Physics and Chemistry of the Earth xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Physics and Chemistry of the Earth

journal homepage: www.elsevier.com/locate/pce

Potential use of dissolved cyanobacterial DNA for monitoring toxic *Microcystis* cyanobacteria in filtered water

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Dissolved DNA
PCR template
Primers
Mcy genes
Toxic *Microcystis* sp.
Water quality

ABSTRACT

Toxic and non-toxic *Microcystis* sp. are morphologically indistinguishable cyanobacteria that are increasingly posing health problems in fresh water systems by producing odours and/or toxins. Toxic *Microcystis* sp. produces toxicologically stable water soluble toxic compounds called microcystins (MCs) that have been associated with cases of aquatic life and wildlife poisoning and kills including some cases of human illnesses/deaths around the world. Thus, the need for rapid detection of toxic *Microcystis* sp. in surface water is imperatively a necessity for early mitigation purposes. Genomic DNA from potentially toxic *Microcystis* sp. comprises of ten microcystin synthetase (*mcy*) genes of which six major ones are directly involved in MCs biosynthesis. In Polymerase Chain Reaction (PCR) methods*mcy* genes can be amplified from intracellular/extracellular genomic DNA using PCR primers. However, little is known about the limitations of sourcing genomic DNA templates from extracellular DNA dissolved in water. In this work, filtered water (0.45 μM) from a *Microcystis* infested Dam (South Africa) was re-filtered on 0.22 μM syringe filters followed by genomic DNA isolation and purification from micro-filtrates (9 mL). Six major *mcy* genes (*mcy*ABCDEG) from the isolated DNA were amplified using newly designed as well as existing primers identified from literature. PCR products were separated by gel electrophoresis and visualized after staining with ethidium bromide. The limitation of using dissolved DNA for amplification of *mcy* genes was qualitatively studied by establishing the relationship between input DNA concentrations (10.0–0.001 ng/μL) and the formation of respective PCR products. The amplification of *mcy*A gene using new primers with as little as 0.001 ng/μL of DNA was possible. Other *mcy* gene sensitivities reached 0.1 ng/μL DNA dilution limits. These results demonstrated that with appropriately optimized PCR conditions the method can provide accurate cost-effective tools for rapid detection of toxic *Microcystis* sp. in water giving early information for water quality monitoring against MC producing cyanobacteria.

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1. Introduction

The World Bank's mid-year 2012 report on drinking water supply and sanitation estimated that over 40% of people with no access to safe drinking water live in sub-Saharan Africa (WHO, 2012). Rural populations are most disadvantaged as they depend on untreated surface water as the main supply for drinking water and sanitation. Rapid increase in the world's population (Xie, 2006) affects both rural and urban surface water resources (such as rivers, ponds, and lakes) due to pollution as a result eutrophication has escalated leading to growth and persistence of noxious cyanobacteria (Anderson et al., 2002; Vasconcelos, 2006). Cyanobacteria, also known as blue green-algae, are photosynthetic gram-negative single cell organisms, which on aggregation form surface algal scums (algal blooms) on water bodies especially during summer seasons (Carmichael et al., 1988). Their occurrences year round have, however, been reported elsewhere in the world (Kumar et al., 2011). Algal blooms are particularly of health concern as they inflict water quality by producing unpleasant smell (odours) and deplete dissolved oxygen as they die off. But, the worst impact feared most are toxic compounds called cyanotoxins produced by some algal species (Carmichael, 1992, 2001). A group of cyanotoxins called microcystins (MCs) are potent poisonous water soluble compounds comprising of more than eighty (80) variants widely distributed in fresh water systems (WHO, 1999). High toxicity levels of microcystins have often caused massive kills of aquatic-life forms, wildlife and domesticated animals around the

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http://dx.doi.org/10.1016/j.pce.2013.07.004

Please cite this article in press as: Mbukwa, E.A., et al. Potential use of dissolved cyanobacterial DNA for monitoring toxic *Microcystis* cyanobacteria in filtered water. J. Phys. Chem. Earth (2013), http://dx.doi.org/10.1016/j.pce.2013.07.004

world (WHO, 1999). Human illnesses (such as diarrhoea, vomiting, headaches, and skin itches) and/or deaths have been reported due to MC intoxication as a result of people drinking, swimming or consuming sea foods harvested from water contaminated with toxic algal blooms (Carmichael et al., 2001).

