Sustainable bioethanol production using agro-industrial by-products

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Abstract: This work aimed to evaluate a sustainable bioethanol production by a laboratorial isolate strain of *Saccharomyces cerevisiae*, along with the use of agro-industrial by-products as carbon source. The effect of several carbon sources and their concentrations was studied using carob pod extract (CPE) and beet molasses (BM) and compared with glucose and sucrose as conventional carbohydrates at different concentrations, 15, 20 and 30 g/l.
No significant difference was found between maximum ethanol production obtained with CPE, BM, glucose and sucrose fermentations profiles. It was obtained values of 10.65 g/l and 10.5 g/l ethanol, respectively for sucrose and CPE at 30g/l, which can be improved using higher substrate concentration.

Key-Words: agro-industries residues, beet molasses, bioethanol production, carob pod extract, *Saccharomyces cerevisiae*

1. Introduction

Over the last few years, an increased interest and attention has been devoted to Bioethanol production and use, mainly due to its potential as a substitute for fossil fuels and the need to reduce global economics dependence on fossil resources [1, 2, 3].
At the present Brazil and the USA are the world's largest producers of bioethanol, with approximately 62% of world production [4, 5]. The major feedstocks used by these countries are sugar cane and corn, respectively. In Europe ethanol production, based in beet molasses, is still very sharp due to the lack of available feedstocks that can support local ethanol productions plants [5].
Several research approaches are being carried out in order to evaluate the possibility of increasing ethanol yields from alternative and available feedstocks [2, 3, 6]. Ethanol produced from lignocellulose and agri-industrial wastes can be seen as the most promising ones, given the great advantage of a bioenergy production that is not competing with food resources and yet a broader spectrum of feedstocks are used when compared to traditional processes [5, 6, 7]. Some of these residues such has, beet molasses or carob pulp, represent an abundant, cheap and readily available source of raw-material to be converted into fuel [8, 9, 10].
In previous studies it was reported the use of conventional carbon sources and industrial residues for ethanol production using the yeast *Saccharomyces cerevisiae* [9, 10, 11, 12, 15]. In Table 1 are summarized bioethanol productivities and yields coefficients

obtained in batch cultures, from recent studies [12, 14, 15, 16, 17, 18] and compared with the coefficients obtained in this work.
The objective of the current work is to contribute to the development of a sustainable 2nd generation bioethanol production, using agri-industrial residues like carob pod extract and beet molasses, rich sugar and cheap feedstocks and to compare it with conventional and known sources, like glucose and sucrose.

2. Material and Methods

2.1. Microbial growth and pre-inoculum

A laboratory isolate of the yeast *Saccharamyces cerevisiae* was used throughout the process. The yeast strain was maintained on solid NYDA medium (Nutrient broth 8g/l, Yeast extract 6 g/l, Dextrose 10 g/l, Agar 20 g/l) distributed on sterile petri dishes.
Pre-inoculum was prepared by growing 4 day old culture on solid NYDA medium for 18h at a 250 ml erlenmeyer with 50 ml of liquid YEPD medium (Yeast Extract 10g/l, Peptone 20g/l, Glucose 20g/l), in an orbital shaker with temperature controller (Neifo Pentlab, Portugal) at 25ºC and 150 rpm

2.2. Fermentation conditions

Growth medium was based on YEPD medium with a variation on carbon source and carbon source concentration according to the by-product under study, beet molasses (BM) or carob pod extract (CPE).
Carbon source concentration effect was studied using three different concentrations, 15, 20 and 30 g/l of total sugar available. All studies were performed in triplicate for 28h, on 250 ml erlenmeyers with 50 ml of medium, in an orbital shaker with temperature controller (Neifo Pentlab, Portugal) at 25ºC and 150 rpm.

2.3. Analytical techniques

Samples were collected throughout fermentation cycle. Absorbance at λ = 554 nm (Genisys 10 vis., Thermo Electron Corporation) and pH were measured (Crison GLP21, Portugal) Samples were then centrifuged, filtered and analyzed. HPLC analyses were performed on a Beckman System Gold HPLC (Beckman, USA) equipped with a Jasco Refractive Index model 1530 (Jasco, Japan). Sugar analyses of the carob pod extract (CPE), beet molasses (BM), glucose and sucrose were performed using a Purospher STAR NH_2 column (Merck KGaA, Germany) in a isocratic system, Acetonitrile:Water (75:25) at 1 ml/min and 35ºC. Ethanol quantification used an OH AY column (Merck KGaA, Germany), in an isocratic system, with H_2SO_4 0,002N at 0.5 ml/ml and room temperature.

2. Results and discussion

A comparison between conventional carbon sources, glucose and sucrose, was made to better understanding and to develop a more efficient the ethanol production, by batch culture of *Saccharomyces cerevisiae*, using agro-industrial by-products, as carob pod extract and beet molasses, in a perspective of optimal yields for biofuels production.

