Biotecnología

Revista Mexicana de Ingeniería Química

Vol. 13, No. 3 (2014) 713-722

FERTIGATION OF SWEET SORGHUM (*Sorghum bicolor* L. MOENCH.) IN LABORATORY AND NURSERY ASSAYS WITH TREATED VINASSES OF HYDRATED ETHANOL OF UASB REACTOR

FERTIRRIGACION DE SORGO DULCE (*Sorghum bicolor* L. MOENCH.) EN LABORATORIO Y ENSAYOS EN VIVERO CON VINAZAS TRATADAS DE ETANOL HIDRATADO DE UN REACTOR UASB

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Received August 19, 2013; Accepted December 31, 2013

Abstract

Vinasse is used in crop fertigation indiscriminately, however, this wastewater generates serious environmental damage as the reduction in crop yield or even its death in the short-term and acidification, decreased oxygenation, increased organic load, accumulation of phenols in soil in the medium and long term. The aim of this study was the fertigation sweet sorghum (*Sorghum bicolor* L. Moench) in laboratory (bioassays germination) and growth in nursery treated with hydrated ethanol stillage from a UASB modified, in order to improve its chemical properties and use them to 50, 75% (diluted) and 100% of treated vinasse (TV50, TV75 and TV100 respectively). The raw vinasse (RV) had the lowest percentage of germination in seeds of sweet sorghum in laboratory tests (14% in 8 days) compared to the control treatment where 94% of germinated seeds. Growth of seedlings in the nursery with RV treatment was lower than in other treatments and after 13 days the plants completely stopped growth and died.

Keywords: energy crop, UASB treated vinasse, distillery wastewater management, soil fertility.

Resumen

Las vinazas se han utilizado en la fertirigación de cultivos de manera indiscriminada, sin embargo, estas aguas residuales generan daños graves al medio ambiente, desde la reducción en el rendimiento o muerte de los cultivos a corto plazo, así como la acidificación, disminución de la oxigenación, aumento de la carga orgánica, acumulación de fenoles en el suelo en el mediano y largo plazo. El objetivo de este estudio fue la fertirrigación de sorgo dulce (*Sorgo bicolor* L. Moench) en laboratorio (bioensayos de germinación) y crecimiento en vivero con vinazas tratadas de Etanol hidratado de un UASB modificado, con la finalidad de mejorar sus propiedades químicas y utilizarlas al 50, 75% (diluidas) y al 100 % de vinaza tratada (TV50, TV75 y TV100 respectivamente). La vinaza cruda (RV) presentó el porcentaje más bajo de germinación en las semillas de sorgo dulce en las pruebas de laboratorio (14% en 8 días) comparado con el tratamiento control donde germinó el 94% de las semillas. El crecimiento de las plántulas en el vivero con la vinaza cruda (RV) fue menor que en otros tratamientos, y después de 13 días las plantas detuvieron completamente su crecimiento y murieron.

Palabras clave: cultivos energéticos, vinasas tratadas UASM, manejo de aguas residuales de destilerías, fertilidad del suelo.

1 Introduction

The production of ethanol for fuel, pharmacy, industrial use and alcoholic beverages has increased in recent years worldwide, its production generates between 9 to 14 liters of waste water per liter of ethanol which are known as vinasse. The raw vinasse have recalcitrant compounds and characteristics like acid pH (3.5-5), high chemical oxygen demand (COD) ranging from 50 to 150 g/L, dark brown color

