POTASSIUM REMOVAL FROM DISTILLERY SLOPS BY *Candida utilis* PROPAGATION

By

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Abstract

YEASTS accumulate varied amounts of most of the minerals present in their growth media. Much of the 7.5–8.1% ash found in the yeast grown for baking or harvested from beer is potassium phosphate, but yeast has the ability to accumulate other ions provided (but not necessarily needed) in high concentration. Distillery slops still contain about 70% of all potassium contributed to the soil in cane fields as chemical fertiliser, thus fertigation with these wastewaters has to be carefully calculated since otherwise soil salinisation can occur. When grown in a medium composed of distillery slops, nutrient salts (ammonium phosphate and sulfates) and a microbial growth enhancer *Candida utilis* shows a great resistance to potassium concentration in continuous culture. Yeast cells were propagated under the above conditions with increasing amounts of K_2O from 2.5 g/L concentration (distillery slops from molasses fermentation) up to 25 g/L in propagation medium. Specific growth rate (μmax) ranged from 0.32 to 0.28 h^{-1} for the extreme values mentioned above, while biomass-substrate yield coefficients were 0.23 to 0.18. These results suggest that yeast propagated on supplemented distillery slops could significantly reduce the potassium content of these wastes making them more suitable for irrigation purposes. According to the nutritional assessment reported, the potassium accumulated has no deleterious effect on animal health.

Introduction

Currently, there are 121 sugar-producing, and 57 ethanol-producing countries in the world. Roughly, 72% of the world's sugar comes from sugarcane, and the rest from sugar beets (2005). For ethanol, 54% comes from grains, and 46% from sugarcane (2005).

Production of ethanol is concentrated mostly in Brazil and the US, which together account for 72% of world's production in 2005. Although for decades Brazil was unrivalled as the world's largest biomass ethanol producer, since 2005 the US has overcome Brazil as the largest producer (Nastari, 2006).

Ethanol makes an excellent motor fuel: it has a research octane number (RON) of 109 and a motor octane number (MON) of 90, both of which exceed those of gasoline. Ethanol also has a lower vapour pressure than gasoline which results in lower evaporative emission. On the other hand, anhydrous ethanol has lower and higher heating values of 21.2 mega joules per litre and 23.4 mega joules per litre, respectively; for gasoline the values are 30.1 and 34.9 mega joules per litre.

Ethanol is a fast growing source of vehicle fuel, and it is likely that it will keep on growing in the future, to be extensively blended with gasoline. Environmentalists hope it could be developed as a cleaner source of fuel than oil or natural gas.

Sugarcane agro-industry exhibits a high ecological impact in all tropical and subtropical countries that operate that production. On the other hand, it should be said that the industry generates many jobs in the Latin American region, as sugarcane is one of the most widespread commercial plants and, at the same time, ranks among the most efficient plants with respect to bioconversion of solar energy into biomass.

Sugar and ethanol are basically the sole physical products that the sugar industry generates and both of them require large amounts of water during processing which led to the evolution of large quantities of liquid wastes (Otero *et al.,* 2008).

Among the most polluting industrial wastes are distillery slops or vinasse from ethanol distillation, due to their content of organic and inorganic substances.

Vinasse is produced at a rate of 10 to 12 cubic metres per cubic metre of distilled ethanol unless special techniques are applied, but all they do is concentrate the same amount of BOD / COD into a smaller volume. Chemical oxygen demand in vinasse is a function of fermentation and distillation efficiencies and ranges from 30 to 85 mg/mL.

To reduce the environmental impact of vinasse disposal, several strategies have been implemented mainly through biotechnological systems in order to match current legal settings.

Among them are fertigation, anaerobic digestion, desalting and concentration, burning and recycling to reduce the amount of dilution waters (Otero *et al.,* 2006; Otero *et al.,* 2008).

However, the upgrading of vinasse through the production of microbial protein makes this wastewater a valuable product that in addition makes an important contribution to the sustainable management of ecosystems (Martinez *et al.,* 2004; Otero *et al.,* 2006).

One of the most aggressive components of vinasse is its inorganic fraction, especially potassium salts that compose the overwhelming majority of ash composition (van Haandel and van Lier, 2006)

Distillery slops still contain about 70% of all potassium contributed to the soil in cane fields, thus fertigation with these wastewaters has to be carefully calculated since otherwise salinisation is a real danger. However, at the same time, potassium is an essential ion for microbial metabolism. The assessment of the removal of potassium content from vinasse by yeast propagation is the main objective of the present paper.

Materials and methods

Microorganism *Candida utilis* NRRL Y-660 was used for propagation in all experiments. Inoculums were prepared from agar-malt slants, grown overnight in a medium at pH 4.5 containing sugarcane molasses at 20 mg/mL of total reducing sugars concentration and nutrient salts (diammonium phosphate and sulfate) to cover cell nutritional requirements using a rotary shaker at 32ºC. A 2.5 L Marubishi MD5 fermenter was used to start batch propagation with a medium composed of molasses-based slops from a local distillery at a COD concentration of 60 mg/mL containing nutrient salts (see above) in the corresponding concentration to ensure that COD was the limiting substrate.