Co-occurrence of MC producing (toxic) and non-toxic algal species in eutrophic water bodies is a growing worldwide problem (WHO, 1999). However, the two species are indistinguishable based on morphological appearances or colours (Kumar et al., 2011). In addition to this, any produced MCs cannot be completely removed by commonly applied filtration processes for drinking water. It is further known that dissolved MCs cannot be degraded through boiling drinking water or cooking contaminated sea food (WHO, 1999). Therefore, low level MCs in water poses serious health hazards including liver cancer developments (Nishiwaki-Matsushima et al., 1992). Hence, the WHO (1999) limits the amount of the most toxic MC (MC-LR) in drinking water to 1.0 μg/L. This limit is, however, difficult to confidently declare that it is being adhered to especially in rural sub-Saharan countries where surface water remains the major source of drinking water from which cases of algal contamination are likely to occur unnoticed as observed by Fosso-Kankeu et al. (2008). This problem calls for development of low cost approaches appropriate to sub-Saharan Africa (Simonis and Basson, 2012; Makurira et al., 2012) for the monitoring of cyanobacteria and their toxins. The integration of such approaches to the Integrated Water Resources Management (IWRM) policy should ensure that rural populations are involved and made aware of the dangers posed by toxic algae in drinking water. The involvement of the community in the fight against water pollution would lead to alleviation of water eutrophication and prevention of algal growth leading to sustainable and accessible water free from MC producing algae, such as *Microcystis* spp.

Molecular-based methods for the identification of toxic *Microcystis* spp. in the water using Polymerase Chain Reaction (PCR) have been developed (Pearson et al., 2010). The methods involve the use of specific PCR primers in the amplification of ten (10) microcystin synthetase (*mcy*) genes. The latter are specialized algal genes which mediate the biosynthesis of MCs in toxic *Microcystis* spp. Unlike analytical methods (e.g. for isolation and LC–ESI–MS analysis of MCs), molecular-based methods require minute amounts of algal biomass for DNA isolation and are relatively cheaper and quicker. Six *mcy* genes named as *mcy*A, B, C, D, E and G are known to be directly involved in MC biosynthesis in *Microcystis* spp. (Pearson et al., 2010). Thus, their identification from a given species signifies its potential for MC production. Almost all toxic *Microcystis* spp. have all six *mcy* genes in their DNA if no mutation has occurred and this signifies their full potential for MC production (Meissner et al., 1996; Ouahid et al., 2005; Kumar et al., 2011). The presence of *mcy* genes in toxic species gives a differentiation tool from non-toxic species (Ouahid et al., 2005) providing vital first hand information on the toxicological status of the bloom for early mitigation purposes. A further analytical confirmation for MC production from potentially toxic species is usually recommended and in particular the use of LC–ESI–MS technique gives reliable information on structural identities of MCs (Msagati et al., 2006; Trojanowicz, 2010; Mbukwa et al., 2012a,b).

To increase the confidence level in the PCR amplification of *mcy* genes from toxic *Microcystis* spp. in the water environment, Ouahid et al. (2005) recommended that various *mcy* genes (basically the six major *mcy* genes) should be amplified from field or cultured *Microcystis* cells. Recently, Kumar et al. (2011) reported a successful PCR amplification of the recommended six *mcy* genes using short fragments of extracellular *Microcystis* DNA dissolved in water. But in practice at any given time the concentration of dissolved DNA in water is dynamically variable. As a result, chances are that the sensitivity and/or quality of PCR products to be formed during PCR reaction would differ for proper amplification of all six *mcy* genes especially from water samples characterized by invisible algal blooms.

In this work therefore, the sensitivity/quality of formation of six major *mcy* PCR products was investigated using various concentrations of extracellular dissolved *Microcystis* DNA to determine a minimal input DNA concentration required in the formation of *mcy*A, B, C, D, E and G PCR products in a standardized PCR reaction. PCR primers used in this study were chosen based on their successful use in the amplification of *mcy* genes from intracellular DNA as reported in Tillett et al. (2001), Mikalsen et al. (2003) and Ouahid et al. (2005).