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(Pant and Adholeya 2007) and different salts and compounds that can pollute the soil and water courses (Sahai *et al*., 1985). The chemical characteristics of the vinasse generated in the production process of biofuel (hydrated ethanol) and the anhydrous alcohol (96% and 99.8% purity respectively) are more complex than that generated by the alcoholic beverages (5-40% purity), because during the distillation process are separated from the raw material aromatic compounds in addition to alcohol, melanoidins, phenols, ketones and esters, and other compounds, which remain in the vinasse when finally discharged to the environment; in the process of alcoholic beverages, the above mentioned compounds are preserved along with the alcohol providing the characteristic flavor and aroma (Janhom *et al*., 2009). However, the vinasses have also nutrients and compounds that can be used for the growth of crops, such as nitrogen, phosphorus and potassium, as well as a high organic matter content, providing them fertilizers properties (Rajkishore *et al*., 2012; Satyawali and Balakrishnan 2008; Pant and Adholeya 2007; Kaushik *et al*., 2005), which are used in distilleries located in the rural zone of countries such as India, Brazil even Mexico, where vinasse has been used for irrigating crops (Chandra *et al*., 2009; Ometto *et al*., 2004; Kalaiselvi and Mahimairaja 2011; Deshpande *et al*., 2012). But the direct disposal of the vinasses (without pre-treatment) may cause soil acidification due to a low pH and the high concentration of organic matter of the vinasse that increases the content of CO_2 in the soil; which in turn reduces their quality, their fertility and affects the development of plants and the microbiota. However, different treatments for this type of wastewater have been developed (España-Gamboa *et al*., 2011) that allow the final disposal to the environment with a lower pollution level or even making them exploitable.

The direct use of raw vinasses in crop fertigation may lead to the acidification of the soil and cause a deficiency in the absorption of nutrients by the plants, or may affect crops irrigated due to its high content of salts and other toxic compounds (Taiz and Zeiger 2006; Kaushik *et al*., 2005). Inhibitory effect on the plant growth have been reported when concentrated vinasse with or without treatment is used on fertigation of many crops: sunflower, pea, tomato, onion, cucumber, pepper, pumpkin, corn and peanut (Kadioglu and Algur 1990; Ramana *et al*., 2002a, b, c), so it is essential to perform a pre-treatment of the vinasse before using them as fertigation in agricultural fields and reduce their impact on the environment, and even turn them into a usable safe resource. The anaerobic treatments reduce the pollutant effect of the vinasse and maintain some nutrient concentration and organic matter beneficial to the growth and development of crops, instead of causing its depletion (Pant and Adholeya 2007, Deshpande *et al*., 2012).

The sweet sorghum (*Sorghum bicolor* L. Moench.) is one of the main energetic crops in the global context of renewable energy with low culture demand, it adapts to a wide range of soil types, have drought tolerance, its water demand can be covered with low volumes of water, tolerates soils with low fertility and tolerates high concentrations of salts in soil (Almodares and Hadi 2009; Chuck-Hernández *et al*., 2011; Rajkishore *et al*., 2012). In the search of a sustainable process of bioethanol production for biofuel based on sweet sorghum in Yucatan, Mexico, the goal of this study was fertigation of sweet sorghum (*Sorghum bicolor* L. Moench) in the laboratory (bioassays germination) and growth in nursery with hydrated ethanol stillage treated in a UASB, in order to improve its chemical properties and using treated 50, 75% (diluted in water), and at 100% of vinasse (TV50, TV75 and TV100 respectively). The proper anaerobic digestion of these wastewaters from the distillery industry could provide a reliable fertigation for crops and safe for the environment.

2 Materials and methods

2.1 Germplasm of sweet sorghum

The bioassays were conducted with sweet sorghum (*Sorghum bicolor* L. Moench.) var. M81E; this variety was developed by classic crop improvement methods, their parents were Brawley and Brauley/Rio. The M81E variety has been used in many basic studies, as well as commercial crop in the USA, other countries and in many states of Mexico; in cultivation reaches heights of plant exceeding 3.5 m, with approximately 12 °B concentration in the stem juice and 110 days to anthesis after planting (Ali *et al*., 2008).

2.2 Treated vinasse, origin and characteristics

The raw vinasse generated in the alcoholic fermentation process and the treated vinasses were used for fertigation.

Table 1. Characterization of hydrated ethanol vinasse of sugar cane molasses before and after treatment in a UASB* reactor and used for fertigation of *Sorghum bicolor* L. Moench.

	Untreated Vinasse	Treated Vinasse
pH	4.51±0.3	7.22±0.3
COD (mg L^{-1})	125,600±13,000	39,810±630
N_T (mg L^{-1})	1,377±140	1,160±70
N-NH_3 (mg L^{-1})	113±60	230±0
PO_4^{3-} (mg L^{-1})	147±40	117±10
S^- (mg L^{-1})	172±35	275±17
SO_4^{2-} (mg L^{-1})	5,433±810	0±0
K^+ (mg L^{-1})	6,706±930	6,838±240

*The UASB reactor was managed following the procedure established by España-Gamboa *et al*. (2012).

