



Removal Cyanobacteria by Diatomite Filter from Raw Freshwater and Potential Use in Watering Domestic Animals

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To cite this article:

Belkacem Behira. Removal Cyanobacteria by Diatomite Filter from Raw Freshwater and Potential Use in Watering Domestic Animals. *Animal and Veterinary Sciences*. Vol. 3, No. 3, 2015, pp. 80-83. doi: 10.11648/j.avs.20150303.11

Abstract: Growing harmful cyanobacteria in freshwater cause several poisoning episodes of livestock, wild and domestic animal. Conventional surface drinking water treatment utilizes coagulation; flocculation, sedimentation, filtration and disinfection are inadequate and require great financial means or application in watering domestic animals. The filtration of raw freshwater by diatomite is very simple without use of chemical products. Samples of locally diatomite were dried and calcined and tested to verify their efficiency to removal cyanobacteria cells from raw freshwater. The results obtained shows that filtration by calcined diatomite improve high efficiency to removal cyanobacteria and decrease the level of microcystin in the filtrate solutions.

Keywords: Cyanobacteria, Diatomite, Removal, Raw Freshwater

1. Introduction

Cyanobacteria are a morphologically diverse group of photosynthetic prokaryotes that occupy a wide range of niches, from freshwater to hydrothermal vents, from desert rocks to Antarctic lakes. They most common toxic cyanobacteria in freshwater are *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Planktothrix* (syn. *Oscillatoria*) *rubescens*, *Synechococcus* spp., *agardhii*, *Gloeotrichia* spp., *Anabaena* spp., *Lyngbya* spp., *Aphanizomenon* spp., *Nostoc* spp., some *Oscillatoria* spp., *Schizothrix* spp. and *Synechocystis* spp. Toxicity cannot be excluded for further species and genera (WHO,2003).

Temperature, light, and nutrients level and composition are the main factors affecting toxin production. Variation of cellular toxin levels under different growth conditions have been studied mainly with the batch cultures of *Microcystis*, *Oscillatoria*, *Anabaena*, *Anabaena*, *Aphanizomenon*, and *Planktothrix* (Rapala 1998, Sivonen and Jones 1999). About 75% of all cyanobacteria sampled contain toxins. It is the huge conglomerations of cells that present a great concern of development of large blooms in late summer and early fall (Chorus *et al.* 2000). The production of cyanotoxins includes risks to human and animal health. Depending on the concentration in the aquatic environment, they can cause

severe poisoning, produce chronic diseases such as cancer, and even lead to death. Cyanotoxins are thus an important group of chemical compounds, from the point of view of ecotoxicology, toxicology, and environmental chemistry (Bláha,2009): Several poisoning episodes of livestock, wild and domestic animals have been associated with the occurrence of cyanobacteria blooms in surface waters used for drinking (Stewart *et al.* 2008). Microcystins are the most frequently occurring and widespread of the cyanotoxins. Due to these adverse health effects, the World Health Organization established a provisional guideline of 1µg/L for microcystin-LR in drinking water (WHO, 1998). Conventional surface drinking water treatment utilizes coagulation, flocculation, sedimentation, filtration and disinfection as basic methods. However, conventional treatment may need to be optimized for cyanotoxin removal, relating to the form of the toxin to be removed (intra- or extracellular), the background water matrix, and possible dissolved toxin release during the treatment process (Falconer, 2005). In agreement with earlier findings that alum flocculation, filtration, and chlorination are ineffective in the removal of cyanobacteria toxins (Vuori *et al.* 1992). Physicochemical water treatment processes have been shown to cause cell lysis and toxin release (James and Fawell, 1991). Diatomite is a natural material formed from

the remains of diatoms, which grew and were deposited in seas or lakes. The structure of diatomite is quite complex. Because there are many fine microscopic pores, cavities, and channels, it has a large specific surface area, high absorption capacity, and low density. Moreover, because of its relative low cost and abundance, it is utilized extensively as a filler, a filtering aid, an abrasive, an insulating material, a conventional catalyst support, and a membrane. Small, highly porous diatomite particles are useful in fabricating highly permeable microporous membrane filters (Vasconcelos et al., 2000). Diatomite was used in purification potable water, contaminated ground- and surface water, decontamination of sewage liquids and waste water. (Grešovnik, 2007).