A microbial growth enhancer QZ-350 (Quimizuk, Havana, Cuba) was added to supply other micronutrients at a concentration of 0.03 mg/mL. Different amounts of potassium sulfate were added to test cell resistance and kinetic behaviour.

Chemical analysis nitrogen was determined according to Kjeldahl (Anon., 1983) in a 1030 Kjeltec Auto System (Tecator AB, Haganas, Sweden). Reducing sugars were estimated by copper reduction (Greenfield and Geronimus, 1985). Dry matter was done by desiccation at 105ºC overnight and lipids by extraction with ethyl ether and desiccation until constant weight in a vacuum oven at 60ºC.

Ash was determined by incineration at 600ºC for 4 hours and referred to dry matter content. Sediment was dissolved in 1N HCl and taken up to 100 mL with distilled water; then the potassium content in ash referred to biomass dry matter was determined by atomic absorption spectrophotometry.

Results and discussion

Alcohol production from sugarcane

Distillery slops or vinasse contains organic matter as well as mineral component. Typical concentration of molasses-based vinasse is about 8–10° bx, while juice-based vinasse hardly reaches 5° bx. Table 1 shows the typical composition of both vinasses.

Table 1—Typical composition of distillery slops (vinasse) from molasses and cane juice ethanol fermentation.

Component	Molasses-based	Juice-based
Total solids, mg/mL	70–80	25
Chemical Oxygen Demand COD), mg/mL	60–65	30–35
Biochemical Oxygen Demand (BOD) at 20ºC, mg/mL	48–55	18–20
pH	4.6	4.1–4.5
Ash, %	1.7–3.5	1.5–3.0
Nitrogen, mg/mL	0.70	0.43–0.50
Phosphorus (P2O5), mg/mL	0.30	0.25
Potassium, mg/mL	3.74–7.83	1.20–2.10
Calcium, mg/mL	1.8	0.51
Magnesium, mg/mL	0.77	0.23

The dominant vinasse ash component is potassium regardless of the original substrate in fermentation. This fact is a consequence of the potassium requirements of sugarcane. It is the most used nutrient and its demand by the plant can reach 800 kg/ha.

A harvest of about 100 tonnes of sugarcane removes from the soil close to 220 kg of K_2O. Around 98% of metallic ions in molasses or juices are K, Ca and Mg. In a typical molasses, potassium content in ash ranges from 70 to 75% of the whole inorganic fraction (Otero *et al.*, 1993).

The use of vinasse for soil fertilisation has evolved from the direct disposal to water streams in the 1970s to the current rational utilisation as fertiliser that prevents, or at least tries to, underground water contamination (Veronez de Sousa, 2008).

However, it has been documented that the main disadvantage of vinasse fertigation lies in the distance from its site of generation to the field. When that distance reaches 50 km, the real cost for irrigation increases far beyond 300 US dollars/ha.

Thus, the area of current disposal of vinasse for this purpose is normally significantly smaller than the original area where cane was harvested with the concomitant accumulation of unused potassium in the soil.

Potassium ions are the most abundant intracellular cations in most types of cells, from yeast to humans. Potassium has been reported to play a crucial role in a variety of cellular processes, including regulation of cell volume, intracellular pH, and protein synthesis, plus the prevention of the deleterious effects of sodium ions (Gomez *et al.*, 1996; Rodriguez-Navarro, 2000; Camacho *et al.*, 2005). Even more, during potassium limitation, ammonium ions become toxic for yeast metabolism (Hess *et al.*, 2006).

The propagation of yeast on vinasse is capable of removing potassium from the growth medium and embodying it in the cell biomass. In doing so, a potentially valuable protein source is also produced.

To assess the inhibition limits of potassium in yeast growth, a set of experiments under different potassium concentrations was done. Table 2 shows yeast kinetic parameters at different potassium concentrations.

Table 2—Growth parameters of *Candida utilis* NRRL Y-660 under different potassium concentrations.

$[K^+]$, mg/mL	μmax, h^{-1}	P, mg/mL–h	Yx/s	K in yeast, mg/mL
0.00*	0.228	1.403	0.226	21.2
12.96	0.225	1.321	0.206	32.7
25.91	0.175	1.236	0.177	42.1

* K^+ = 5 mg/mL

The table shows the kinetic behaviour of yeast cells under K^+ concentration between 5 and 30 mg/mL. This concentration is far beyond the normal content in vinasse. Up to 13 mg/mL, there is no significant effect of potassium concentration on specific growth rate, productivity or biomass-substrate yield for *C. utilis*.

The values for μmax are quite similar to those previously reported by the authors of the present paper (Martinez *et al.,* 2004; Otero *et al.,* 2008) for the same medium without potassium addition.

The fact that yeast is capable of growth in high potassium concentration is important but not enough for vinasse decontamination. Yeasts accumulate varied amounts of most of the minerals present in their growth media and have the ability to accumulate other ions provided (but not necessarily needed) in high concentration.

Therefore, the mineral content of cells harvested from a particular propagation medium could be adjusted to be of significance for the removal of undesirable metals in liquid wastes (Duncan *et al.,* 1995; Otero *et al.,* 2000). The last column in Table 2 shows the potassium content accumulated in yeast cells under the different experimental conditions.

