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Short communication

Bio-concentration of vinasse from the alcoholic fermentation of sugar cane molasses

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Abstract

A concentration-incineration process of vinasse has been in use for several years in order to deal with pollution resulting from the industrial production of ethanol by fermentation and distillation. However, as vinasse concentration has a high energy demand, a bio-concentration method with no energy consumption is reported in this paper. Vinasses was used instead of water in the preparation of the fermentation medium and repeatedly recycled. A final solid concentration of 24% dry matter was produced, an amount that positively modifies the energy balance of the concentration-incineration process. A decrease of 66% in nutrients addition, 46.2% in fresh water and 50% in sulfuric acid requirement was achieved together with an improvement in the efficiency of the fermentation. The final vinasse had a significant amount of non-volatile by-products of commercial importance such as glycerol. A mathematical model is proposed for the prediction of the final solids concentration in vinasse under various working conditions.
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Keywords: Ethanol production; Fermentation; Distillation; Vinasse; Sugar cane molasses; Bio-concentration

1. Introduction

Because of pollution problems, the treatment of distillery wastewater, generally known as vinasse, is one of the most significant and challenging issues in the industrial production of ethanol.

A distillery with a daily ethanol production of 100 m^3 has a vinasse discharge of 1300 m^3 having a high pollution load with BOD values ranging from 30 to 60 g O_2/l [1]. Vinasses, which hold the remaining soluble matter after the fermentation-distillation process of sugar cane molasses as well as the non-volatile fermentation by-products, is one of the most recalcitrant wastes [2].

Some of the existing methods for the disposal of vinasse are direct land application [3], and methane production [4]. However, if vinasse is discharged on land, the alkalinity of the soil is reduced so that crops may be destroyed [5], a manganese deficiency in the soil occurs [6] and seed germination can be inhibited [7]. Another option, the concentration-incineration of vinasse, is the only system that can provide a satisfactory solution to the pollution problem, its only drawback being its expensiveness [2].

In this investigation, we worked on the alcoholic fermentation of sugar cane molasses and studied the possibility of bio-concentrating vinasse, using it instead of water in the preparation of the fermentation medium. We worked with different vinasse percentages in the medium and repeated the process in several successive batches, and then designed a mathematical model to predict the final solids content in vinasse. In addition, we determined the effect of this system on acid, water and nutrients consumption as well as the efficiency of the process.

2. Materials and methods

2.1. Micro-organism

Saccharomyces cerevisiae CMI237, obtained from the Biotechnology Department stock collection, was used in the experiments. The yeast was kept at 4°C and transferred monthly into an agar Sabouraud medium. The

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inocula for the experiments were prepared by culturing the yeast in liquid Sabouraud medium and then separating it by centrifugation at 2800 ***g*** for 10 min.

2.2. Fermentation medium

In g/l: 320 sugar cane molasses containing 50% fermentable sugars; 0.3, $MgSO_4 \cdot 7H_2O$; 1.0, $(NH_4)_2SO_4$; 0.5, KH_2PO_4. The mixture was adjusted to pH 4.5 with a 20% (p/v) solution of sulfuric acid.

2.3. Fermentation conditions

All fermentations were carried out at 30°C in 1 liter Erlenmeyer flasks containing 500 ml fermentation medium each in sterile conditions at an initial cell concentration of 3 g/l dry weight (d.w).

The determination of reducing sugars was effected by the Fehling–Soxhlet method [8]. Ninety-five per cent of reducing sugars are fermented by the yeast used in the experiments.

The ethanol content was measured using a gas chromatograph equipped with a flame ionization detector and a column packed with a Chromosorb 101. Glycerol was measured with a triglicerids assay kit from Wiener Lab. according to the supplier's instructions.

The determination of yeasts concentration was made by the dry weight method. The viable cell count was effected using methylene blue stain [9] and total solids according to standard methods for the examination of water and wastewater [10].

2.3.1. Experiment I: 100% re-use of vinasse

The first fermentation (F1) was carried out using the fermentation medium described above. When the fermentation was completed, alcohol was separated by distillation and yeasts were removed by centrifugation. The residual liquid (vinasse), which was used to prepare the medium for the second fermentation, was supplemented with molasses and salts, brought to its original volume with water, and adjusted to pH 4.5 with a 20% (p/v) solution of sulfuric acid. Identical operations reusing vinasse were carried out three times, hereafter referred to as F2, F3 and F4.