The aim of this work is to test the natural and calcined diatomite as filter without chemical products use to removal cyanobacteria from raw water in watering domestic animals.

2. Materials and Methods

The raw diatomite obtained from (Sig deposit, Algeria) under investigation was subjected to treatment under various conditions. The first sample is the natural sample (S1). The second one (S2) is treated by 0.5 N HCl, then washed by distilled water and finally dried in drying oven at 110 °C for 5 hours. The third one (S3) is calcined diatomite at 500 °C using muffle furnace. While the fourth (S4) is calcined at 700 °C. All samples milled in a ball mill machine and sieved below 200 µm to remove all particles larger than 200 µm. ideally, these fractions contain sand, rocks, clay, and other impurities. Raw water collected from eutrophic lake was used in this experiment. A pre-filtration was occurred on fine sand to removal microalgae and other large particular matters. To achieve filtration, 10 grams of diatomite were added in funnel and the pre-filtered water and one liter was filtered through the diatomite filter. The quantification and identification of cyanobacteria was determined by inverted microscopic. The morphological identification of cyanobacteria was done according to (Komárek and Zapomelova 2008). The determination of intracellular and the extracellular microcystin concentration in extracted samples was determined by HPLC following the method of Lawton et al, (1994). All chemicals used were HPLC grade. The stationary phase was a Symmetry C18 column (250 x 4.6 mm I.D., 5 µm particle size, Waters) at a flow rate of 1ml min⁻¹ and column temperature 38 °C. The mobile phase were Milli-Q water (solvent A) and acetonitrile (solvent B) both acidified with 0.05 % v/v TFA. MC-LR stock solution was prepared by mixing MC-LR standard (Sigma) with methanol, and then it was centrifuged in 2000 RPM for 15 min. MC-LR standard solutions were prepared in different concentrations by adding desired volumes of stock solution to methanol. The prepared standard solutions were 0,01, 0.1,0.2, 0.4 ,0.6, 0.8, 1.0 , 2.0 , 4.0 , 5.0, 10.,20, 40, 80 and 100 µg/L of concentrations.

All experiments were repeated three twice.

Table 1. cyanobacteria count in filtrate solutions (cell/ ml).

Sample	cyanobacteria spp.			
	<i>microcystis</i> spp.	<i>anabaena</i> spp.	<i>Oscillatoria</i> spp	<i>Synechococcus</i> spp
S1	2.10 ⁴	2.10 ³	2.10 ²	50
S2	5. 10 ³	2.10 ²	20	10
S3	80	20	10	4
S4	10	4	0	0

S1: natural diatomite, S2: Diatomite trated by 0.5 N Hcland drying at 110 °C; S3: Diaomite calcined at 500°C; S4: Diatomite calcined at 700 °C.

