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A combined process to treat lemon industry wastewater and produce biogas

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Abstract We studied a process employed for treating lemon industry effluents, using the macrophyte *Eishhornia crassipes* (water hyacinth) in a phytoremediation tank with a 6000-L workload. The diluted effluents BOD and COD were reduced to 70 and 61%, respectively, working with a 1.5-h hydraulic residence time (HRT). We investigated the effect of adding every 12 h an inoculum consisting of a consortium of microorganisms isolated from the macrophyte roots and recirculating 30% of the outflow. In this way, we achieved a volumetric removal rate (VRR) of BOD = 354 g/m^3 day. Plants were daily harvested from the tank to maintain growth rate and the density originally planted. We studied their use for biogas production in an anaerobic digester working with 12 and 16 days of hydraulic residence time. The yield obtained was 0.87 L/g and productivity 0.87 L/L day with a loading rate of 5 g/L day. Integrating both processes on an industrial scale would solve the effluent pollution problem and generate an energy source that could be used by the industry itself to lower its production costs.

Keywords Biogas · Treatment · Lemon industry · *Eishhornia crassipes*

Introduction

The treatment of industrial effluents is one of the important areas to be investigated to help us arrive at a solution for the environmental crisis the world is going through. Drinking water, the most precious element for the survival of all species, is currently undergoing an unrelenting contamination.

It is a priority to develop low-cost technologies for the treatment and recovery of the water used by industry. Developing such technology would be very useful so that the treated water may be used by recycling it again, by the same industry.

The citrus industry is one of the largest water consumers. It requires 17 m^3 to process 1 t of lemon to manufacture 60 kg of lemon concentrate (50°Bx), 4 kg of essential oil, and 59 kg of dehydrated peel. A factory that processes 25 t/h lemon consumes more than 10 million liters of water per workday.

Although the negative environmental impact of the lemon industry waste water is very large, very little is known about the use of low-cost technology like phytoremediation employed to treat this effluent.

Among all the techniques studied, the use of aquatic macropohytes is the most successful to eliminate organic and inorganic compounds (Valderrama et al. 2002; Mishra and Tripatki 2008; Ayyasamy et al. 2009). One of the best-known species is *Eishhornia crassipes*, a rapidly growing rooted macrophyte (Abbasi and Ramasani 1999; Malik 2007), known to grow profusely in polluted water bodies, (Gupta and Sujatha 1996) and eutrophic lakes. It has a great potential for heavy metal accumulation (Mishra et al. 2008; Agunbiade et al. 2009).

During the treatment with aquatic plants, it is necessary to harvest old plants systematically. A proposal to use that residual biomass is to produce biogas by anaerobic fermentation.

There are many reports on the production of biogas from plant waste (Badawi et al. 1992; Math-Alvarez et al. 1993; Lehtomäki et al. 2007; Nallathambi Gunaseeland 2004; Ward et al. 2008; Zhu et al. 2008).

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This biogas is combustible and may be used as an inexpensive source of energy to generate electricity and/or vapor in the production process (Jewell et al. 1993; ICITI 1983; Bauer et al. 2009). Besides, as the slurry left over after the anaerobic digestion is rich in nitrogen, phosphorus, and potassium content, it may be used as a fertilizer to improve soil quality in agricultural applications (ICITI 1985).

This study is aimed at designing an ecological process for the treatment of lemon industry effluents and biogas production as a concrete contribution toward solving the pollution problem.

Two objectives are proposed in this study. First, to reduce the contaminant load of the citrus industry effluents by a continuous phytoremediation process using *E. crassipes* and second, to use the daily harvested plants from the phytoremediation tank to produce methane in a biodigester. This biogas is combustible and may be used as an inexpensive source of energy to generate electricity and/or vapor in the production process. Besides, as the slurry left over after the anaerobic digestion is rich in nitrogen, phosphorus, and potassium content, it may be used as a fertilizer to improve soil quality in agricultural applications.

Materials and methods

Phytoremediation

Plants of *E. crassipes* (water hyacinths) with an average weight of 80 g were collected from a pond located in the Biological Park of the University of Tucumán, Argentina and washed with deionized water to remove the particles adhering to the plant.

The assays were carried out in a phytoremediation tank of 15 × 1 × 0.5 m ($l \times w \times h$) divided in 15 equal sections with a workload of 6000 L. Figure 1 shows the phytoremediation tank scheme. The divisions are necessary to improve effluent contact with the macrophytes and to maintain a uniform quantity of plants per surface unit.

To start assay No. 1, the tank was filled with water, and plants were placed in it with a density of 720 g wet weight (ww)/m^2. Plants were fed with an increasing percentage of citrus industry effluents to adapt them to the new substrate until reaching loads of COD = 2265 g/day and BOD = 1950 g/day with an HTR = 1 day on day 20 of the assay. The medium pH was adjusted to 7, according Wilson et al. (2005) optimum pH and temperature for the growth of water hyacinths is 6–8 and 25–27.5°C, respectively .

Work HTR was low (1 day) to have good liquid circulation in the tank. There is a consensus that water circulation is beneficial because it prevents thermal and chemical stratification (Turrel and Leeds-Harrison 2004).

