Evaluation of new bio-stimulation technology as a solution for the pollution of Rio de Janeiro lagoons

Bruno Corrêa Meurer [1*]
Oliver Alexandre F.C. Pereira [1]
Edgar P. David Santos [1]
Amanda Rosa J. Rodrigues [1]
Marcos Rosa [2]
Marina Bastos de Souza Penetra [1].

[1] Instituto de Ciências Biológicas e Ambientais da Universidade Santa Úrsula. Laboratório de Ecologia Marinha. Rua Fernando Ferrari 75, Botafogo, CEP- 22231-040 Rio de Janeiro, RJ, Brasil.
E-mail: *bcmeurer@hotmail.com.
[2] Faculdade Maria Thereza. Av. Visconde do Rio Branco, 869 - São Domingos, Niterói - RJ, CEP - 24210-006.

ABSTRACT

The Rodrigo de Freitas Lagoon is located in the southern region of the city of Rio de Janeiro, and is a main tourist attraction, with great ecological importance. However, it has been undergoing a pollution process for several years, due to the contribution of *in natura* sewage. In February 2016, USU's Marine Ecology Laboratory initiated a partnership with Curtin University (Australia) and O2ECO, the company representing The Water Cleanser® (TWC) product. To verify the possibility of improving water quality by the treatment of polluting sources, the Marine Ecology Laboratory at USU set up a system with six 100 L tanks, assembled on April 26, 2016. Water was collected from the Rodrigo de Freitas Lagoon, and TWC was cut into 30cm x 5cm pieces and distributed in the four experiment tanks. Twelve replicates of TWC were evaluated and no negative impact was recorded on aquatic biota. The meroplankton larvae of barnacles and mussels developed until the adult phase and fixed to the walls of the tank. The control tanks, without TWC, presented lower numbers of organisms fixed to the walls. Ammoniacal nitrogen, nitrite, nitrate and phosphate reached values close to zero, indicating water quality improvement in all TWC-containing tanks.
Keywords: Eutrophication; Desnitrification; The Water Cleanser®; Water quality, Water bodies.

RESUMO

A Lagoa Rodrigo de Freitas está localizada na região sudeste da cidade do Rio de Janeiro e é uma grande atração turística, com grande importância ecológica. No entanto, ela está passando por um processo de poluição por muitos anos, com a contribuição de esgoto em natura. Em fevereiro de 2016, o laboratório de Ecologia Marinha da Universidade Santa Úrsula (USU) iniciou uma parceria com a Universidade de Curtin (Austrália) e a O2ECO, companhia representante do produto The Water Cleanser® (TWC). Para verificar a possibilidade de melhorar a qualidade de água pelo tratamento do nível de poluição, o laboratório de Ecologia Marinha da USU montou um sistema de seis tanques de 100L em 26 de abril de 2016. A água foi coletada da Lagoa Rodrigo de Freitas, e o WC foi cortado em pedaços de 30 cm x 5 cm e distribuído em quatro tanques. Em 12 réplicas com TWC não houve impactos na biota aquática. Larvas de cracas e mexilhões que já vieram com a água coletada se desenvolveram até o estagio adulto. Os valores de nitrogênio amoniacal, nitrito, nitrato e fosfato chegaram próximos de zero, indicando a melhora da qualidade da água em todos os tanques com TWC.

Palavras-chave: Eutrofização; Desnitrificação; The Water Cleanser®; Qualidade de água, Corpos hídricos.

INTRODUCTION

The Rodrigo de Freitas Lagoon is located in the southern region of the city of Rio de Janeiro, and is a main tourist attraction, with great ecological importance. However, it has been undergoing a pollution process for several years, due to the contribution of *in natura* sewage (Weerelt et al. 2012; Assunção e Rohlfs, 2012).

According to the limits permitted by Brazilian CONAMA Resolution 274/2000 and CONAMA 357/2005 regarding water quality, results obtained in 2015 and in February 2016 indicate that certain parameters are well above those allowed for class 2 (brackish water) used in secondary contact activities. Thermotolerant fecal coliforms reached 2,400,000 thermotolerant fecal coliforms MPN /100 mL, when the limit is 2,500 MPN thermotolerant fecal coliforms /100 mL. This may have been a consequence of the rainy period, since during the rainy season the sampling system is overflowed with sewage due to rain (Oliveira e Contador, 2016).