2. Materials and methods

2.1. Study area and sample collections

Water samples and algal cells used in this study were collected from the *Microcystis* dominated Hartbeespoort Dam (GPS: 25°43′44.56″S, 27°51′30.35″E) located in North-West Province, South Africa. The samples were from four sites (S1–S4) identified in our previous publication (Mbukwa et al., 2012b). Algal cells were collected and treated as described in Mbukwa et al. (2012b) prior to DNA isolation. Water samples were collected between 1 and 5 m below water surface into 250 mL bottles and all samples were transported to the laboratory in cooler boxes filled with ice blocks. On arrival, water samples were first filtered on glass-fiber filters (0.45 μM) to completely remove algal cells. To minimize cell lysis during filtration the procedure was performed at low pressure using small portions (20–50 mL) of water samples per time.

2.2. Isolation of DNA from Microcystis algal cell samples

Ten (10) mg of freeze dried algal cells were accurately weighed and DNA isolated using a modified proteinase K/chloroform-phenol procedure described in Mbukwa et al. (2012b).

2.3. Isolation of dissolved Microcystis DNA from water samples

Ten (10) mL of the water filtrates were re-filtered through 0.22 μM membrane syringe filters and the micro-filtrates (9 mL) subjected to DNA extraction using DNeasy min plant Kit (Qiagen, Germany Gmbh) following the manufacture's protocol, albeit with slight modifications as explained in Mbukwa (2013a). All DNA extracts and unutilized water filtrates and/or algal cells were stored at −25 °C till further use.

2.4. Determination of the quality/purity and quantity of extracted DNA

The quality/purity and quantity of the isolated DNA were determined using a NanoDrop ND-1000-UV–Vis Spectrophotometer, v3.7 (Thermo Scientific, USA) according to Sambrook et al., 1989.

2.5. PCR primers and the amplification of mcy genes and 16S rDNA

The choice of PCR primers used in this study was based on their previous success in the amplification of *mcy* genes elsewhere as cited below. However, since universal primers do not exist (Valério et al., 2010) a pilot investigation was conducted to test the suitability of the chosen primers prior to a full scale experimental work for the amplification of *mcy* genes. Primers were sourced accordingly for *mcy*A (Tillett et al., 2001); *mcy*B (Mikalsen et al., 2003) and *mcy*C, -D, -E and G (Ouahid et al., 2005). In addition four newly de-

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Table 1
Additional PCR primer pairs for the amplification of *mcy*A and *mcy*B from a toxigenic *M. aeruginosa* dominating the Hartbeespoort Dam.

Target gene	Primer ID	Primer sequence 5′ → 3′	Annealing temp. (°C)	Diagnostic PCR product size (bp)	Reference
mcyA	u-102F u-620R	CGATGAACAAATCGGGCAATGGCA TGCAAGTTTCGCACATCTCCAAGG	55	518	(Mbukwa et al., 2013c)
mcyB	P5102F P5853R	AGTCATCATCTTCCTTCACCGCGT TGTCCTGCCATCCGTTCAATCGTA	55	751	″

Table 2
Quantity and quality/purity of *Microcystis* DNA extracted from algal cells and water samples using two extraction methods ($n = 2$) for the February 2011 samples collected from the Hartbeespoort Dam.