The molasses from sugar cane was fermented and then was distilled to produce hydrated ethanol (96% purity); after that, the vinasses obtained were treated in a modified UASB reactor (España-Gamboa *et al*., 2012) for the treatment of high organic loads, the UASB Reactor was operated at an organic load of 17.05 Kg COD/m^3 d^{-1}, with a hydraulic retention time of 7.5 days, that produced methane (0.263 m^3/Kg COD_{added}) (España-Gamboa *et al*., 2012). The vinasse was characterized before and after treatment in the UASB reactor (Table 1).

2.3 *Germination and growth experiments*

The study was conducted in two phases; the first one phase was the seed germination behavior under controlled conditions in the laboratory to observe the direct effect of the treated and raw vinasses; in the second one, the effect of these fertigation treatments were evaluated over germination and the vegetative growth phase in the nursery.

2.3.1. Germination bioassay

The seeds were placed in a petri dish between two double layers of paper (Brown paper and absorbent paper) dampened with 5 ml of the irrigation treatment, the humidity was maintained all time. The Petri dishes were kept in a germination chamber at 28±1 °C in darkness (Ramana *et al*., 2002a). The germinated seeds were counted every day in a sterile air laminar flow cabinet for eight days and the germination percentage (GP) was calculated; the germination criterion was the emergence of the radicle through the seed coat (Ramana *et al*., 2002a). However, as the germination process can be interrupted or inhibited at the biochemical or physiological levels at different stages (imbibition, when metabolic activity start and growth initiates), the germination value (GV), mean daily germination (MDG), peak value of germination (PV), period of energy (PE) and germinative force (GF) were analyzed according to Cabello *et al*., (2002).

2.3.2. Plant growth in the nursery

Pots of plastic with soil (6 kg of the first 10 cm of the superficial soil) native from the peninsula of Yucatan, Mexico were used; prior to the beginning of the experiment a mixture of soil sample was taken for the physical and chemically laboratory analysis. The experiment was conducted in a nursery protected by a plastic tunnel; this avoided the effect of the rain on the experimental units but allowing the passage of the light of the sun, leaving plants exposed to the natural photoperiod. The plant height, leaves number and length of the leaves were weekly evaluated.

At the start of the experiment the soil was irrigated till to saturation point and three seeds per bag were planted 3 cm depth; the soil humidity was monitored three times a week with a Lincoln tensiometer (9" length), if the moisture decreased below 50% of the field capacity all the treatment was irrigated again till to the field capacity.

2.3.3. Soil analysis.

The soil used in the pots of the nursery experiment comes from superficial native soil (10cm) of the north-west central region of Yucatan peninsula, classified as

Alfisol. At the end of the experiment, the soil of each treatment was chemical analyzed. The soil samples were dried at environmental temperature (26 °C) in the shade, they were ground and sifted through a sieve of 0.5 mm diameter for analyses of soil particle by the method of Bouyoucus hydrometer; the content of organic matter (OM) and the total nitrogen (Nt) by the Kjeldahl method; the pH was measured in a 1:2 soil:water ratio, the cation exchange capacity (CEC), potassium (K), calcium (Ca) and magnesium (Mg) interchangeable were determined by the method of NH_4OAc (ammonium acetate 1 N, pH 7) in a 1:20 ratio. The P determination was chemically analyzed by the methods: Bray P1 and Olsen for extra phosphorus. All these soil analyses were conducted following the methods established in the Official Mexican Standard 021 (NOM-2000).

2.4 Analysis of the data

In all experiments four treatments of irrigation were applied: Raw vinasse (RV), treated vinasse 100% (TV100) and diluted vinasse 75% (TV75) and 50% (TV50), with four replications, the control treatment was distilled (lab experiments) or tap water (nursery experiments). The experimental unit of the bioassays of germination (phase 1) consisted of Petri dishes with 25 seeds; the nursery experiments (phase 2), the experimental unit was a pot with three seeds and had in turn four replications. The information was analyzed by the completely randomized design (one way ANOVA) and when statistical differences among treatments were detected, a Tukey test was applied.