In February 2016, USU's Marine Ecology Laboratory initiated a partnership with Curtin University (Australia) and O2ECO, the company representing the The Water Cleanser® (TWC) product, that contains trace elements that stimulate nitrogen cycle bacteria to reduce ammoniacal nitrogen, nitrite and nitrate, while also improving BOD and other important parameters required for adequate water quality. The product, thus, promotes bioremediation through biostimulation.

Biochemical degradation of contaminants in a certain medium can be performed through bioremediation, which, in general, consists of increasing the local microbial population (Bernoth et al. 2000; Silva et al. 2004).

Among bioremediation strategies is biostimulation, which is applied *in situ* and seeks to stimulate the growth of the native microbial population by creating favorable environmental conditions for its development in the contaminated site, with nutrients and co-substrates, in order to accelerate degradation of contaminating compounds. However, heavy metals such as cadmium and lead are not absorbed or captured by microorganisms, but can be transformed into less harmful compounds. An important factor for the success of this technique is that bacteria capable of degrading the target pollutant should already be present in the environment (Lee e De Mora, 1999; Lima et al. 2011; EPA, 2004; Gaylarde et al. 2005).

The Water Cleanser® (TWC) product has been applied successfully in other countries such as Australia, New Zealand, and Mexico, among others. However, the question arose as to whether this product could impact the aquatic biota of the Rodrigo de Freitas Lagoon.

In this context, the present study aims to evaluate the possible impact of the The Water Cleanser® (TWC) on the barnacles and mussels of the Rodrigo de Freitas Lagoon, by evaluating its effects on water quality.

MATERIAL AND METHODS

To verify the possibility of improving water quality by the treatment of polluting sources, the Marine Ecology Laboratory at USU set up a system with six 100L tanks, assembled on April 26, 2016. Water was collected from the Rodrigo de Freitas Lagoon, stored in 100L containers until arriving at the laboratory and then distributed on the same day between six tanks.

The Water Cleanser is a probiotic culture block with trace elements and carbon. TWC is a wax plate that was cut into 30cm x 5cm pieces and distributed in the four

experiment tanks. The experiment was repeated three times, totaling 12 replicates, including 6 controls.

Ammoniacal nitrogen, nitrite nitrogen, nitrate nitrogen, phosphorus, and the planktonic community were monitored.

The analyses were performed weekly and the variations of each tank were monitored. The tanks were maintained at 23 ° C and salinity at 10.

The parameter values obtained in the experiments were compared with the maximum permissible limits accepted by Brazil's National Environmental Council of Resolutions, CONAMA 357 and CONAMA 430.

The nutrients were measured by photometric methods at the Chemistry Laboratory at USU. Ammonia Nitrogen was measured by the Nessler method. Nitrite was analyzed by the Naphthylamine method, while Nitrate was determined by the N-(1-naphthyl)-ethylenediamine method. Phosphorus were determined by the Ascorbic Acid digestion Method.

An NMDS (Non-metric Multidimensional Scaling) statistical test by Euclidean distances was performed to analyze the differences between the experiment tanks.

RESULTS

The TWC-containing tanks presented variations over time for all analyzed parameters, decreasing during the fourth week of the experiment. The control tanks presented low variation with significant difference compared with TWC-containing tanks (F=34, 1; *p*=0,03).

Increases in ammoniacal nitrogen were registered mainly during the second week (Figure 1). Ammoniacal nitrogen was well below the limit determined by CONAMA, of 20 mg. L^{-1} in all tanks.

Mean nitrite concentrations were also below the limit determined by CONAMA 357 and CONAMA 430, in all tanks. The highest values were recorded during the second week. In the fourth week, all tanks showed nitrite concentrations below 0.1 mg.L^{-1}.

Mean nitrate concentrations were also below the limit determined by CONAMA 357 and CONAMA 430, in all tanks. The highest values were recorded during the third week, following the transformation of ammonia to nitrite and from nitrite to nitrate. With the exception of control tank, all tanks presented values below 0.1 mg.L^{-1} during the fifth week.

Mean phosphorus values were below the limit determined by CONAMA 357 and CONAMA 430 in all tanks, with the highest concentrations recorded in the controls. The TWC-containing tanks presented values below 0.05 mg.L^{-1} after the fourth week.