Extraction method	Source of cyanobacterial DNA	DNA yield (ng/μL)	Observed typical% DNA purity	
		Approx. range 0–1400 ng/μL	260/280	260/230
Proteinase K treatment followed by Chloroform-phenol extraction	a. Nuclease free water (−ve control)	0	0	0
	b. Intracellular DNA of *M. aeruginosa* extracted from freeze-dried cells (10 mg):[†]	Average values		
	S1 (surface)	1417.1	1.96	2.0
	S2 (surface)	1099.1	1.91	2.0
	S3 (surface)	817.3	1.98	2.0
	S4 (surface)	857.0	1.86	2.1
DNeasy min plant extraction Kit (Qiagen, Germany)	c. Extracellular/dissolved DNA of *M. aeruginosa* extracted from micro-filtrate water (9 ml):[a]	Average values		
	S1 (5 m)	29.8	1.98	2.0
	S2 (5 m)	25.2	1.94	2.0
	S3 (1 m)	15.4	1.97	2.1
	S4 (1 m)	11 > 5	1.96	2.0
	d. Tap water	<0	0.03	–

[a] Site map (S1–S4) is detailed in Mbukwa et al. (2012b).

signed primers (Mbukwa, 2013a) suitable for the amplification of *mcy*A and *mcy*B from *Microcystis aeruginosa* dominating the Hartbeespoort Dam (Table 1) were also used in this study. The cyanobacterial *16SrDNA* was amplified using a cyanobacterial specific 16SF/16SR primer pair with a base pair sequence of 5′-CTGAAGAAGAGCTTGCGTC-3′ and 5′-CCCAGTAGCACGCTTTCG-3′, respectively. All primers were synthesized at Integrated DNA Technologies (Leuven, Belgium). The PCR reaction was programmed as described in Mbukwa (2013a) after which PCR products were separated using 1% gel electrophoresis and visualized after staining with 5 μL of ethidium bromide.

2.6. Determination of the sensitivity of the amplification of mcy genes from diminishing dissolved Microcystis DNA

To simulate the dynamism of the dissolved *Microcystis* DNA in natural water environment, the isolated dissolved DNA was serially diluted in nuclease free water into 10.0, 3.0, 1.0, 0.33, 0.1, 0.05, 0.001 ng/μL DNA aliquots (duplicates) and used to study the relationship between the formation of PCR products (amplified *mcy* genes) and the input DNA in a standardized PCR reaction (Promega polymerase Gotaq™ Green Master Mix 2x protocol, Promega Corporation, USA).

3. Results and discussion

3.1. Site selection

The Hartbeespoort Dam is a recipient of the effluent from wastewater treatment plants serving highly populated areas including Johannesburg, Pretoria, Midrand and Krugersdorp metros. Often wastewater is rich in phosphates and nitrogenous compounds which lead to water eutrophication and algal bloom proliferation (Oudra et al., 2000; Vasconcelos and Pereira, 2001; Tanada et al., 2003) and so is the Hartbeespoort Dam. While a diversity of algae constitute blooms in wastewater effluents, the Hartbeespoort Dam is uniquely dominated by *M. aeruginosa* as the only toxic algal bloom found in this reservoir (Harding and Paxton, 2001; Oberholster and Botha, 2010) dating back since the 1970s. Since the concentrations of substances in water are naturally dynamic, the uniqueness of the Hartbeespoort Dam in terms of the presence of a single dominant toxic algal bloom in a 20 km^2 sized Dam provided the unique natural water environment where concentration variation of the dissolved DNA from the same *Microcystis* sp. would be expected. The results from this study demonstrate application and limitation of use of variable concentrations, and in particular the diminishing amounts of dissolved DNA from natural waters for a successful PCR amplification of all recommended target *mcy* genes.

3.2. Isolation, quantity and quality/purity of DNA extracts

Two approaches for DNA isolation were employed in this study and these were the proteinase k/phenol–chloroform and DNeasy Plant Mini Kit (Table 2). The former method usually involves a number of steps and therefore the method is well suited where large samples of extraction material are available (e.g. freeze dried algal cells). Therefore, for the isolation of dissolved DNA from water samples, DNeasy Plant Mini Kit (Qiagen) were used in this case, because, these Kits are well known for their extraction efficiency and recovery of high purity DNA and thus they are very useful where low DNA yields would be expected (Qiagen DNeasy Handbook, 2000).

