Physicochemical parameters and cytogenotoxicity evaluation of two methodologies applied in a municipal wastewater treatment plant

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Abstract

The intense urbanization combined to the release of domestic sewage into water resources constitutes a potential risk to ecosystems and human health, which leads to the need of using technologies in the sewage treatment. In the present study, physicochemical parameters and the *Allium cepa* assay were carried out in order to assess the potential cytogenotoxic effects of effluents from two sewage treatment systems: activated sludge and floating emergent macrophyte. For cytogenotoxic evaluation, seeds of *A. cepa* were exposed to different concentrations (25, 50 and 100%) of the effluent samples treated with both technologies. The physicochemical analyses revealed both treatment systems met the legal criteria for chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), suspended solids and termotolerant coliforms. Despite the floating emergent macrophyte system did not reach the necessary efficiency for ammoniacal nitrogen removal, it presented a better removal of phosphorous when compared to the activated sludge system. For cytogenetic endpoints, there was no difference between both treatments and the negative control were found in mitotic index and frequencies of micronuclei and chromosomal aberrations. These results suggest that the floating emergent macrophyte system is a promising alternative methodology in the domestic sewage treatment.

Keywords: Allium cepa. Sewage treatment. Macrophytes. Activated sludge.

Theme Area: Clean technologies

Parâmetros físico-químicos e avaliação da citogenotoxicidade de duas metodologias empregadas em uma estação municipal de tratamento de esgoto doméstico

Resumo

A intensa urbanização combinada à descarga de esgoto doméstico em recursos hídricos constitui em um risco aos ecossistemas e à saúde humana, o que gera a necessidade de utilização de tecnologias no tratamento do esgoto. No presente estudo, a caracterização físico-química e o bioensaio com Allium cepa foram realizados para avaliar os possíveis efeitos dos efluentes de dois sistemas de tratamento de esgoto: lodo ativado e macrófitas flutuantes. Para a avaliação citogenotóxica, sementes de A. cepa foram expostas a diferentes concentrações (25, 50 e 100%) das amostras de efluentes tratados com ambas as metodologias. A análise físico-química mostrou que ambos os sistemas de tratamento de esgoto atenderam à legislação para demanda química de oxigênio (DQO), demanda

biquímica de oxigênio (DBO), sólidos suspensos e coliformes termotolerantes. Apesar de o sistema com macrófitas flutuantes não ter alcançado a eficiência de remoção necessária para nitrogênio amoniacal, este sistema apresentou melhor remoção de fósforo quando comparado ao lodo ativado. Para a análise citogenética, não foram observadas diferenças entre ambos os sistemas quando comparados ao controle negativo quanto ao índice mitótico, frequências de micronúcleo e aberrações cromossômicas. Estes dados sugerem que o sistema com macrófitas flutuantes é uma alternativa promissora no tratamento de esgoto doméstico.

Palavras-chave: Allium cepa. Tratamento de esgoto. Macrófitas. Lodo ativado.

Área Temática: Tecnologias limpas

1 Introduction

Human activities are provoking great impacts on aquatic ecosystems. Discharges of industrial and domestic effluents in the rivers are the most common pollution source of chemical compounds. Several effluents contain toxic mixtures such as heavy metals, industrial products, pesticides and others that, together, can aggravate the environmental conditions and, consequently, all the aquatic ecosystems (HOSHINA et al., 2009). In recent years, with the consolidation of legislation and regulatory control, companies have begun to treat the water used in their manufacturing process (FIGUEIREDO et al., 2010). However, municipal wastewater still remains a problem, since only a small portion of domestic sewage is treated in Brazil before being released into water resources.

The most successful and widely used technology for municipal wastewater treatment is the biological system with activated sludge, in which aerobic microorganisms metabolize organic matter. Alternatively, the use of a floating emergent macrophyte treatment wetland, in which emergent plants are grown in a hydroponic matter on floating rafts, offers the advantage of providing a relatively passive, low-maintenance, and operationally simple wastewater treatment system (HEADLY & TANNER, 2012). Thus, studies on physicochemical characterization of effluents combined to bioassays can provide information about the toxicity of effluents which contains complex mixtures of substances.

The *Allium cepa* L. assay is acknowledged as an excellent genetic model to assess the presence of environmental pollutants in water samples (GRANT, 1994; FRANKLIN et al., 2002). Moreover, this species also presents other advantages, including low costs, easy handling, and suitable chromosomal features; this plant bears large and few chromosomes (2n=16) which facilitates the evaluation of chromosome damages and/or disturbances in cell division cycle (FISKESJO, 1985). The cytotoxicity levels of an agent or a complex mixture of substances can be determined by the decrease or increase in the mitotic index, i.e. number of cells in division (FERNANDES et al., 2007), while the observation of micronuclei and chromosomal aberration, such as bridges, fragments and vagrant, allows estimating the genotoxic effects of agents. This test system has shown high sensitivity in detecting environmental chemicals (LEME & MARIN-MORALES, 2009), and has successfully been used to evaluate the genotoxicity of sludge samples (RANK & NIELSEN, 1998), hospital wastewaters (MAGDALENO et al., 2014), herbicides (FERNANDES et al., 2007), petroleum refinery wastewaters (HOSHINA et al., 2009) and rivers (GOMES et al., 2015).

