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Efficiency of physicochemical and biological treatments of vinasse and their influence on indigenous microbiota for disposal into the environment

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ABSTRACT

Molasses-based distilleries are one of the most polluting industries generating large volumes of high strength wastewater called vinasse. Different processes covering anaerobic, aerobic as well as physicochemical methods have been employed to treat this effluent. This study evaluated the microbial communities present in the vinasse during different stages of its treatment by traditional and molecular methods. The analysis of the efficiency of each treatment was performed by physicochemical parameters and toxicity analysis. The treatment of vinasse was performed in the following steps: high flow fermentation; filtration; chemical flakes; low-flow fermentation; filtration; and neutralization. The physicochemical analysis in different stages of the vinasse treatment demonstrated that phases of treatment influenced the performance of the evaluated parameters. Among the 37 parameters, 9 were within the limits established by the Commission for Environmental Policy of Minas Gerais, Brazil (COPAM), especially BOD (96.7% of pollution reduction), suspended solids (99.9%), pH, copper (88%), iron (92.9%), and manganese (88%). Some parameters, even after treatment, did not fit the maximum allowed by legislation. The microbial population decreased reaching 3 log CFU/ml present in the steps of the flakes chemical and disinfection treatment of vinasse. *Lactobacillus brevis* and *Pichia kudriavzevii* were present in all stages of the treatments, showing that these microorganisms were resistant and demonstrated that they might be important in the treatment of vinasse. The vinasse showed a significant reduction of pollution load after the disinfection treatment however still should not be discarded into water bodies because the high values of tannins and sediment solids, but suggest the use of the effluent in the cooling coil during the distillation process of the beverage.

1. Introduction

The source of pollution is the main cause of the impact of water quality in urban areas, which can be changed by the impacts of human activities (Silva et al., 2011; Santos et al., 2013). Among the organic pollutants of great environmental impact are the agro-industrial residues such as vinasse. Vinasse is a liquid residue considered an organic pollutant produced after the distillation of fermented sugar cane broth to obtain alcoholic beverages such as brandy, rum, cachaça, and also bioethanol (Doelsch et al., 2009). The production of cachaça and fuel ethanol generates 4–10 L of vinasse per liter of final product (Silva et al., 2011). Basically, this residue becomes pollutant when it reaches the water bodies due to the presence of a high load of organic matter and high rates of biochemical oxygen demand (BOD) (Navarro et al., 2000). The nutrients (especially potassium) and organic matter present in the vinasse have been used in the sugar cane fields in partial or total substitution of mineral fertilizers (Ferreira et al., 2010). The indiscriminate application of vinasse is related to soil salinization, and possible sources of pollution to surface and groundwater. The harvest of 2007/2008, the year of this work, produced approximately 24 billion liters of ethanol producing 312 billion liters of vinasse in Brazil, a country that is among the major producers of alcohol (Demattê et al., 2004; Walter et al., 2008).

The physicochemical treatment of vinasse is expensive, such as those based on natural evaporation in ponds or on chemical oxidation, mainly by the large amount of water (about 95%), which requires high expense of energy to be separated from the part of interest. However, the vinasse treatment using microbial fermentation might be economically viable, and could reduce the BOD

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of vinasse with biomass production and allowing the dumping of effluent into water courses or used for other purposes (Satyawali and Balakrishnan, 2008).

The use of vinasse in aerobic fermentation processes is feasible because vinasse is rich in carbon and some salts like potassium and calcium, becoming an important source of microbial growth. The challenge of better utilization of waste is not a new subject, but it can still be improved (Navarro et al., 2000; Silva et al., 2011).

In developing technology and improving processes of decomposition of organic matter, the knowledge of microorganisms present in the substrate and their role during storage of vinasse is essential. A correlation between economic and ecological advantages of agro-industries residues treatment is necessary before proposing a new technology applicable to small, medium, and large producers of spirits, and bioethanol industries.

The aim of this study was to establish an eight-stage physicochemical and biological treatment targeting the reuse of vinasse. The persistent microbiota in each step and the assessment of toxicity parameters regulated by environmental laws for disposal of liquid waste in the environment were evaluated.

2. Material and methods

2.1. Vinasse

The vinasse was obtained from a cachaça producer from Perdões city (21° 6′ 4″ South, 45° 5′ 21″ West), Minas Gerais, Brazil. Samples were collected immediately after the distillation of spirit and 40 L of vinasse were transported to the Microbial Physiology and Genetics Laboratory, Department of Biology (UFLA) in aseptic container and stored at 4 °C until use.