The most significant decrease occurred during the fourth week, with all nutrients presenting values below 0.05 mg.L^{-1}, except for nitrate, that maintained mean values of 0.14 ± 0.09 mg.L^{-1} (Figure 1).
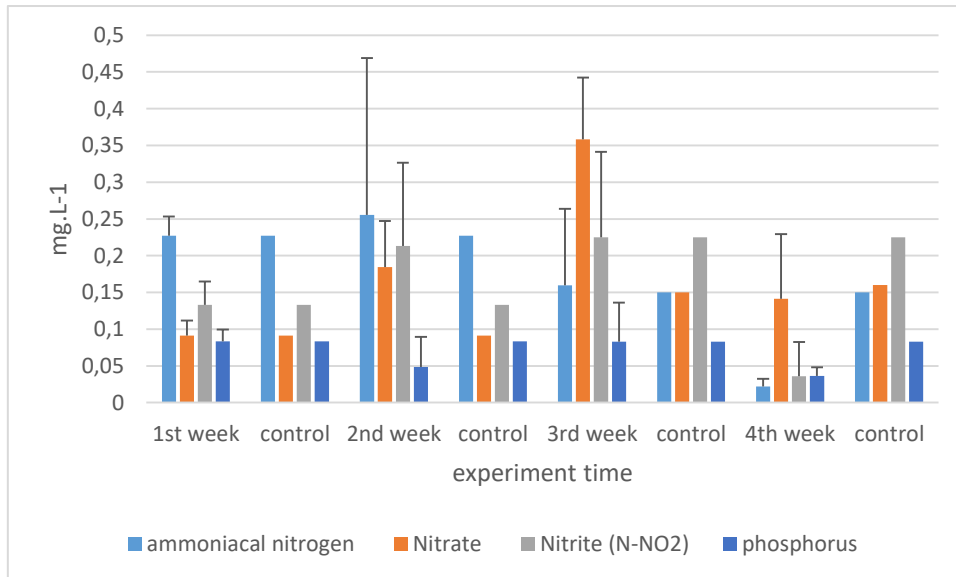
Figure 1 - Means and standard deviations of the nutrient in TWC-containing tanks.

The TWC-containing tanks presented significant improvements in water quality in one week, providing conditions for the development of aquatic biota, and filtering strata (barnacles), which were recorded in all tanks (Figure 2). These barnacles came in the form of larvae when the Rodrigo de Freitas Lagoon water was collected, so the plankton present in the water found good conditions for development to the adult form.

Figure 2 – Barnacle adult and larvae attached to the glass in a TWC-containing tank.

The TWC-containing tanks presented mean number 38,5± 21,3 barnacles and 17,5 ± 8,9 mussels. The control tanks presented only five organisms, four barnacles and one mussel (Figure 3).
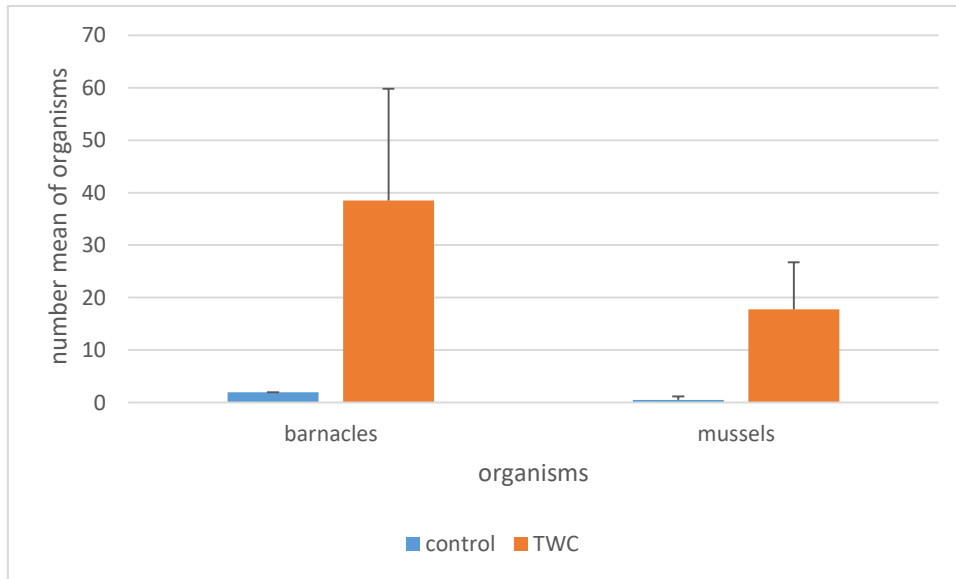
Figure 3- Means and standard deviations of barnacles and mussels.