3 Results and discussion

3.1 Effect of the vinasse in sweet sorghum seed germination

The raw vinasse (RV) was deleterious for germination (14% to 8 days) of sweet sorghum seeds compared to the Control treatment where germinated 94% of seeds (Figure 1). This result agrees with the reports of Kadioglu and Algur (1990) and Ramana *et al*., (2002 a, b, c) where the concentrated and untreated vinasses completely inhibited the germination of seeds of sunflower, pea, tomato, onion, cucumber, pepper and pumpkin. On the other hand, this result is also consistent with the observed by Kaushik *et al*., (2005) and Bharagava *et al*., (2008), the GP descended as the concentration of the treated vinasse in the anaerobic

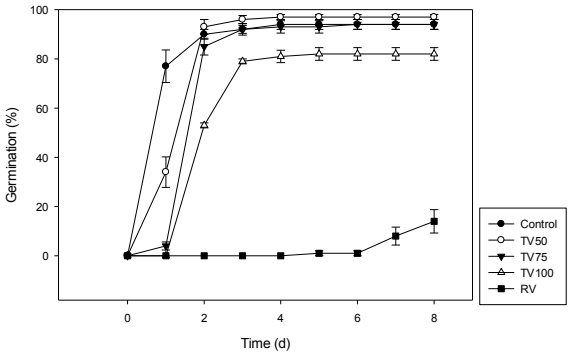

Fig. 1. Percentage of germination of *S. bicolor* L. Moench var. M81E seeds imbedded for eight days in untreated and treated vinasses in a UASB. Mean ± Std. error.

digestor increased, achieving a GP of 97, 94 and 82% for TV50, TV75 and TV100, respectively. Did not exist statistical differences of the GP among treatments (including the Control, 94%), suggesting a good effect of the treated vinasse, this fact supported that the vinasse treatment applied is very efficient, surpassing the reported until today (Kadioglu and Algur 1990; Ramana *et al*., 2002 a, b, c; Deshpande *et al*., 2012); in these works, the treated vinasse has to be diluted 50% to reach the GP value obtained in the germination with water. The results suggested that this system for treating vinasse will permit the direct application of treated vinasse (undiluted) as fertigation since the beginning of planting, which will allow a substantial saving of water for crop irrigation as compared to reported systems of vinasse treatments that require large quantities of water for the dilution of raw vinasse or dilution of the treated vinasse.

Sahai *et al*., (1985) found that seeds of *Phaseolus radiatus* reduced 67% the GV compared with the control (water imbibed) when applied untreated vinasse diluted 50%, and there was complete inhibition of germination (GV = 0) when applied concentrated vinasse; Kannan and Upreti (2008) noted that *Vigna radiata* declined to 46% the GV when seeds were imbibed for 6 hours in untreated vinasse diluted 20%, as well as a total lack of germination when the imbibition lasted 30 h. The results of this work indicated that for sweet sorghum, as in the studies mentioned before the GV values diminished. The germination was not inhibited when the seeds were imbibed in the RV for 8 days, but the GV declined from 23.67 to 2.99 (87.5%) in the Control treatment (Table 2).

Table 2. Physiological germination seed indexes *S. bicolor* L. Moench var. M81E under the effect of hydrated ethanol stillage in a UASB treated and untreated vinasse.

Index	Treatment				
	Control	TV50	TV75	TV100	RV
GV	23.67±1.52^{a}	18.69±1.26^{ab}	14.31±0.89^{bc}	11.47±0.74 ^{c}	2.99±0.67 ^{d}
MDG	0.77±0.07 ^{a}	0.465±0.015 ^{b}	0.425±0.017 ^{b}	0.265±0.005 ^{c}	0.018±0.006 ^{d}
PE (d)	1^{c}	2 ^{b}	2 ^{b}	2 ^{b}	8 ^{a}
GF	9.04±0.79 ^{a}	5.64±0.23 ^{b}	5±0.25 ^{b}	2.75±0. 13 ^{c}	0.04±0.015 ^{d}

GV, germination value; MDG, mean daily germination; PE, period of energy; GF, germinative force (1×10^{-2}). Values through lines with the same letters are statistically similar. PE is expressed in days. Mean ± Std. error.