2.3.2. Experiment II: 70% re-use of vinasse

The same procedure used in Experiment I was carried out, this time using 70% of the vinasse obtained from the preceding fermentation plus 30% water to prepare the medium. This operation was repeated until five reuses (F2–F6) had been made.

2.3.3. Experiment III: 70% re-use of vinasse with yeast recycling

The same procedure as in Experiment II was followed, this time re-using the yeast from each fermentation.

Up to the fifth fermentation yeast was re-used; after that, 20% yeast was discarded. Eight fermentations with re-use of vinasse (F2–F9) were made.

2.3.4. Experiment IV: 60% re-use of vinasse with yeast re-use

The same procedure as in Experiment III was followed, this time 60% vinasses was re-used. Fourteen vinasse re-usages were effected.

After the fifth fermentation, the added amount of salts was reduced to a third of that indicated under fermentation medium.

3. Results and discussion

In Experiment I, the final values of ethanol in each fermentation decreased with the number of vinasse re-used. In the fourth fermentation (F4), the fermentation rate was very slow and the ethanol concentration reached a maximum of only 18 g/l (Fig. 1). The concentration of soluble solids was 35% dry matter (d.m.) this being the probable reason for the fermentation inhibition. The soluble solids present in vinasse, which proceed mainly from molasses, are non-fermentable carbohydrates and salts as well as growth inhibitory compounds such as furfural [11]. Vinasse also has soluble solids originated as by-products of the metabolism of yeasts such as glycerol, propanol and lactic acid, which also inhibit fermentation and growth rate [12–14]. Solid content of vinasse after the first fermentation is 9.6% (p/v).

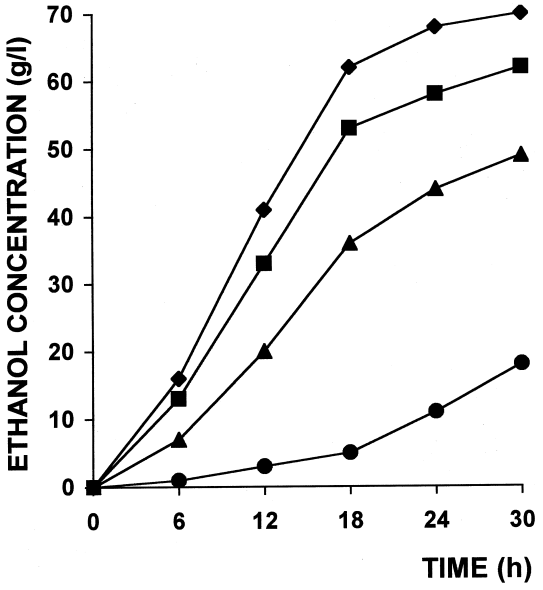

Fig. 1. Experiment I, ethanol concentration as a function of time in successive fermentations with 100% re-use of vinasse. Fermentation 1 (F1), ◆; F2, ■; F3, ▲; F4, ●. F stands for fermentation, so that F1 refers to the first fermentation, F2 to the second and so on. For a full explanation refer to Section 2.

Experiment II showed results similar to those obtained in Experiment I. However, in this case, it was possible to carry out five additional fermentations with no significant loss in either fementation rate or ethanol concentration. This was due to the fact that, when 70% vinasse was used, the accumulation of solids was lower, reaching a maximum of 29% (d.m.). In the sixth one, the fermentation rate was very slow and the ethanol concentration dropped almost to 15 g/l (Fig. 2).

In Experiment III, both the fermentation rate and the ethanol concentration increased during the first eight fermentations and then decreased in F9 (Fig. 3). The increased number of re-using vinasse with the associated high ethanol production in this experiment were not related to the accumulation of solids, which reached a slightly higher value (30.8%) than in the previous assay, but due to yeast recycling, since cell recycling produced a progressive adaptation of the yeast to the medium's unfavorable conditions.

During Experiment IV the final ethanol concentration was higher than 70 g/l in all fermentations. The solids content in vinasse reached a value of 24% d.m. in the last fermentation. In this case, it seems that the inhibitory compounds in the medium did not reach concentrations high enough to inhibit yeast growth. In the sixth fermentation (F6), the addition of salts and acid to the medium was reduced; this allowed us to determine that an amount of 66% salts and 50% sulfuric acid lower than in the original medium did not alter the performance of the following fermentations.