Therefore, the aim of this work was to assess the physicochemical parameters and cytogenotoxicity from effluents treated with two distinct methodologies – activated sludge and a floating emergent macrophyte system, in a municipal sewage treatment plant.

2 Material and methods

The municipal sewage treatment plant selected for this study is located in the city of Novo Hamburgo, state of Rio Grande do Sul, Brazil. This treatment plant serves a population of about 5000 people and receives an average of 520 m^3 of domestic sewage a day (Goldoni et al., 2014). There are two technologies operating simultaneously in this plant: activated sludge system and a floating emergent macrophyte system with *Typha domingensis* (developed and patented by the University of Polytechnology in Madrid). However, since this technology was applied in a wastewater treatment plant that already existed, it was not possible to implement all components of this system, which include three ponds. The first pond runs through anaerobic zone; the second pond runs through the use of diffused air acting as an aerobic system and the third one has the function of clarification and phosphorous removal. The dimensions of the reactors are 2,5mx17mx17m. The feeding in the floating macrophyte

system has Q = 0,7 $L.s^{-1}$ at a constant speed. The activated sludge is a batch reactor that operates with cycles of four hours: 2'45", fed with Q = 3,2 $L.s^{-1}$; 30", decanting and 45", output.

Samples for physicochemical and *A. cepa* assays were collected in May 2015. Physicochemical analyses of treated sewage samples were carried out for the following parameters: biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammoniacal nitrogen, phosphorous, suspended solids, aluminum, chromium, zinc and termotolerant coliforms (*Escherichia coli*).

Seeds of *A. cepa* from the same lot and variety ("Baia Periforme" onion) were submitted to germination at 22ºC in Petri dishes (10 cm of diameter), each dish covered with filter paper and individually poured with 3mL of distilled water. When roots reached 1 cm in length (approximately five days after the beginning of the assay), they were transferred to a dish containing the sample and then, exposed for 24h. A control group was maintained in distilled water. The samples of domestic sewage treated with the two technologies were diluted in distilled water in the following concentrations: 25, 50 and 100%.

After the exposure period, onion roots were collected (10 per group), fixed in alcohol-acetic acid (3:1-v/v) for 24h, transferred to 70% ethyl alcohol and stored in a refrigerator. To prepare the slides, roots were rinsed in distilled water, hydrolyzed in 1N HCl at 60ºC for 8 minutes and rinsed again. The meristematic regions were covered with coverslips and carefully squashed into a drop of 1% acetic orcein. Stained root tips were analyzed under light microscopy (Olympus DSC2000) for cytogenetic endpoints.

For the mitotic index, 500 cells per slide were analyzed, which was used as microscopic parameter for cytotoxicity. To assess genotoxicity, 500 interphase cells were analyzed to estimate the frequency of micronucleus and 50 cells in anaphase-telophase were analyzed to determine the frequency of chromosomal aberrations, such as chromosomal fragments and losses and bridges, following the criteria used by Leme and Marin-Morales (2008). Five slides were analyzed per treatment, totaling 2500 cells for mitotic index and micronucleus analysis, and 250 cells for chromosomal aberrations analysis. The mitotic index, chromosomal aberrations and micronuclei frequencies were calculated according to the formula: frequency=(A/B) x 100, where A is equivalent to the total number of cells with a parameter to be analyzed, and B corresponds to the entire number of analyzed cells.

Statistical analysis was performed using ANOVA one-way followed by Tukey multiple comparison when appropriate. Significant difference was considered when $p<0.05$.

3 Results

Physicochemical parameters of activated sludge and floating emergent macrophyte systems effluents are shown in Table 1.

Table 1 – Physicochemical and microbiological analysis of raw domestic sewage, activated sludge and floating emergent macrophyte filter

Parameter ($mg.L^{-1}$)	Raw domestic sewage	Activated sludge system[1]	Floating emergent macrophyte system[2]	Emission Pattern[1]	Emission Pattern[2]
BOD_5	320.0	36.0	115.0	80	150
COD	573.0	45.7	155.5	260	360
$N\text{-}NH_3$	54,5	ND	50.3	20	20
Phosphorous	8.3	4.8	3.1	-	-
Suspended Solids	102.5	15.5	16.0	80	180
Aluminum	0.6	ND	ND	10	10
Chromium	ND	ND	ND	0.1	0.1
Zinc	0.2	0.1	0.02	2.0	2.0
Termotolerant coliforms	29,000,000	500,000	3,300,000	10^5	-

ND – Not detected
[1] – According to CONSEMA (State Environmental Council) Resolution no. 128 (Rio Grande do Sul, 2006): $500 \leq Q < 1000$ ($m^3.d^{-1}$)
[2] – According to CONSEMA (State Environmental Council) Resolution no. 128 (Rio Grande do Sul, 2006): $20 \leq Q < 100$ ($m^3.d^{-1}$)

The State Environmental Council provides the limits for release of sewage into waterways, according to the flow of each season. By having a larger flow, the discharge limits for activated sludge system is much more restrictive than for the floating emergent macrophyte filter system.