The GV decreased from 18.7 to 11.5 as the concentration of treated vinasse increased from 50% to 100% (Table 2), the GV was significantly lower than the Control treatment when the seed was imbibed in vinasse with concentrations of 75% and 100%. However, the vinasse treated in the UASB employed here was significantly less harmful than the RV treatment, which showed the smaller GV (2.99), similar to what was observed by Kannan and Upreti (2008).

The seed germinated in water (Control treatment) showed the maximum value of the daily average of germination, 0.77 the first day, this value correspond to a period of energy (PE) of one day (Table 2); the treatments with treated vinasse (TV50, TV75 and TV100) reached the maximum values of the daily average of germination on the second day, which suggested a delayed of the germination process although not detrimental effect; this inhibitory effect observed in the MDG (Table 2) was greater (0.47 to 0.27) the more increased the concentration of the TV (50% to 100%). The third day of germination, the Control, TV50 and TV75 did not differ in the MDG (0.307, 0.32 and 0.307, respectively), although TV100 still showed a significantly lower value (0.263). The reduction of the MDG of the control, TV50, TV75 and TV100 continued the following days (4-8); however, from the day five the differences between Control and treatments with treated vinasse disappeared (Figure 2). The seeds imbibed in RV showed the lowest MDG over the evaluation time with values close to zero and reached a peak until the eight day (0.018), which corresponded to their PE.

The little inhibitory effect on the treated vinasse decreased on dilution with water; however, the inhibitory effect of untreated vinasse in the physiological processes (GV, MDG and PE) was very high compared with the vinasse that has received a treatment in the modified UASB (Figure 2, Table 2).

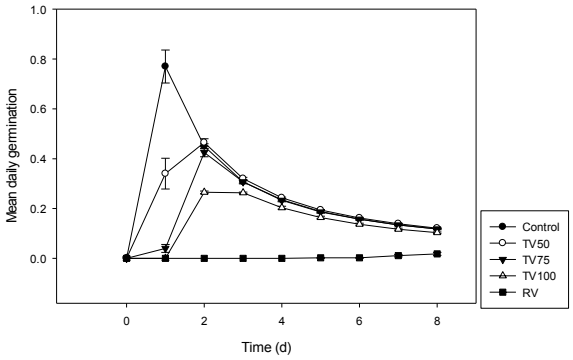

Fig. 2. Mean daily germination over time of seeds of *S. bicolor* L. Moench var. M81E, imbedded in untreated and treated vinasses in a UASB. Mean ± Std. error.

The highest germinative vigor was achieved by seeds germinated in water (Control), followed by TV50, TV75 and TV100 (Table 2), where TV50 and TV75 were statistically similar to the Control treatment. The RV showed the smaller GF (almost zero) indicating its deleterious effect; Kannan and Upreti (2008) reported that the GF decreased as increased the concentration of treated vinasse treated with anaerobic digestion; a similar effect was obtained in this study with UASB (anaerobic digestion) but less inhibitory effect was observed, suggesting that the treatment applied here to the vinasse detoxified more efficiently.

Many studies have indicated that the exposure of the seeds to unfavorable environmental conditions can reduce and even completely inhibit germination (Kranner and Colville 2011; Ahmad *et al*., 2009; Kannan and Upreti 2008). These results indicated that the process of germination is affected since the initial

stages (the first two days, perhaps hours) possibly since the phase of imbibition of water, which involves the process of osmosis that allows the hydration of the cells, its volumetric growth and the softening of covering tissue that allow the oxygen diffusion at the beginning of the seed breath (McDonald *et al*.,1987); According to Bharagava *et al*., (2008), the inhibition of germination when used vinasse as fertigation can be attributed to the high concentration of salts, which produces an increase in osmotic pressure and anaerobic conditions, affecting many biochemical processes, such as the transport of solutes, the seed breath process, as well as the enzymatic processes involved in germination. Similarly, it has been observed that the potential water of untreated vinasse is very low, due to the presence of salts and metals, causing a deficit in the water absorbed by the seeds, affecting the biochemical processes mentioned above (Kranner and Colville 2011; Kannan and Upreti 2008). However, these inhibitory effects of the vinasses can vary among plant species, for example, the tomato (*Solanum lycopersicum*) showed inhibition of germination since 5% of vinasse concentration; in contrast, onion seeds (*Allium cepa*) showed a greater and faster germination in the vinasse 10% concentration when compared with germination in water (Ramana *et al*., 2002a). The sweet sorghum was successfully germinated with treated vinasse in our UASB till to 75% concentration, this could be due partly to the fact that the specie tolerate high concentrations of salt (Almodares and Hadi 2009); however, it also suggests that the use of such device decreased in high quantity the inhibitory effect on the germination of sweet sorghum, due to the removal of large amount of salts and recalcitrant compounds initially present in the vinasse (Table 1).