2.2. Steps of vinasse treatment

The steps of the vinasse treatment were performed in plastic containers with capacity of 60 L arranged in decreasing height, allowing the flow of effluent under gravity. Two liters of sample were collected for physicochemical and microbial (biochemical and molecular methods) evaluation at each stage of the treatment. The samples were preserved at – 80 °C for later use.

The arrangement of tanks and the sequence of the treatment steps are illustrated in Fig. 1. The vinasse treatment consisted of eight successive stages involving physicochemical and biological (fermentation) processes. The activated sludge was used as inoculum for the fermentation. The descriptions of the formation of the activated sludge and the treatment steps of the liquid residue are described below.

2.2.1. Obtaining the activated sludge

Re-inoculation of the microorganisms naturally present in activated sludge after cell multiplication was done in all fermentation stages. The amount of inoculum used in the stages of fermentation (high and low flow) corresponded to 10% (v/v) of the total volume used.

In the formation of the activated sludge, the vinasse was maintained at rest and the decanted biomass was used for formation of the activated sludge. The cell increase was obtained in 70 mL of decanted biomass removed every 24 h for 8 days and maintained under stirring at 150 rpm until the formation of inoculum or activated sludge as recommended by Freire and Cortez (2000).

In the fermentation of high flow, the activated sludge was added to this step immediately after the formation of the inoculum. In the fermentation of low flow, the activated sludge was stored at −80 °C and then inoculated in this treatment step.

2.2.2. Fermentation with high flow system (activated sludge batch)

This step consisted in the inoculation of 4 L of activated sludge in 40 L of vinasse to be treated in the fed-batch process with flow rate of 4.4 L min^{-1}. The fermentation was carried out for 24 h (Araujo and Zaiat, 2009; Nandy et al., 2002).

2.2.3. Filtering

The removal of suspended particles was performed by filters consisting of layers of gravel (0.19 g), crushed stone (0.21 g), coarse sand (0.19 g), and fine sand (0.18 g) in descending sequence (Fig. 1). The filtration processes were performed by two interconnected filters. Filtration occurred after the fermentation processes (with high and low flow) and chemical flakes (Blonskaja et al., 2003).

2.2.4. Chemical flakes

The chemical flocculation was performed by addition of calcium oxide (30 g L^{-1}), aluminum sulfate (5 g L^{-1}) and ferric chloride (0.3 g L^{-1}) (Amudaa and Amoob, 2006; Satyawali and Balakrishnan, 2008). After this addition, the vinasse was left to rest for 6 h.

2.2.5. Fermentation of low flow system (activated sludge batch)

The fermentation of low flow system was conducted by the method described in Section 2.2.2 with flow rate of 0.4 L/min. The fermentation period was of 24 h (Araujo and Zaiat, 2009).

2.2.6. Neutralization

The neutralization was performed to minimize the corrosive power of the liquid waste treated. Orthopolyphosphate (5 mg L^{-1}) was added to reach a pH value of 7 (Prianti Jr. et al. 1998). This stage lasted 6 h.

2.2.7. Disinfection

The final treatment step consisted of the addition of sodium hypochlorite (4%) in the supernatant derived from the previous step. The holding time was of 6 h (Tomida et al., 1999).

2.3. Sampling

The first sample was removed from the fresh vinasse (untreated, sample 1) from the supernatant and from the sludge (composed of granular material of organic matter and microorganisms, sample 2). Further samples were taken after each stage of treatment of vinasse, successively: Fresh vinasse (supernatant + sludge) (sample 3); activated sludge (sample 4); supernatant from the fermentation of high flow (sample 5); silt from the fermentation of high flow (sample 6) and filtration I (sample 7); supernatant flakes chemical (sample 8); supernatant from the fermentation of low flow (sample 9); sludge from the fermentation of low flow (sample 10) and filtration III (sample 11); neutralization supernatant (sample 12); disinfection supernatant (sample 13) (Fig. 1).

All samples were subject to microbiological analysis and identification of microorganisms by traditional and molecular techniques. The physicochemical analyses were performed on supernatants from all recovery process of vinasse (samples 3, 5, 8, 9, 12 and 13).

2.4. Physicochemical analyses

The samples belonging to each step (Section 2.3) were evaluated according to the recommendations of the American Public Health Association (APHA, 1992) for pH, color, turbidity, bicarbonate alkalinity, conductivity, total dissolved solids, hardness, dissolved oxygen , BOD, COD, suspended solids, sediment solids,