The floating emergent macrophyte system did not reach the necessary efficiency for ammoniacal nitrogen disposal, unlike the activated sludge system. In contrast, the floating emergent macrophyte system showed better phosphorous removal, compared to the activated sludge system. COD, BOD_5, suspended solids and termotolerant coliforms met the legal criteria for both treatments. Activated sludge system removed 88.7% for BOD_5, 92% for COD, 42.2% for phosphorus, 85% for suspended solids and 98.3% for termotolerant coliforms. Moreover, all the ammoniacal nitrogen was removed. Concerning the floating emergent macrophyte system, it removed 64% of BOD_5, 73% of COD, 63% for phosphorus, 84.4% for suspended solids and 88.6% for termotolerant coliforms. Concentrations of aluminum and chromium were not detected in any effluents after both treatments, while zinc was detected in both of them.

The results from the *A. cepa* are shown in Table 2. The mean mitotic index was 53.64 for control group. In seeds exposed to different concentrations of the activated sludge treatment, the mitotic index ranged from 38.21 to 53.13, while a variation of 46.8 to 49.13 was found in seeds exposed to the effluent from the floating emergent macrophyte treatment. In general, low frequencies of micronucleus and chromosomal aberrations were found in meristematic cells of *A. cepa* exposed to the sewage treatment plant samples and in control group. No significant differences in mitotic index, micronucleus and chromosome aberrations were observed among the negative control and effluent samples from the sewage treatment plant.

Table 2 – *Allium cepa* mitotic index and frequency of micronuclei and chromosome aberrations in seeds exposed to different concentrations of effluents from two different sewage treatment systems.

Treatment	Mitotic index	Micronucleus	Chromosomal aberrations
Negative control	53.64±12.57	0.0±0.0	0.40±0.90
Activated sludge 25%	54.46±25.07	0.0±0.0	0.0±0.0
Activated sludge 50%	53.13±29.37	0.0±0.0	0.0±0.0
Activated sludge 100%	38.21±9.78	0.0±0.0	1.00±1.15
Macrophyte system 25%	49.80±26.73	0.50±2.15	2.00±8.62
Macrophyte system 50%	51.26±19.38	1.00±4.30	1.00±4.31
Macrophyte system 100%	46.80±17.03	0.0±0.0	1.33±4.84

Data expressed as mean±standard deviaton.

4 Discussion

Due to the discharge of treated and untreated sewage into rivers and lakes, there is concern that chemical substances may harm organisms in the ecosystems as well as humans, by accumulation in the food chain (GRISOLIA et al., 2005). It has been shown that in large metropolitan areas, municipal wastewaters constitute in complex mixture of substances, containing a wide range of pollutants from a variety of sources (WHITE & RASMUSSEN, 1998). Thus, in addition to physicochemical characterization, the *A. cepa* test has been successfully used to detect the potential induction of cytogenetic alterations on wastewater from different sources.

Regarding to physicochemical analyses, despite the floating emergent macrophyte system had presented better removal of phosphorous and met the legal criteria for BOD_5, COD, suspended solids and termotolerant coliforms, in general, the activated sludge system showed better efficiency than the floating emergent macrophyte system. In a study carried out in this same municipal wastewater treatment plant previously, Goldoni et al. (2014) found that termotolerant coliforms and phosphorous did not meet the legal criteria for the floating emergent macrophyte system. Besides, the study was conducted during the startup of the system (within the first year of its implementation). The better results obtained by the current study for these parameters demonstrate an improvement in this system. However, since its efficiency is not suitable yet, continuous chemical monitoring and changes in the operating dynamics are planned in order to optimize and improve this system.

Because the use of a floating macrophyte system is recent, there is a lack of studies assessing this technology using bioassays. Firbas and Amon (2013) observed a decreased level of genotoxicity in meristematic onion cells exposed to effluents from a constructed wetland which received domestic sewage. Grisolia et al. (2005) also did not observe significant differences between the frequencies of chromosomal aberrations in onion bulbs exposed to crude sewage and effluents from a municipal wastewater treatment plant. Accordingly, in this study, there was no significant difference among groups regarding the *A. cepa* assay. However, a decrease in the mitotic index in the most concentrated samples from both treatments when compared to control was observed, especially in the activated sludge, which suggests the presence of cytotoxic agents in the sample. However, since micronucleus

and chromosomal aberrations were rare in both samples, it indicates that effluents from both treatments were not genotoxic, despite the differences in the removal efficiency of substances evidenced by the physicochemical characterization.

Therefore, in the present study, the physicochemical characterization combined with plant assay provided additional knowledge about two sewage treatment systems, demonstrating that the floating emergent macrophyte system is a promising alternative technology in sewage treatment.

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