In standard vinasse, yeasts remove about 50% of the potassium present in the growth medium. As potassium content in the propagation broth increases, the amount of potassium removed by yeast cells also increases but in a lesser proportion with respect to the amount provided. However, for all practical purposes yeast propagation not only reduces the organic load of vinasse by 60–70% (Otero *et al.,* 2007) but also half of its potassium content.

Reports about vinasse-yeast use in animal nutrition showed no deleterious effect on animal health (Lezcano and Mora, 2008; Llanes-Iglesias *et al.,* 2008; Rodriguez *et al.,* 2008).

Conclusions

Yeast propagation offers a unique method for vinasse decontamination, reducing both the organic fraction and the potassium content to a significant extent. According to the results presented above, the propagation of yeast on vinasse medium is an excellent pretreatment for further irrigation. Vinasse with less than 30 mg/mL of COD and potassium content below 2.5 mg/mL offers no ecological impact when used for irrigation of cane fields.

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ELIMINATION DU POTASSIUM DES VINASSES DE DISTILLERIE AU MOYEN DE *CANDIDA UTILIS*

Par

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MOTS-CLÉS: Décontamination des Vinasses de Distillerie, Protéines Unicellulaire, Protéines d'Alimentation, Suppression de Potassium.

Résumé

LES LEVURES sont connues pour accumuler des montants variés de la plupart des minéraux présents dans leurs milieux de culture. La majeur partie de 7.5-8,1 % de cendres trouvée dans la levure boulangère ou de brasserie est composée de phosphate de potassium, mais les levures ont le moyen d'accumuler d'autres ions qui s'y trouvent en concentration élevée, mais ces ions ce sont pas nécessairement requis. La vinasse de distillerie contient encore environ 70 % de potassium provenant des engrais chimiques utilisés dans les champs de canne à sucre d'où la nécessité d'utiliser ces rejets par fertigation avec précaution afin d'éviter des problèmes de salinité. Lorsqu'elle est cultivée dans un milieu composé de vinasse de distillerie, éléments nutritifs tels (phosphates et sulfates d'ammonium) et un promoteur de croissance, *Candida utilis* montre une grande résistance à la concentration de potassium en milieu de culture continue. Des cellules de cette levure ont été propagées dans les conditions sus-mentionnées avec des concentration de K2O variant de 2.5 g/L à 25 g/L en prevenance de la vinasse résultant de la fermentation de mélasse. Des taux de croissance spécifique (µmax) allant de 0.32 à 0.28 h-1 pour les valeurs extrêmes sus-mentionnées ont été obtenus, alors que les coefficients de rendement de la biomasse-substrat étaient 0.23 à 0.18. Ces résultats suggèrent que la levure propagée sur des vinasses de distillerie enrichies peut réduire considérablement la teneur en potasse de ces rejets en les rendant plus aptes à des fins d'irrigation. Selon l'évaluation nutritionnelle qui est rapportée, le potassium accumulé n'a aucun effet nuisible sur la santé animale.

REMOCIÓN DEL POTASIO DE LOS RESIDUALES DE LA DESTILACIÓN MEDIANTE LA PROPAGACIÓN DE *CANDIDA UTILIS.*

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PALABRAS CLAVE: Decontaminación, Residuales de Destilería, Proteína Unicelular, Proteína Alimenticia, Remoción De Potasio.

Resumen

LAS LEVADURAS acumulan diferentes cantidades de la mayoría de los minerales presentes en sus medios de cultivo. La mayor parte del 7,5-8,1% de cenizas encontradas en la levadura de panificación ó cultivada para cervaza está constituida por fosfato de potasio, pero las levaduras tienen la habilidad de acumular, en altas concentraciones otros iones suministrados (pero no requeridos). Los residuales de destilerías contienen todavía cerca del 70% del potasio que se aporta a los suelos cañeros como fertilizante químico, de aquí que la fertirrigación con estas aguas residuales debe ser cuidadosamente calculada, porque de lo contrario puede producirse salinización del suelo. La *Candida utilis* cuando es cultivada con técnicas de fermentación contínua en un medio compuesto por residuales de destilería, sales nutrientes (fosfatos y sulfatos de potasio)y un estimulador del crecimiento microbial, muestra una alta resistencia a las concentraciones de potasio. Se propagaron células de levadura, bajo las condiciones anteriormente descritas, con cantidades crecientes de óxido de potasio desde 2.5g/L (residuales de destilerías que emplean melazas) hasta 25 g/L en los medios de cultivo. La velocidad específica de crecimiento (μ max) osciló entre 0.32 y 0,28 h^{-1} para los valores extremos referidos anteriormente, obteniéndose también coeficientes de rendimiento biomasa-sustrato de 0.23 a 0.18. Estos resultados sugieren que levaduras propagadas en residuales de destilerías suplementados, pueden reducir significativamente el contenido de potasio de estos residuales, haciéndolos más apropiados para la irrigación. De acuerdo con los resultados nutricionales reportados , el potasio acumulado no tiene ningún efecto negativo en al salud animal.