In Experiment IV, after successive re-use of the yeast, morphological changes occurred, producing in some cases yeast cells with a "rabbit head" shape, that might be attributed to the presence of acetic acid in the medium. Acetic acid inhibits phosphate transport through the membrane by chemical interference, a process that leads to a membrane disruption, which alters cell morphology, producing irregular elongated cells [12]. In this experiment, the percentage of dead yeasts did not exceed 3%.

The maximum value obtained for glycerol in the concentrated vinasse was 12.6 g/l. Glycerol production increased together with vinasse concentration, probably due to the hypertonic medium. This fact can be of interest for industrial exploitation.

The number of repeated fermentations with no decrease in the fermentation rate seems to depend on the experimental conditions, i.e. vinasse solids concentration, percentage of vinasse recycled and adaptation of the yeasts to the medium.

The inhibitory effect on growth rate because of vinasse concentration caused a decrease in the consumption of the substrate, so that its availability for ethanol production increased. This effect, added to the fact that non-fermented residual sugars can be used in the next batch fermentation and to the increased maintenance of energy requirement for the yeasts in a stressed environment [12], resulted in an increase from 0.445 to 0.462 in the yield coefficient ($Y_{p/s}$).

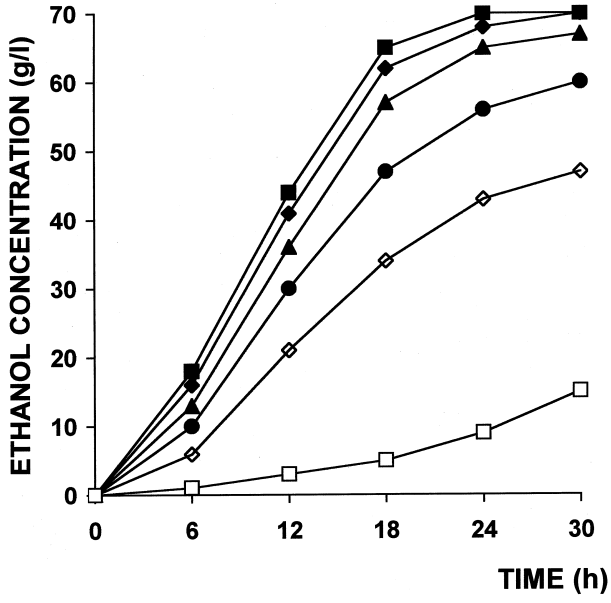

Fig. 2. Experiment II, ethanol concentration as a function of time in successive fermentations with 70% re-use of vinasse. Fermentation 1 (F1), ◆; F2, ■ ; F3, ▲ ; F4, ● F5, ◇ ; F6, □. F, stands for fermentation, so that F1 refers to the first fermentation, F2 to the second and so on. For a full explanation refer to Section 2.

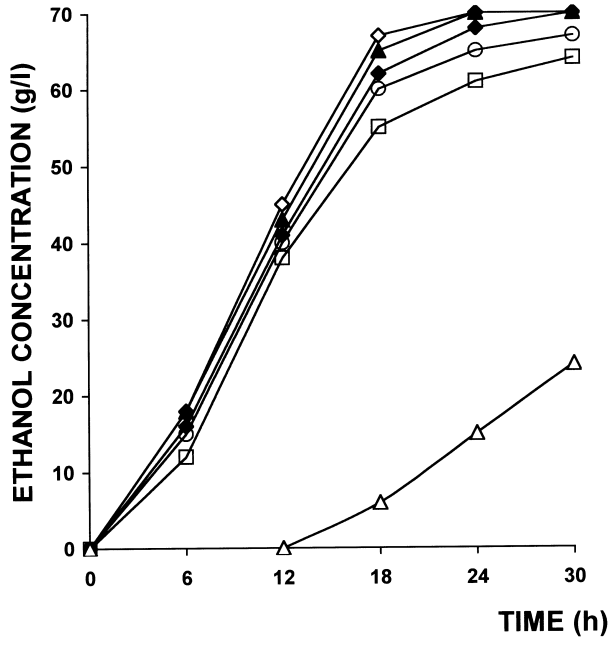

Fig. 3. Experiment III, ethanol concentration as a function of time with reutilization of yeasts and 70% re-use of vinasse. Fermentation 1 (F1), ◆; F3, ▲; F5, ◇; F7, ○; F8, □; F9, △. F, stands for fermentation, so that F1 refers to the first fermentation, F2 to the second and so on. For a full explanation refer to Section 2.

