium for KKU25s cell immobilization was successful in removing [Dha7]MC-LR at the concentration of 25 μg l⁻¹ within 24 h in the batch experiment and within 56 h in the continuous flow experiment. The degradation rates for the immobilized cells in the batch experiments and the continuous flow experiments were 1.04 μg l⁻¹ h⁻¹ and 0.7 μg l⁻¹ h⁻¹, respectively. The immobilized bacteria in the batch experiment can degrade the toxins more rapidly than the continuous flow experiment. In the batch experiments, the toxin was circulated within the bioreactor by internal air lift for the 48 h duration of the experiment. During this time, the biomass of bacteria within the bioreactor would probably have increased. In contrast, with a single pass of the synthetic wastewater in the continuous flow experiment, with only an 8 h residence time, it is perhaps not surprising the degradation rate was slightly less. From these results, it appears that the removal of microcystins in a bioreactor (in which KKU25s cells were immobilized onto plastic medium) is an effective and promising process for microcystin biodegradation. In addition, a pilot-scale system would be recommended for further study.

**CONCLUSIONS**

A bacterium, KKU25s, isolated from the Bueng Nong Khot reservoir in Khon Kaen, Thailand, is capable of degrading [Dha7]MC-LR. On the basis of morphological and biochemical characteristics as well as the 16S rRNA gene sequence, the isolate is likely a member of the genus *Novosphingobium*. Based on the detection of two intermediate by-products of [Dha7]MC-LR and the identification of a cluster gene involved in microcystin degradation, the degradation of [Dha7]MC-LR by KKU25s is suggested to occur by a similar mechanism as the *Sphingomonas* strain MJ-PV. The biodegradation of [Dha7]MC-LR by KKU25s in an internal airlift loop bioreactor is established. In the batch experiment, KKU25s break down [Dha7]MC-LR at the concentration of 25 μg l⁻¹ at 30°C within 24 h, whereas in the continuous flow experiment, KKU25s break down the same concentration of the toxin within 56 h.

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