



Integrated system with constructed wetlands for the treatment of domestic wastewaters generated at a rural property – Evaluation of general parameters ecotoxicity and cytogenetics



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ABSTRACT

The present research investigated the efficiency of an integrated system for the treatment of wastewaters generated at a rural property located in the city of Vera Cruz do Sul, southern Brazil. The integrated system was composed of an anaerobic unit (UASB/AB), 4 subsurface constructed wetlands (SSFCWs), and two photoreactors (UV-254 nm). The evaluation of the performance was based on the reduction of the load parameters and mainly on the detoxification of the wastewaters. The ecotoxicological analysis included short-term assays with the microcrustacean *Daphnia magna* whereas the cytogenetic potential of the wastewaters was assessed by using the *Allium cepa* test system. On the one hand, the raw wastewaters presented a strong pollution potential due to high COD, BOD₅, TKN, N-NH₃ and total P values. On the other hand, the effluents treated by the integrated system showed high reductions of the abovementioned parameters and fully attend the Brazilian and International legal requirements. Furthermore, the treated wastewaters presented a complete absence of the acute ecotoxicity (100%) as well as significant reductions of the cytotoxicity, genotoxicity, and mutagenicity when compared to the raw wastewaters. Therefore, the investigated system can be considered an interesting low-cost sanitation alternative, especially for wastewaters produced in rural areas, where decentralized systems are more suitable. Besides, the results obtained in the present work demonstrate the importance of including toxicity analysis in the monitoring of waters and wastewaters in order to obtain a broader evaluation concerning the efficiency of different treatment systems.

1. Introduction

The sustainable use of the water resources is a crucial issue that has been worldwide discussed over the last decades. Due to some factors such as climate change and resulting drought, population growth, and increased pollution, water shortage will reach increasingly people around the world (Pintilie et al., 2016). The results of a report of the World Health Organization (WHO) and the United Nations International Children's Emergency Fund (UNICEF) reported that almost 750 million people have no access to potable drinking water and around 1.8 billion people use a source of drinking water that is faecally contaminated (WHO/UNICEF, 2015).

In this sense, the polluted water resources without proper treatment are directly responsible for many illness and deaths worldwide. Therefore, the establishment of appropriate wastewater management

systems is a vital issue to protect the environment and the water bodies that serve as drinking water sources, especially in developing countries (Lam et al., 2015).

In order to minimize the environmental impacts caused by the disposal of untreated wastewaters, several treatment systems causing different impacts to the environment have been designed over the past decades (Horn et al., 2014). However, mostly, traditional wastewater treatment plants (WWTPs) while effective systems to remove some pollutants, commonly require large capital investments as well as high operation and maintenance costs (Machado et al., 2017).

Constructed wetlands (CWs) are something among the proven efficient technologies for wastewater treatment and have a strong potential for application in developing countries (Vymazal and Kröpfelová, 2009; Hijosa-Valsero et al., 2010). Because of the low energy consumption and the low maintenance costs, these systems may constitute an

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interesting alternative for the decentralized treatment of wastewaters in rural communities. Furthermore, CWs have a lower visual impact and lead to the production of smaller quantities of sewage sludge when compared to traditional WWTPs and to the production of biomass that can be used in animal feed (Vymazal and Kröpfelová, 2008; Sezerino et al., 2003).

Nevertheless, wastewater loading factors such as total Kjeldahl nitrogen (TKN) and total phosphorus (P) that have large surface area requirements, as well as strict standards of water quality for wastewater treatment and water reuse are some factors that can limit the application of these systems (Horn et al., 2014). In this sense, the integration of different treatments methods with CWs appears as an interesting alternative to minimize the limitations of the processes and add potential for improving the efficiency (Machado et al., 2015).

In general, urban wastewaters are composed of a great variety of environmental contaminants which might be toxic and only partially or even not eliminated by wastewater treatment systems (Radić et al., 2010; Hemachandra and Pathiratne, 2017). So, traditional physicochemical analyses are insufficient to determine the potential adverse effects that wastewaters might inflict on wildlife and humans due to the additive, synergistic, or antagonistic interactions between the chemicals present in wastewaters. In this way, the biomonitoring using different species might be considered an interesting alternative to complement physicochemical analyses, as test organisms respond to all the compounds in wastewaters (Prasse et al., 2015).

Therefore, the biomonitoring using aquatic organisms is an important tool in aquatic ecotoxicology that allows for the assessment of the level of pollution and degree of toxicity of wastewaters into the environment (Kern et al., 2015). *Daphnia magna* is a microcrustacean with worldwide distribution in freshwater that has been widely used as a test organism in ecotoxicological assays involving several chemical compounds present in aquatic ecosystems. Because of its importance in the food chain, sensitivity to toxic agents, and easy handling in the laboratory, standard methods with *D. magna* have been recommended by international environmental agencies such as the Organisation for Economic Co-operation and Development (OECD) and the United States Environmental Protection Agency (USEPA) (Kern et al., 2015).