If the physiological germination indices (high germination, faster seed germination in shorter periods of time and higher germinative force) are not affected by the fertigation or they are improved, in agronomic terms helps in the crop establishment and the development of the plants in the field, making easier the management of the crop and prevents the economic loss.

3.2 Effect of the vinasse in the vegetative growth in the nursery

When sweet sorghum seeds were sown in soil and irrigated with water showed 100% germination (criteria: the emergence of the epicotyl, cotyledon and first apical meristem above the soil surface); the seeds irrigated with TV50 showed 91.7% of germination and TV75 and TV100 100% of germination. By involving the soil factor, germination was less affected compared with that observed in germination tests in the lab by direct imbibition in TV100 and RV treatments, suggesting a buffering effect on the inhibitory factors.

Seedlings irrigated with RV showed the lowest percentage of germination (83.33%) and a delay in the germination time. The growth of seedlings in this treatment was lower than in other treatments and after 13 days the plants completely stopped growth and died (Figure 3a), so this treatment was eliminated from subsequent analyses.

After 94 days of cultivation in pots in the nursery (vegetative phase), it was noted that the trends in growth (plant height, number of leaves per plant and leaf length) did not differ between the different irrigation treatments (Control, TV50, TV75 and TV100); thus, at the end, the plants were between 53.5 and 64.7 cm height (Figure 3a); the number of leaves per plant was between 5.8 and 6.8 (Figure 3b), and the leaf length were between 29.1 and 29.9 cm (Figure 3c).

Kadioglu and Algur (1990) applied fertigation in pea plants (*Pisum sativum*) and sunflower plants (*Helianthus annus*) with untreated vinasse, observed a sharp decline in growth at concentrations of 25% and higher. A similar effect was observed by Sahai *et al*., (1985), mung bean plants (*Phaseolus radiatus* L.) irrigated with untreated vinasse (from distillery); the vinasse diluted at 30% of concentration or at higher concentrations showed deterioration. In both works, the seedlings did not survive the irrigation with concentrated raw vinasse; this effect also was observed in this study, so the vinasse without treatment or diluted but at high concentrations it is not suitable for use as fertigation in any crop, since plants do not have the ability to survive and can even be contaminating soils and surface water and groundwater.

Bharagava *et al*., (2008) irrigated black mustard plants (*Brassica nigra*) with treated vinasse digested anaerobically (from distillery); the vinasse was diluted at different concentrations and found that 50% concentration led to an increase in the height of plants, root length and leaves number, but when plants were irrigated with higher concentrations (75-100%), the variables increased only during the initial phase of growth, followed by a deterioration in the final phase coupled with delays in flowering and fruit formation in the plants. Bharagava and Chandra (2010) observed an increase in the height of bean plants (*Phaseolus mungo*