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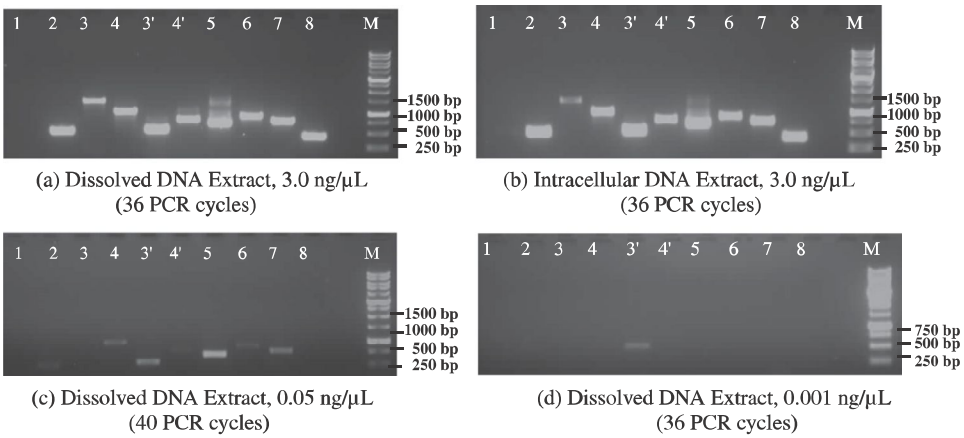


Fig. 1. (a–d): 1% agarose gel electrophoresis of PCR products showing amplified *mcy* genes from DNA extracts of a toxigenic *Microcystis aeruginosa* from the Hartbeespoort Dam, South Africa. Lane 1 = negative control; lane 2 = 16SrRNA (positive control); lane 3 = *mcy*A (1300 bp); lane 4 = *mcy*B (955 bp); lane 3′ = *mcy*A (518 bp); lane 4′ = *mcy*B (751 bp); lane 5 = *mcyC* (674 bp); lane 6 = *mcy*D (859 bp); lane 7 = *mcy*E (755 bp); lane 8 = *mcy*G (425 bp); lane M = Standard DNA ladder (1 kb).

In this study, both extraction methods resulted in high quality and purity DNA extracts suitable for PCR reactions based on spectrophotometric measurements (Table 2). From algal cells, good yields (>400 ng/μL) of *Microcystis* DNA was extracted from 10 mg of algal cells, whereas as water samples yielded between 5 and 29 ng/μL of DNA extracted from 9 mL of micro-filtered water samples (Table 2). There were observable differences in the amounts of DNA isolated from different sites in the Dam using both methods (Table 2). The variation in the amounts of dissolved DNA isolated from water samples seemed to be directly related to the mass of algal blooms prevailing on the water surface during sampling time. For instance, in mid-summer (February, 2011) relatively higher amounts of dissolved DNA (15–29 ng/μL) were extracted from sites S1 and S2 where higher algal masses prevailed on water surfaces than sites S3 and S4 with sparsely distributed algae cells as observed earlier (Mbukwa et al., 2012b). However, both methods yielded high quality/purity of the same *Microcystis* DNA extracted from all sites/depths (Table 2). The extracted *Microcystis* DNA from both water and algal cells was identified as described in Section 3.3 below. From Table 2, the variations of the dissolved DNA concentration among sites (S2 > S1 > S4 ⩾ S3) indicated that the concentration at a particular area is probably related to the number of possible cellular deaths from surface algal cells and water dynamics. For instance, algal cell deaths due to overpopulation and competition over resources are expected to be higher where massive algal cells dominate in the Dam than where cells are sparse, thus, lower DNA concentration is expected in the latter case. Low concentrations of dissolved DNA were observed at sites S3 and S4 compared to densely populated sites S1and S2 as supported by literature with regard to cell density (Mbukwa et al., 2012b; DWA, 2012). Tap water (Table 2) did not yield any detectable dissolved *Microcystis* DNA a possible indication that the studied tap water was free from *Microcystis* sp.