The use of assays with higher plants to evaluate the cytotoxic, genotoxic and mutagenic effects of environmental pollutants has increased during the last years (Lutterbeck et al., 2015). Among them, the test system with *Allium cepa* root-tip cells has been widely used in assays to evaluate chromosome damages and disturbances in the mitotic cycle (Leme and Marín-Morales, 2009). Besides being an easy handling assay and don't require previous treatments of tested samples, as well as the addition of exogenous metabolic system, as in the Ames test, the *A. cepa* bioassays present a good correlation with other test systems, e.g. mammals (Rank and Nielsen, 1994) and allows the assessment of different endpoints. In this test, the screening of the cytotoxic potential is determined by the alterations of the Mitotic Index (MI), while the evaluation of the chromosome aberrations (CA) has been used as a parameter to detect potentially genotoxic agents. Furthermore, the *A. cepa* test also enables the evaluation of the mutagenicity through the formation micronucleus (MN).

Therefore, aiming to seek alternatives for treatment of wastewaters generated in rural communities, the present work investigated the performance of an integrated system in a rural property located in the city of Vera Cruz do Sul, Rio Pardo Valley, southern Brazil. The evaluation of the performance was based on the reduction of load parameters and mainly on the detoxification of the wastewaters.

2. Materials and methods

2.1. Configuration of the integrated system

Fig. 1 presents the studied wastewater treatment unit (WWTU). This experimental system, constructed in a rural property located, was

conceived in order to develop a decentralized model aiming the reuse of the wastewaters treated in situ. The choice for an individualized WWTP per property is justified by the low occupation density of the rural area.

The anaerobic reactor (UASB) and the anaerobic biofilter (AB) used in this study are composed of fiberglass and have useful volumes of, respectively, 400 L and 210 L. The AB reactor was filled with gravel n° 4 (diameter between 64 and 100 mm). The draining of the gas generated in the anaerobic process was done through a 24 mm PVC pipe with a dissipation height of 6 m. The constructed wetlands were disposed of in a subsurface horizontal flow arrangement (SSF CWs). Four sequential wetlands beds were constructed on the soil using a HDPE sealing membrane of 1.6 mm thickness. The filter support medium is composed of 30 cm of gravel n° 4 (64–100 mm) and overlapped by 30 cm of gravel n° 1 (20–40 mm). The hydraulic accessories used to form the distribution and collection areas consisted of perforated PVC pipes and flanges with 40 mm diameter.

Because of characteristics such as easy acclimatization to the study site, supporting load factors of total P of at least $0.3 \text{ g m}^{-1} \text{ day}^{-1}$, to have a root system of at least 40 cm in length and allow the pruning every three or four months, *Hymenachne grumosa* was the macrophyte selected in our study (Machado et al., 2015).

The sizing of the SSFCWs took into account the organic loading and the results from previous studies, which indicated a range of 3–5 m^2 per person (Vymazal, 2005; Sousa et al., 2004). The study conducted by Sousa et al. (2004) was consulted for the design of the present system because the applied load of COD in that study ranged from 5.01 to $9.45 \text{ g COD m}^{-2} \text{ day}^{-1}$.

Tables 1 and 2 present design specifications of the integrated system, which include data of the load factors with maximum values according to the characteristics of the studied effluents.

The photochemical unit was composed of two PVC photoreactors connected in series, with a diameter of 100 mm and equipped with two UV lamps of 30 W (=254 nm), which were concentrically arranged in the tubular reactors. The photovoltaic cell used to supply the power to the system has a capacity of 75 W, storing energy in a battery of 170 Amp-hr.

Five sampling points were defined at the treatment system: (P1) raw wastewaters, (P2) output of the anaerobic unit, (P3) input of the 3rd CW, (P4) output of the 4th CW and (P5) after photochemical unit (Fig. 1). The project was carried out over a period of one year.

2.2. Wastewater analytical characterization

At each collection point, the following characterization parameters of the raw and treated effluents were determined: Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), pH, total phosphorus (P), total Kjeldahl nitrogen (TKN) and ammonia nitrogen. All the analysis were carried out according to APHA/AWWA/WPCF (2012). Samples were taken weekly and analyzed thereupon the collection.

2.3. Acute ecotoxicity assays

The acute toxicity tests involving the microcrustacean *Daphnia magna* were performed at the ecotoxicology laboratory of the University of Santa Cruz do Sul (UNISC), through six sample replicates ($n = 10$). The assays were performed according to the methodology established in ABNT NBR 12713 (2004). A more detailed description of the test procedures and methodology can be found elsewhere (Lutterbeck et al., 2014).

Raw effluents and treated samples collected at different treatments stages of the integrated system (anaerobic unit/SSF CWs/UV) were tested and prepared with volumetric precision at a geometric progression ratio of $\frac{1}{2}$. Due to the variation in the composition of the raw effluents, 9 different concentrations were tested (100–0.390%).

To estimate EC_{50} , the non-parametric statistical method (Trimmed

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