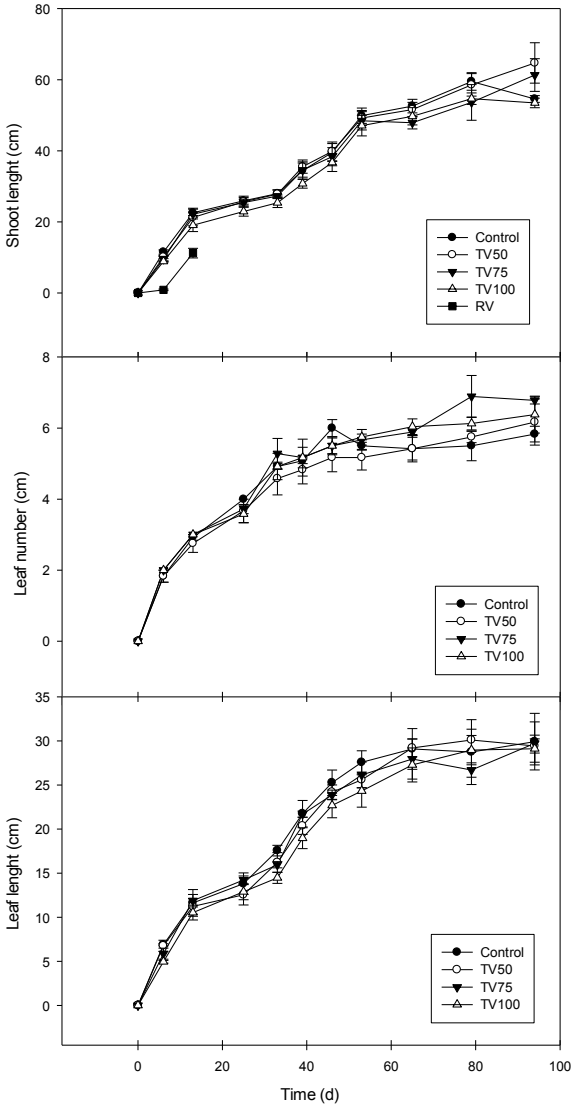

Fig. 3. Growth of *S. bicolor* L. Moench var. M81E irrigated with untreated and treated vinasses in a UASB. a) Plant height, b) Number of leaves and c) Leaf length.

L.) when were irrigated with vinasse treated with anaerobic digestion (from distillery) and when were diluted 5-10%, and they saw a sharp decrease in the plant height when the concentration was higher than 40%. In the same way, Kaushik *et al*., (2005) found that pearl millet plants irrigated with treated vinasse (from distillery) with anaerobic digestion and diluted to 50% had no important differences in their growth compared to Control (water irrigated), in contrast to those plants irrigated with undiluted treated vinasse, which showed a decline in its growth rate. Unlike the cited works where the highest concentration of vinasse treated in anaerobic digestion supported by involved crops was 50%, this study showed that the vinasse treated (hydrated ethanol) in the UASB did not produce negative effects on the growth of sweet sorghum plants, var. M81E, even when the treated vinasse was applied concentrated. This will allow the use of the treated vinasse concentrated as fertigation, this means that no water is required to dilute it before its application in the field and mean an additional saving of water as no clean water is needed for irrigation.

3.3 Effect of the treated vinasse in the soil

The pH of the treated vinasse in the UASB was always found near neutral value, it is an appropriate range for the use of wastewater in agriculture (6.5-8.4). The pH of the soil increased progressively as the treated vinasse was applied; it rose from 6.43 to 7.17 as the vinasse increased in concentration (up to 100%). When the soil pH surpass the value of seven there is the possibility for the crops present difficulties in the absorption of certain nutrients (P, B and Mn), being critical the pH equal to or greater than 7.5 (Taiz and Zeiger 2006).

The extractable phosphorus in the soil, found in the labil part (aqueous fraction, usable by plants) decreased from 33.8 % in TV75 to 25.45% in TV100 as compared to Control treatment (Table 3), this fact can be mainly attributed to the natural consumption by plants and not necessarily means that the phosphorus was exhausted dramatically; since the largest amount of phosphorus in soil is immobilized in the solid fraction of soil. In soils with an alkaline pH, the union of the ion phosphate (the extractable phosphorus) to the calcium ions present in the soil is promoted, causing its precipitation in the form of calcium phosphates (Lee *et al*., 2011; McDowell *et al*., 2003); in this study, the available calcium concentration must be high because of the calcareous nature of the soil of the peninsula of Yucatán, Mexico, as well as the vinasse application, that had a significant concentration of calcium (0.45-5.18 g L^{-1} CaO). It is important to mention that the sweet sorghum is a crop adapted to alkaline soil that supports up to 8.5 pH (Almodares and Hadi 2009; Patil and Sheelavantar 2004).