The relative growth rate was determined by the relation (ln P1 – ln P2)/*t*. where P1 and P2 are fresh weights of the plant at different times between samplings (Walkley and Black 1934).

COD, BDO_5, TSS (total soluble solid), TVS (total volatile solid), color, and TKN (total kjeldahl nitrogen) were determined according to standard methods (APHA 1998). Carbon was analyzed according to Walkley and Black's technique (Walkley and Black 1934).

The percentage removal was calculated using the average inflow and outflow concentration of three samples collected on consecutive days.

Plants' weight measurement was done every week, carefully trying to avoid great disturbance to the system.

To improve process performance, 500 mL of a 6 g/L microorganisms consortium was inoculated every 12 h (assay No. 2). To produce it, the plant root was submerged and shaken in a medium prepared with 5% citrus plant effluent. It was next enriched with 10 g/L peptone, mono and disodic phosphate buffer for pH 7 (0.76 and 2.54 g/L, respectively) and incubated in rotary shaker (250 rpm) at 30°C. Finally, 30% of the tank outflow was recycled keeping HRT and organic load values (assay No. 3).

Biogas production

A 30-L, plug flow horizontal reactor of continuous load was used. The digester was intermittently shaken (5 min/h) at 30 rpm. It was originally fed with 7–12 mm pieces of macrophyte, but as many problems arose in the biodigester performance, all the assays for biogas production were carried out with the substrate in powder form. To obtain it, all the plants were cut into 2–3 cm pieces that were dried in a heater until reaching 8.4% humidity. Next, the pieces were ground and sieved through an ASTM E11 2000.

Biogas production was measured everyday by employing the water displacement technique (Singhal and Rai 2003). Two hydraulic residence times were assayed: 12 and

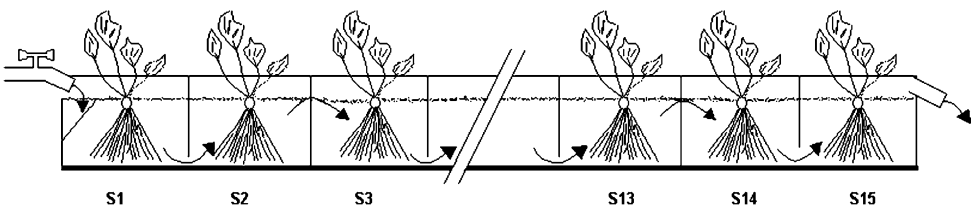

Fig. 1 The phytoremediation tank scheme

16 days. The production medium was a suspension of the powdered macrophyte dissolved in effluent, 8% total solids (TS). Initial pH was 6.8, C/N ratio 29, and temperature was kept at 35°C.

The ideal C/N ratio proposed for methanogenesis varies from 12 to 72 (DeRenzo 1997; Huang and Shih 1981; Ghosh et al. 1981).

The medium added was liquid cow manure in a 1/10 dung/water ratio. The liquid cow manure was obtained mixing and filtering water and manure in equal weights. All the assays were performed at ambient temperature that ranged from 24 to 30°C.

Results and discussion

Phytoremediation

The growth of *E. crassipes* was slow during the first few days of the assay, but it increased until day 30 when it stabilized. This was probably a period of adaptation to the substrate supplied (citrus effluent). Afterward, *E. crassipes* grew at a steady rate of 103 g(ww)/m^2 day. The fastest development occurred at the far end of the phytoremediation tank with values between 130 and 150 g(ww)/m^2 day, while the lowest values were detected in sector 1 with a growth rate of 60 g(ww)/m^2 day. As an average, the macrophyte doubled its wet weight after 7.2 days.

Eishhornia crassipes accepted the effluent as a substrate in the studied concentrations, reaching growth rates of 11.85 g(dw)/m^2 day, comparable with 10.57 g(dw)/m^2 day: data obtained by Olguin et al. (2008).

The relative growth rate for the whole system was 0.07 g/g day, average value taken from three samples on consecutive days starting on day 30 of the assay.

To keep the original plant density of 720 g(ww)/m^2, plants were daily harvested with an average value of 1500 g(dw). The specimens selected were those that showed some damage or the largest adults. Humidity plants content was 91.9%. Young plants had black roots with white ends which became violet when they bebome adult. The daily harvest keeps plants permanently growing which maximizes contaminant removal. The system in a stationary state achieved reductions of 70% for BOD, 61% for COD, and 61% for TKN with an HRT of 1.0 days. The process was more efficient when the treated effluent was recirculated than when laboratory-cultured microorganisms were inoculated (see Table 1, assay 3).

Residual BOD values during treatment kept a similar pattern to that of COD, but with lower values of about 0.32 and 0.89 g/L, respectively.

pH increased slightly throughout the experiment, reaching values of over 7.