When applying the NMDS analysis through Euclidean distance, it was demonstrated that tanks TWC2 (T2), TWC3 (T3) and TWC4 (T4) had the largest number of organisms reaching the adult stage, followed by tank TWC1 (T1). The control tanks showed much lower densities of organisms reaching the adult stage (Figure 4).

Figure 4 - NMDS analyses of the Euclidean distance of the composition of benthic filtering organisms in the experiment tanks.

DISCUSSION

Most studies on bacteria dynamics in the aquatic environment analyze factors that vary on a time scale, such as months or seasons. In temperate ecosystems, seasonal changes in temperature, organic matter and nutrients, such as inputs from melted snow or water column mixtures, and phytoplankton blooms, among others, lead to changes in the abundance and production rates of bacterioplankton and, consequently, nitrogenous compounds (Sondergaard, 1997; Biddanda e Cotner, 2002; Mc Manus et al. 2004). In tropical ecosystems, bacterioplankton growth seems to be related mainly to seasonal alternations between allochthonous and autochthonous resources, with a correlation between bacteria and nitrogenous compounds. Reducing the source of nutrients from

domestic sewage sludge can reduce the number of pathogenic bacteria and favor increases in the diversity of the aquatic biota.

Increasing interest in solutions applying different technologies and strategies for the improvement of water quality in environments polluted by anthropogenic sources has been observed. One of these solutions is the use of genetically modified microorganisms that would present better catabolic potential than native species that could be used for bioremediation (Wrobleski, 2014). However, there is concern about the use of these microorganisms, since there may be uncontrollable dispersion and irreversible impacts (Papinazath e Sasikumar, 2014).

The TWC product is a nitrifying bacteria biostimulator, composed of trace elements that allow for the action of these organisms. The product stimulates the already existing bacteria in the environment to complete the nitrogen cycle, and does not bring any new bacteria at all. This type of solution has lower costs when compared to chemical solutions or solutions that use equipment with electric power sources.

Kamath (2007) collected information on bioremediation applications in Central European and Indian lakes, and indicated satisfactory results, but raised the issue that there is need for the application of other types of techniques in conjunction with bioremediation for better control of the pollutant factors of lakes in large lakes. Coelho et al. (2015) states that, in order to obtain large scale and higher efficiency, it is necessary to apply techniques to overcome the limitations of the bioremediation process, such as genetic engineering, to develop more efficient microorganisms.

All TWC-containing tanks presented decrease in the evaluated nutrient levels. Nitrate was present in the highest concentrations following the nitrification process in most of the tanks, from the decomposition and release of ammoniacal nitrogen, its transformation to nitrite and then to nitrate.

The control aquaria presented reduced nutrients, because they present bacteria in the water responsible for nitrification. However, they did not show growth of meroplankton species until the fixation phase as observed in TWC-containing tanks.

The main difference between the control tanks and the TWC-containing tanks was aquatic biota fixation to the tank walls. The control tanks did not present organisms, while the others formed a benthic biota that arrived in the form of larvae at the beginning of the experiment. From the NMDS analysis through Euclidean distances it was possible to observe the control tanks were separated from the others when comparing the abundance of filtering organisms fixed in the tanks. Thus, it can be concluded that the plaque that stimulates nitrification bacteria did not negatively influence the aquatic biota; on the contrary, the water quality in the TWC-containing tanks favored organism development until the adult phase. In this scenario, this new biostimulation technology could be used to reduce the pollution of Rio de Janeiro lagoons, used alongside strategies for the treatment of domestic and industrial sewage. Studies should be conducted to analyze the best strategy for each ecosystem, since biota composition varies from lagoon to lagoon.

CONCLUSION

The control tanks presented low variation with significant difference compared with TWC-containing tanks that presented variations over time for all analyzed parameters, decreasing during the fourth week of the experiment.

Twelve replicates of TWC were evaluated and no negative impact was recorded on barnacles and mussels. The meroplankton larvae of barnacles and mussels developed until the adult phase and fixed to the walls of the tank. The control tanks, without TWC, presented lower numbers of organisms fixed to the walls.

Ammoniacal nitrogen, nitrite, nitrate and phosphate reached values close to zero, indicating water quality improvement in all TWC-containing tanks.

Biostimulation techniques can offer lower costs compared to traditional remediation and can be implemented on a large scale, being considered an efficient solution for the decontamination of Rio de Janeiro lagoons.