3.1. Ethanol, vinasse and energy

An energy balance of the process of evaporation-incineration was carried out using the data from Experiment IV. The solids content present in the vinasse obtained in that experiment (24% d.m.) was not the highest throughout our assays. Nevertheless, the working conditions in that experiment would allow the re-use of vinasse indefinitely with none of the inhibitory effects observed in Experiments I, II and III.

A distillery with a daily production of 100,000 liters of ethanol consumes 270×10^6 kcal/day (2700 kcal/l anhydrous ethanol) for distillation and produces as waste approximately 1300 ton/day vinasse with a solids content of about 9% d.m.

The bio-concentration procedure carried out in Experiment IV would permit the treatment of 450 ton/day vinasse with a solids content of 24% d.m. The amount of energy required to concentrate that quantity of vinasse to 56% (d.m.) is 33×10^6 kcal, and the result would be 192 ton/day concentrated vinasse with a combustion heat of 1800 kcal/kg [15]. By incinerating the vinasse thus concentrated, it would be possible to produce all the energy necessary for the final vinasse concentration process and for ethanol distillation. A flow chart of the ethanol production process with bio-concentration and incineration of vinasse can be seen in Fig. 4.

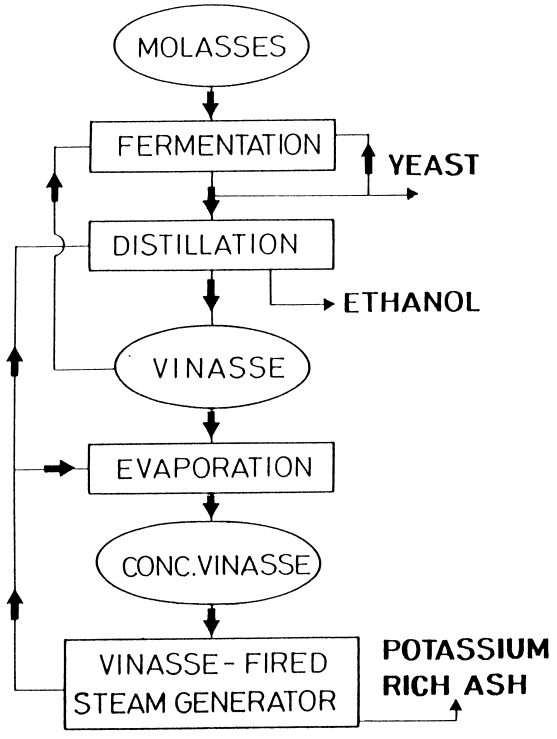

Fig. 4. Flow chart of the ethanol production process with bio-concentration and incineration of vinasse.

3.2. Mathematical model

In order to find out the final solids concentration (C_f) in vinasse we propose, the following mathematical model:

$$C_f = \frac{C_o(1 - r^{n+1})}{1 - r}$$

where C_o is the initial solids concentration in the vinasse, n is the number of batches re-using vinasse. r is the recycling ratio and is the difference between the amount of vinasse used and the amount of vinasse produced. The values for the final solids concentration, C_f, obtained with this model are very similar to the experimental ones. The statistical analysis of the data yielded a value of goodness of fit test $\chi^2_{(9)} = 0.12632$ ($P = 1$).

4. Conclusions

The vinasse recycling system results in the build-up of yeast by-products and compounds that inhibit yeast fermentation. This problem can be overcome by recycling a certain percentage of the total vinasse in order to keep the concentration of undesirable compounds below the level of toxicity that appears in a vinasse with 26% solids content. The recycling of 60% of the generated vinasse is technically feasible and may enhance the ethanol production process with no inhibitory effects. The bio-concentration process proposed in this paper positively modifies the energy balance of the evaporation-incineration process and also provides an energy surplus that can be used for ethanol production. Moreover, this scheme decreases the nutrients and sulfuric acid consumption. As a consequence of the recycling of vinasse, the fermentable sugars and nutrients still present in it from an incomplete fermentation process can be used in subsequent fermentations, thus improving the yield coefficient. An incidental advantage is that fermentation by-products such as glycerol become more concentrated together with the vinasse, reaching values that can be of interest for their industrial exploitation.

Considering that the amount of water required for the preparation of the fermentation medium in an alcohol-producing plant is about 77% of the total water consumption, the re-use of 60% vinasse reduces 46.2% of the quantity of water required.

All these facts can make the bio-concentration system proposed in this paper a process of economic significance for industrial application, with the added advantage of the non-polluting disposal of potentially contaminating wastes, an issue of growing importance in the world nowadays.

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