Figure 1 depicts the ethanolic production during batch fermentation processes, using *Saccharomyces cerevisiae* at different carbon sources concentrations (15 g/l, 20 g/l, 30 g/l) glucose, sucrose, BM (beet molasses) and CPE (carob pod extract). Ethanol production was significantly improved at 30 g/l initial carbon source concentration, for any of the assayed raw-material, except for beet molasses that showed a slight decrease.

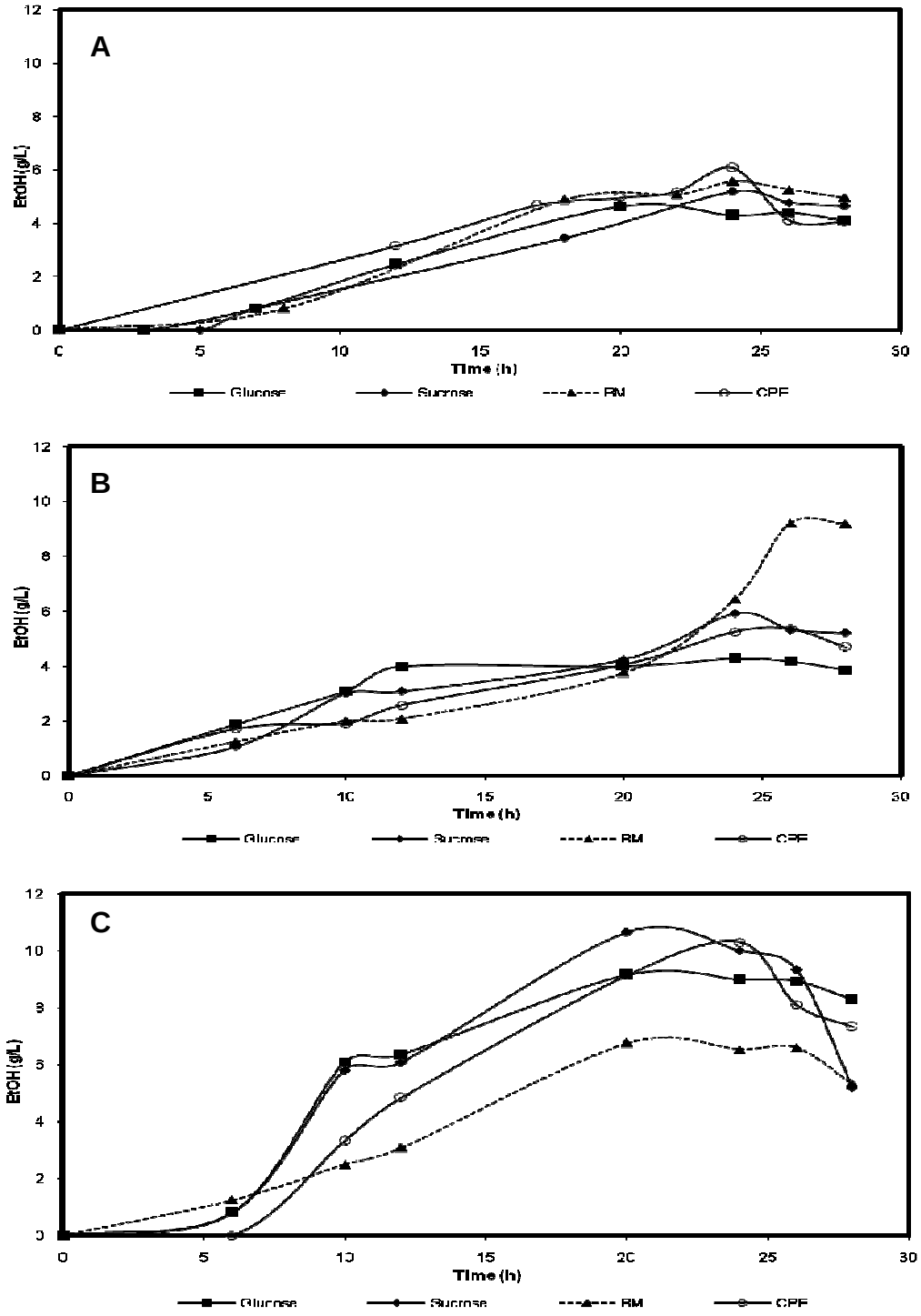

Figure 1. Ethanol production, using *S. cerevisiae* BBE-1 in batch system for different carbon sources, glucose, sucrose, beat melasses (BM) and carob pod extract (CPE) at different concentrations: A – 15 g/l, B – 20 g/l and C – 30 g/l.

At 15 g/l carbon source, a maximum of ethanolic production was obtained, in general after 24 hour inoculation, but glucose promoted an ethanolic maximum after 20 hour inoculation. Probably this occurs due to *S. cerevisiae* higher affinity to glucose than to others carbohydrates. In these conditions maximum concentration of ethanol (6 g/l) was achieved for 15 g/l of carob pod extract and 9 g/l of ethanol for 20 g/l beet molasses growth. For cultures grown at 30 g/l of carbon source values of ethanol formation are between 8 and 10 g/l and the maximum ethanol formation achieved within the first 20 hours of culture for any of the studied carbon sources, at a less period of time than for the others carbon sources concentrations.