REFERENCES

Assunção LPG, Rohlfs DB. Biorremediação em áreas contaminadas. Goiás. Tese [Doutorando em Biociências forenses] – PUC/IFAR; 2012.

Bernoth L, Firth I, Mcallister P, Rhodes S. Biotechnologies for remediation and pollution control in the mining industry. Miner Metall Proc 2000; 17 (2): 105 – 11.

Biddanda BA, Cotner JB. Love handles in aquatic ecosystems: the role of dissolved organic carbon drawdown, resuspended sediments, and terrigenous inputs in the carbon balance of Lake Michigan. Ecosystems 2002; 5: 431- 45.

Coelho LM, Rezende HC, Coelho LM, Souza PAR, Melo, DFO, Coelho, NMM. Bioremediation of Polluted Waters Using Microorganisms. In: Shiomi N. Bioremediation of Wastewater and Polluted Soil. Vienna: Intech; 2015. p. 1 – 22.

Conselho Nacional do Meio Ambiente (Brasil). Resolução nº 357, de 18 de Março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. DOU 053, 18 de Março de 2005; pag. 58-63. Disponível em: <http://www.mma.gov.br/>.

Conselho Nacional do Meio Ambiente (Brasil). Resolução n° 430, de 13 de maio de 2011. Dispõe sobre as condições padrões de lançamento de efluentes,complementa e altera a resolução n° 357, de 17 de março de 2005, do Conselho Nacional do Meio Ambiente. DOU 92, 16 e Maio de 2011; pag. 89. Disponível em: http://mma.gov.br/port/conama/legiabre.cfm?codlegi=646.

Conselho Nacional do Meio Ambiente (Brasil). Resolução n° 274, de 29 de Novembro 2000. Define os critérios de balneabilidade em águas brasileiras. DOU 18, 25 de Janeiro de 2001, Seção 1, pag. 70-71. Disponível em: http://www.mma.gov.br.

Environmental Protection Agency, United States (EPA) [homepage na internet]. How to Evaluate Alternative Cleanup Technologies for undergrond storage tank sites [acesso em 10 maio 2017]. Disponível em: http://www.epa.gov.

Gaylarde CC, Bellinaso MDL, Manfio GP. Biorremediação: Aspectos biológicos e técnicos da biorremediação de xenobioticos. Biotecnologia Ciência e Desenvolvimento 2005; 34: 36 - 43.

Kamath G. Bioremediation of Lakes: Myths and Realities. In: Sengupta M, Dalwani R. Proceedings of Taal 2007 - 12th World Conference. Mubai: Organica Biotech; 2007. p. 44-49.

Lee K, De Mora. In situ bioremediation strategies for oiled shoreline environments. Environ Technol 1999; 20 (8): 783- 79.

Lima DF, Oliveira OMC, Cruz MJM. Utilização dos fungos na bioremediação de substratos contaminados por petróleo: estado da arte. Caderno de Geociências 2011; 8 (2): 113-21.

Mc Manus GB, Griffin PM, Pennock JR. Bacterioplankton abundance and growth in a river-dominated estuary: relationships with temperature and resources. Aquat Microb Ecol 2004; 37: 23-32.

Oliveira CR, Contador LS. Avaliação das condições dos indicadores microbiológicos de balneabilidade da Lagoa Rodrigo de Freitas - RJ. Rio de Janeiro. Monografia [graduanda em ciências biológicas] – USU; 2016.

Sasikumar CS, Papinazath T. Environmental management: bioremediation of polluted environment. In Bunch MJ, Suresh VM, Kumaran TV. Proceedings of the Third International Conference on Environment and Health. Chennai: York University; 2014. p. 464 – 69.

Silva E, Fialho AM, Sa-Correia I, Burns RG, Shaw LJ. Combined bioaumentation and biostimulation to cleanup soil contaminated with high cocentraions of atrazine. Environ Sci Technol 2004; 38: 632-37.

Sondergaard M. Bacteria and dissolved organic carbon in lakes In: Sand- Jensen K, Petersen O. Freshwater biology: priorities and development in Danish research. Copenhagen: Gad; 1997. p. 138-61.

Weerelt MD, Signori C, Enrich-Prast A. Balneabilidade da Lagoa Rodrigo de Freitas – variação temporal e espacial. Oecologia australis 2012 set; 16 (3): 566 – 80.

Wrobleski CM. Bioremediation and Genetic Modification. Depart Biol Sci Davidson College 2014 nov; 23: 18-28.