Table 1 Initial and final values of COD, BOD, TKN, ST and volumetric removal rate (VRR) and %Removal for those variables

Assay		Initial (g/L)	Final (g/L)	%Removal	VRR (g/m^3 day)
1	COD	378	149	61	229
2		380	154	62	241
3		550	169	69	381
1	BOD	325	98	70	227
2		321	98	73	239
3		449	95	79	354
1	TKN	16.9	5.4	68	11.5
2		16.3	5.7	70	13.4
3		24.3	6.1	75	18.2
1	TS	405	95	76	31.0
2		405	102	77	35.3
3		590	116	80	47.4

Los data correspond to assays 1, 2 and 3 (see Materials and methods)

In the macrophytes, there is an important amount of root-associated microorganisms (1.5 x10^7 CFU/mL) that contribute significantly to a reduction of the contaminant load since they use the oxygen transported from the leaves to the roots.

In Table 1 the results obtained are shown with the addition of microorganisms to the system and along with 50% recycling of the phytoremediation tank effluent outflow. The removal percentages of the different variables increased between 5 and 13%. The increment percentage of volumetric removal rates (VRR) was still higher, ranging from 53 to 66%.

Microphyte treatment had another important effect: it reduced the original color up to 17.5%.

The number of microorganisms at the far end of the phytoremediation tank 10 days after beginning the assay was 1.5 × 10^6 CFU/mL; on day 30, the microbial population had grown to 3.3 × 10^7 CFU/mL. These values increased to 2.8 × 10^8 CFU/mL, when 30% of the tank water was recycled.

Anaerobic digestion

The macrophyte ground to powder solved the problems that the digester had experienced with the substrate so far. Similar problems were reported and explained by other authors thus: since *E. crassipes* biomass is lighter than water, it floats and clogs the digester. Hence, it is not feasible to feed it to the conventional biogas digester (Abbasi and Ramasani 1999) even after chopping/mincing. Moorhead and Nordstedt (1993)) reported that finer particles resulted in greater biogas production.

The results obtained with powdered *E. crassipes* as a substrate with two hydraulic residence times, 12 and 16 h

Table 2 Results obtained for biogas production with two hydraulic residence times (HTR)

RT (day)	TS_{in}/TS_{out} (g/day)	Biogas production (L/day)	Yield (L/g)	Productivity (L/L day)	Veloc.carga (g/L day)
12	173/67	27.0	0.255	0.90	6.65
16	130/43	26.7	0.307	0.87	5.00

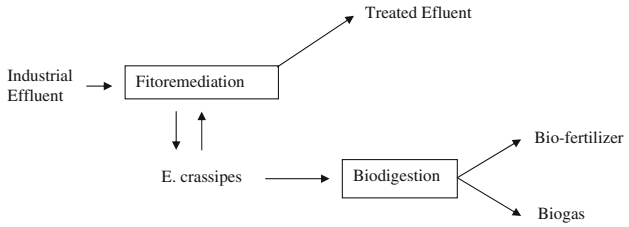

Fig. 2 Scheme of the mixed process "phytoremediation–anaerobic digestion" for the treatment of the lemon industry wastewater

and a load rate of 6.67 and 5.00 (g/L day), respectively, can be seen in Table 2. In both cases there is a solids surplus coming out of the digester that suggests that it is working at full capacity. This is confirmed by the results of volumetric productivity expressed in liters of biogas/L day, in both HRT's are similar. The calculated yield in connection with the volatile solids in the substrate is 0.44 L/g, taking into account that they represent 64% of TS.

The C/N ratio decreased from 29.0 to 20.3 during the anaerobic process as a consequence of decreasing total carbon values (30%). Nitrogen values remained constant. Methane content in the biogas was 63.2%.

The results obtained were agreeing well with the literature and surely the addition of liquid dung to the process helped our cause since a better yield of biogas is obtained using a mixture of animal waste and water hyacinth according to Kumar (2005).

Conclusions

The results obtained confirm the technical feasibility of phytoremediation employed to reduce and keep low the contaminant load of the citrus industry liquid effluents and to produce biogas with the macrophytes harvested during the process.

The scheme of the proposed process is shown in Fig. 2.

Recycling of the effluent treated in the phytoremediation tank favored removal rate by increasing microorganism population and their synergistic activity with plants in the rhizosphere.

The importance of plants supplying oxygen and nutrients to the rhizosphere microbes via fine roots and the beneficial effects of microorganisms on plant root growth have been sufficiently explored (Harvey et al. 2002; Ramos et al. 2005), but the macrophyte treatment showed yet another advantage of reduction in the effluent color due to the metabolic action of the plants on pigments.

The use of the powdered plant as a substrate not only favored process performance, but also improved its use and preservation for later assays that were not carried out during production time. This working methodology was also successfully used by Singhal and Rai (2003).

The powder could also be used in feed production because of its nutritive value (Malik 2007).

Applying the proposed technology, a lemon-processing plant, like the one described in "Introduction", which grinds 25 t lemon/h with an effluent BOD of 7 g/L was used and the yield obtained was 307 L biogas per BOD/g, besides reducing contaminant load by 70%, would produce around 1000 m^3 of biogas per day.

We will continue our research to inquire into the relationship between the microorganisms that live in the microenvironment of the root and the macrophytes. We will also try to improve biogas production yields by composting vegetable waste harvested from the phytoremediation pool before anaerobic digestion.

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