3.3. Identification of cyanobacterial DNA from complex matrices

In natural water systems exists highly complexed mixture of dissolved DNA fragments of different origins; including different bacterial cells, plant, animal and aquatic life tissues. However, the DNA sequence of a particular group of organisms is unique and therefore species identification based on molecular methods has been successfully used in taxonomy (Pearson et al., 2010). One of the straightforward ways to identify cyanobacterial DNA is the amplification of cyanobacterial 16S rRNA gene using gene specific PCR primers. From Fig. 1a–c (lanes 2), it was shown and confirmed that DNA extracts used in this study were of cyanobacterial origin by the formation of a PCR product (500 bp) pertaining to a cyanobacterial 16S rRNA gene. The product resulted from the PCR amplification using 6SF/16SR PCR primers. The product (500 bp) was consistently formed from all dissolved DNA extracts (Fig. 1a). These results were backed up by the amplification of the same gene using algal cell-based DNA extracts (Fig. 1b) from *M. aeruginosa* cells collected from water surface at the same sampling site. The absence of a 16S rRNA band (lane 2) in Fig. 1d is discussed in Section 3.5.

3.4. Amplification of mcy genes from M. aeruginosa DNA extracts

The absence of visible algal blooms on water surfaces presents a deceiving health safety to water users and the general public due to possibilities of presence of toxigenic algal species in surface water, storage tanks (Fosso-Kankeu et al., 2008) or on sediments (Preston et al., 1980). Six *mcy* genes were successfully amplified from *M. aeruginosa* DNA extracts from both water (short fragments of dissolved DNA) (Fig. 1a) and surface floating cells (intracellular) (Fig. 1b). The above results showed that all six mcy genes were amplified from all samples sourced from different sites and depths presented in Table 2. *Mcy* bands observed on lanes 3–8 were comparable to previously reported amplicons for *mcy* genes from *M. aeruginosa* cells corresponding to*mcy*A (Tillett et al., 2001); *mcy*B (Mikalsen et al., 2003); *mcyC*, -D, -E and -G (Ouahid et al., 2005), respectively, (Fig. 1a and b). On the other hand the bands on lane 3′ (*mcy*A) and lane 4′ (*mcy*B) (Fig. 1a and b) resulted from the use of the most recently designed primers (Mbukwa, 2013a). Unlike other studies which amplified *mcy*A gene with difficulties from this Dam (Oberholster and Botha, 2010), probably due to primer weaknesses, the new primers for the amplification of *mcy*A gene (Table 1) successfully and consistently amplified the *mcy*A gene from all samples of a toxigenic *M. aeruginosa* studied from this Dam. The same phenomenon was observed for *mcy*B using its new set of a primer pair (Table 1). Thus, the amplification of all six *mcy* genes from all samples studied indicated that the isolated DNA dealt with in this study belonged to the potentially toxic *Microcystis* sp. (Ouahid et al., 2005; Ouahid and Del Campo, 2009). These results collaborate with reports of Oberholster and Botha (2010) who amplified *mcy*B and -D genes and partly *mcy*A gene. The occurrence of this toxigenic *Microcystis* sp in this Dam is further supported by a more recent publication of Conradie and Barnard (2012) who amplified *mcy*B and -E genes from algal cells. Respective sizes (bp) of PCR products mentioned in this sec-

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tion are indicated in Fig. 1 caption. The obtained PCR products were of good quality (Fig. 1a and b) and matched very well with those reported in literature (Tillett et al., 2001; Mikalsen et al., 2003 and Ouahid et al., 2005).