The nitrogen in the soil was slightly increased, being higher in the soil irrigated with treated vinasse concentrated (Table 3), this is considered beneficial to

Table 3. Soil analysis fertigation with vinasse treated in a UASB during vegetative growth of *S. bicolor* L. Moench var. M81E.

	Control	TV50	TV75	TV100
pH	6.43	6.61	6.97	7.17
N_T (g L^{-1})	6.675	7.032	7.033	7.178
P_{Extra} (mg Kg^{-1})	36.86	9.49	12.46	9.38
K_{Inter} (mg Kg^{-1})	581.71	2397.06	2476.81	3344.66
Organic matter (%)	11.68	11.42	12.29	9.95
Sulphtes (mg L^{-1})	245.78	367.23	358.61	505.16
Texture	Silt loam	Silt loam	Silt loam	Silt loam
%Sand	24.6	22.04	20.04	20.04
% Silt	59.28	58.56	58.56	54.56
% Clay	16.12	19.4	21.4	25.4

pH, in water 1,2, N_T, total nitrogen, P_{Extra}, extractable phosphorous, K_{Inter}, interchangeable potassium.

the soil because even with the application of nitrogen fertilizers the concentration of nitrogen in the soils decreases after cropping. This means that the vinasse supplied the nitrogen needed by plants and prevented the loss of the nitrogen (Table 3), so it is considered that here it has had fertilizer properties.

The exchangeable potassium was greatly increased with the application of treated vinasse, rising from 581.71 (Control treatment) up to 3344.66 mg Kg^{-1} with undiluted treated vinasse (Table 3). This increase was due to fertigation, vinasse treated in the UASB had a high amount of potassium (6838 mg L^{-1}).

It was also observed an increase in the amount of sulfates in soil, the raise was based on the increase in the concentration of the treated vinasse from 245.78 (Control treatment) to 505.16 (mg L^{-1}) for TV100 (Table 3). The treated vinasse applied in the irrigation had no sulphates, but had a significant concentration of ion sulfide (275±17 mg L-1), which could be transformed to sulphate by effect of sulphide oxidizing bacteria commonly present in the treatment of wastewater in anaerobic digestion (Cirne *et al*., 2008) and that therefore they are also in the vinasse treated in the UASB and could come into the soil by irrigation, causing a beneficial effect on the fertility of the soil.

The percentage of organic matter remained without major changes throughout the treatments, this behavior is normal because the vinasse suffered a treatment that reduced to almost one-third the COD (43,450 mg L^{-1}) (Table 1), and also the significant changes in the content of organic matter in the soil lasts one or two decades of cultivation to observe.

After the vegetative phase of growth and development, there were no differences among the Control and the treatments, it means that treated vinasses were innocuous; also based on the soil analysis and the characterization of the treated vinasse the fertigation helped the environment replenishing, increased soil mineral constitution and fed the plants while growing.

The consensus in the literature (as mentioned here) suggest that both the raw and treated vinasse need to be diluted with large amounts of water before irrigation to avoid crop damage, even it is suggested that both kinds of waste water should be applied once just before sowing (Rajkishore *et al*., 2012), but this will causes pollution problems to the soil at mid or long-time, leading to the loss of fertility and increasing the salinity of the soil, among other problems.

Conclusions

This work suggests that vinasses treated by this method can be used concentrated, so partly or completely could replace the fresh water for Sweet sorghum irrigation from which ethanol is obtained, contributing to the sustainability of the bioethanol production system.

The raw vinasse (RV) was deleterious for germination (14% to 8 days) of sweet sorghum seeds compared to the Control treatment where germinated 94% of seeds and a delay in germination time (6 days) in the lab test. Growth of seedlings in nursery with RV was lower, and after 13 days the plants completely stopped growth and died.

The modified UASB reactor, properly decontaminated the vinasses, it was inferred from the normal physiological and developmental behavior in germination and growth of plants and the chemical analyses of the treated vinasses with the advantage of retaining nitrogen, potassium, sulfide and organic matter to healthy levels for the soil, which promoted the partial restoration of soil nutrients and prevented its loss.

Acknowledgments

The authors gratefully acknowledge the contribution of I.A.H. Daniel A. León Gómez for the technical assistance in the nursery. This research was partially financed by Fondo Sectorial CONACYT-CONAVI, Scientific Project: No. 101284.

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