3. Result and Discussion

The identification and quantification in sample of raw water before filtration show that the *microcystis* spp. was the predominant genus followed respectively by *anabaena* spp., *Oscillatory* sop.,*Synechococcus* spp. The colonial diameter of *microcrystals* spp. range from 300 to 400 µm. The length of filaments *anabaena* spp. range from 100 to 200 µm. *Oscillatoria* spp. The filament length range from 90 to 150 µm. The quantification of cyanobacteria was 6. 10⁴ cells/ml of *microcystis* spp, 4.10³ cell/ml of *anabaena* spp., 5.10² cell/ml of *Oscillatoria* spp. and 1.10² cells / ml of *Synechococcus* spp. The quantification of cyanobacteria in the filtrate solution flirtd through the naturally diatomite (S1) shows that the number of microcystiis spp. decrease at 8.10² cell/ ml, *anabaena* spp. at 2.10³ cell/ ml , *Oscillatoria* spp. at 2.10² cell/ ml and *Synechococcus* spp. at 50 cell/ ml. For the diatomite treated by 0.5 Hcl and dried at110 °C (S2), the numeration of cyanobacteria in the filtrate solution shows that the *microcystis* spp. was 6. 10² cell/ml , following by *anabaena* spp. 2.10² cell/ml and 20cell/ml of *Oscillatoria* spp . The counting of the filtrate solution filtered through the calcined diatomite at 500 °C (S3) was 50 cell / ml of *microcystis* spp. , 20 cell/ml of *anabaena* spp., 10 cell/ml of *Oscillatoria* spp., 4 cell/ ml of. For the filtrate solution collected from the filtration by calcined diatomite at 700 °C, was detected only 10 cell/ml of *microcystis* spp. and 4 cell/ml of *anabaena* spp. The content of total microcystin LR in raw water reached 90 µg/L in the same sample the extracellular microcystin LR was 1.6 µg/L. The results of Total and extracellular microcystin in filtrate solutions were summarized in Tab. 2. the majors groups of cyanobacteria found in raw water from this eutrophic lake were *microcystis* spp. as the predominant genus. *Microcystis* genus is one of the most common bloom formers in freshwater systems on every continent except Antarctica. This genus can produce a suite of potentially harmful compounds (Fristachi and Sinclair, 2008). The Tab.1 shows that the retention of cells increases with diatomite processing method. The highest value was observed in the diatomite calcined at 700 °C. median pore size of a calcined diatomite. Lange et al. (1986) found that the grade of diatomite used affected filter performance. For the finest diatomite grade with a median particle size of 7.5

μm , turbidity reduction was close to 100%; however, for coarser grades with a median particle size of 22 μm , a 10% reduction was observed. The percent of retained cells of *microcystis* spp. increase at 33% of initial microbial charge in raw diatomite filter (S1), this value increase at 80 % in dried diatomite filter to reaching 0.01 % by calcined diatomite filter (S4). The high retention was observed at *ossillatoria* spp. 96%was removal by dried diatomite, 98% by calcined diatomite at 500 °C and 100% by calcined diatomite calcined at 700 °C. This is probably explained by the morphology of the filament that can reach 100 μm of long and the mucilage sheath. The estimation of total microcystin LR and the intracellular microcystin shows that this toxin was concentrated in the cells. By subtracting, the intracellular microcystin was very high (88.4 $\mu\text{g/L}$) compared to extracellular microcystin LR content (1.6 $\mu\text{g/L}$). The high rates of dissolved microcystin in filtered lake water at the end of blooming season suggest that release of cyanotoxins from cells occurs during the senescence and the decomposition periods of Microcystis cells (Park et al. 1996). The results shows that the filtration by diatomite was not contributed to increase of microcystin content in all filtrates. However the use of chemical products increases the extracellular microcystin level in the freshwater treatment by lysis of cyanobacteria cells. In similar work Zhang et al.(2011) were tested bombon-based charcoal adsorbent modified with chitosan to removal microcystin from drinking water and their conclusion was the efficiency this product depends of the pH and the organic matters dissolved in water. The diatomite filter efficiency depending of the size of the pores of the diatomite filter and the size of cyanobacterial cell, so not affected by the chemical or the physical parameters of water. Compared to sample (S4), the difference between total microcystin LR and the extracellular microcystin LR in the three samples (S1, S2, S3) is highly significant. the result of filtration shows that was conform to the guidance value for the maximal acceptable concentration of microcystin-LR in drinking water, 1 $\mu\text{g/L}$ recommended by the WHO.

Table 2. content of total microcystin LR and extracellular in filtrate solutions ($\mu\text{g/L}$).

Sample	Total	Total microcystin LR	Extracellular microcystin LR
S1	4.5	0.9	
S2	10.5	0.7	
S3	4.0	0.5	
S4	0.6	0.5	

4. Conclusion

The diatomite is a product of choice to remove toxic cyanobacteria in raw water. Filtration through natural diatomite calcined at 700 ° C exhibits a high efficiency of filtration of all the colonial and filamentous forms of cyanobacteria. This method reduces the amount of

microcystins in filtered water. This simple method can be applied in the reservoirs watering domestic animals. In rural areas this method is economic and easy.

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