3.5. Determination of the sensitivity of the amplification of mcy genes from diminishing dissolved Microcystis DNA

The concentration (29 ng/μL) of a high purity *Microcystis* DNA (260/280 = 1.98; 260/230 = 2) extracted from water (Table 2) was diluted in nuclease free water to give various DNA concentrations (Section 2.5). The DNA dilution approach used in this study simulates a dynamic diminishing and concentration variation tendencies of any dissolved substance in water from a point source (PS) to a non-point source (NPS) in a real natural water environment. In a study of Mbukwa (2013a) using DNA extracts (≈200 ng/μL) from algal cells, the quality of *mcy* PCR products formed were obtained after optimizing PCR cycles to 36. Therefore, the same PCR settings were used in the current study to determine the sensitivity differences in the formation of six *mcy* gene PCR products and 16S rRNA (Fig. 1a and b). From Fig. 1a, c and d, it was observed that the quality of PCR products of all *mcy* genes decreased proportionally to the DNA dilution trend. Fig. 1a showed quality PCR products for all *mcy* genes and 16S rRNA gene were clearly observable after 36 PCR cycles at 3.0 ng/μL concentrations of input DNA. At 0.1 ng/μL DNA concentration however, all respective *mcy* and 16S RNA gene PCR products were formed but there was visible variations in the intensity among individual bands (not shown) of which some showed weak bands with characteristic features of lane 3 in Fig. 1b. This variation was further intensified when the input DNA template was reduced to 0.05 ng/μL at which several PCR products were not observed after 36 PCR cycles (not shown). At the same DNA concentration (0.05 ng/μL) however, (Fig. 1c) increasing the number PCR cycles from 36 to 40 cycles led to three interesting observations with regard to the sensitivity in the formation of *mcy* gene PCR products. First, only four clear PCR products for *mcy*B (lane 4), *mcyA*′ (lane 3′), *mcy*C (lane 5) and *mcy*E (lane 7) were observed. Secondly, the sensitivities of two *mcy* genes; *mcy*D (lane) and *mcyB*′ (lane 4′) as well as 16S rRNA (lane 2) were slightly enhanced by the increase in the number of PCR cycles, however, resulting in faint looking bands of their PCR products (Fig. 1c). And thirdly, the sensitivity of *mcy*A (lane 3) and *mcy*G (lane 8) were completely lost at this concentration and their PCR products did not form even when the PCR reaction was increased to 40 PCR cycles. From Fig. 1d, it was observed that the most sensitive *mcy* gene in this study was *mcyA*′ (lane 3′) which formed PCR products after 36 PCR cycles at a concentration of up to 0.001 ng/μL of DNA template in the PCR reaction. The DNA sequence of the amplified *mcyA*′ gene was reported in our recent publication (Mbukwa et al., 2013b).

4. Conclusions

This study demonstrated a real life application of a PCR method in the detection of toxic *Microcystis* spp. in water using short fragments of trace levels of dissolved *Microcystis* DNA for the amplification of six *mcy* and cyanobacterial 16S rRNA genes. The approach used is robust, accurate and has high sample throughput requiring short operational time and uses relatively low cost equipments, reagents/materials and very small sample sizes. In this study, the quality/purity of the extracted DNA was not affected by the methods of extraction used as both methods yielded high quality DNA extracts suitable for PCR reactions. However, under a given extraction method the concentrations (quantities) of extracted DNA were found to be dynamically variable depending on a sampling site. The studied *mcy* genes showed variable sensitivity towards the formation of PCR products amplified from their respective PCR primers in a given PCR reaction condition. Differences in *mcy* gene sensitivities were particularly observable as input DNA concentration was serially reduced in a PCR reaction. The sensitivity of several *mcy* genes were however enhanced by increasing the number of PCR cycles. The latter increases the copy numbers of PCR products. At very low concentrations of input DNA *mcyA*′, *mcy*B, *mcy*C and *mcy*E gave better PCR amplicons than other studied genes. However, based on the observed differences in PCR product intensities at a given input DNA concentration, it is not clear if the primer's chemistry contributes to the sensitivity of a given *mcy* gene. Thus, further investigations (including real-time PCR) are needed to establish if such relationship exists. Results from such a study will in future give guidance in the selection of appropriate primers for the PCR amplification of *mcy* genes from low level dissolved DNA. Interesting enough however, as part of contributing to IWRM missions for quality drinking water for all, the current approach presented in this study could be used in routine monitoring of invisible MC producing *Microcystis* spp. particularly from surface water used to supply drinking water in rural and non-piped sub-urban areas.

Acknowledgements

Financial supports from the University of Johannesburg (South Africa) and the World Bank-Science, Technology and Higher Education Project, WB-STHE project (Dar es Salaam, Tanzania) are highly appreciated.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pce.2013.07.004.

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Please cite this article in press as: Mbukwa, E.A., et al. Potential use of dissolved cyanobacterial DNA for monitoring toxic *Microcystis* cyanobacteria in filtered water. J. Phys. Chem. Earth (2013), http://dx.doi.org/10.1016/j.pce.2013.07.004