Table 1 presents results for ethanol production, product yields (Yp/s) and productivities achieved in this study and establishing a comparison with results already described by other authors.

Table 1. Ethanolic production by *Saccharomyces cerevisiae* in batch culture, with different substrates

Substrate	Microorganism	Substrate (g/l)	Ethanol Concentration (g/l)	Productivity (g/l.h)	Yp/s (g ethanol/g subst)	Reference
Glucose	*S. cerevisiae*	15	4.63	0.25	0.31	This work
		20	4.28	0.17	0.21	
		30	9.16	0.50	0.31	
Sucrose	*S. cerevisiae*	15	5.19	0.26	0.34	This work
		20	5.92	0.24	0.31	
		30	10.65	0.57	0.35	
Sucrose	*S. cerevisiae*	220	96.71	1.01	0.44	[15]
Glucose	*S. cerevisiae*	200	82.1	---	0.41	[16]
BM	*S. cerevisiae*	15	5.57	0.25	0.37	This work
		20	9.21	0.31	0.46	
		30	6.75	0.34	0.22	
CPE	*S. cerevisiae*	15	6.08	0.25	0.43	This work
		20	5.36	0.20	0.31	
		30	10.30	0.48	0.34	
Mahula (Madhuca latifolia L.)	*S. cerevisiae*	fermentable sugars (28.1–36.3 g /100 g)	31.84	0.33	0.54	[17]
Beet molasses	*S. cerevisiae*	242 – 276		0.48 – 2.97	0.59 – 0.76	[12]
Water hyacinth	*S. cerevisiae*	30.1 g /l glucose	14.4	---	---	[14]
Water lettuce	*S. cerevisiae*	33.3 g/l glucose	14.9	---	---	[14]
Potato starch	*Aspergillus Níger* + *S. cerevisiae* (SSF)	180 g/l glucose	92	---	0.4	[18]

Atiyeh & Duvnjak [12] and Roukas [13] reported fermentations of *S. cerevisiae* with beet molasses, in which the sugar concentration varied between 0.98 to 276.2 g/L, with a maximum ethanol of 0.48 and 3.5 g/l, respectively which are lower than the obtained in this work. For initial sugar concentration at 30g/L, CPE fermentation profile achieves an ethanol production, productivity and yields very similar to the assayed carbon sources, glucose and sucrose. Although a higher yield is achieved with half the concentration (0.43 with 15 g/l) it requires almost two fold the amount of time to produce nearly 70% of the ethanol produced with 30 g/l (10.30 g/l). Mishima *et al* [14]

report 14.9 g/l ethanol for water hyacinth (30g/l) as substrate. However, higher carbon source concentrations, 200 g/ sucrose and 220 g/l glucose can produce 96.7 g/l and 82.1 g/l ethanol concentration respectively, as verified by ζaylak and Sukan [15] and Borzani [16].

S. cerevisiae is able to get high rates of glycolysis and production of ethanol when optimal conditions are presented, by producing 2.5 g/l more ethanol per h and per g of cellular protein. However, this high rate is kept only by brief periods of time during the batch fermentation and decreases gradually while ethanol accumulates in the nutrient medium [11]. Although the yield is slightly higher with a lower substrate concentration, it is relevant due to the the fact that when the carbon source increases ethanol production also increases and the maximum peak of ethanol appears earlier in the fermentation.

In fermentations performed with carob pod extract and beet molasses it was observed that maximum ethanol production increased with sugar concentration as reported by several authors (Table 1). CPE, as feedstock showed the overall best results for product yield at 15 g/l and 30 g/l of total sugar available and similar to the conventional traditional sources, like glucose and sucrose.

The ethanol productivities obtained (g/l.h), in this work, at different concentrations are in the same range of values of results referred by other authors (table 1).

Further experiments will be done to explore the potential use of these industrial by-products with higher carbon source concentration and in a process of carbon source enrichment with the objective of maximizing ethanol production.

Conclusions

Both industrial residues, CPE and BM, used as carbon sources are potentially adequate feedstocks for bioethanol production. Productivities and ethanol yields are similar to those obtained with conventional carbon sources, glucose and sucrose and may attain high product yields. The use of agricultural wastes is a valuable contribution for ethanol production in the near future, as a 2nd generation bioethanol, promoting, in this way, a sustainable biofuels production, that overcomes the problematic depletion of agriculture resources. This alternative 2nd generation production of bioethanol is a determinant strategy that circumvents the known negative impact on food production.

Ethanol produced from renewable and cheap agricultural products provides reduction in green house gas emission, carbon monoxide, sulfur, moreover it helps to eliminate smog from the environment. Bioethanol, both renewable and environmentally friendly, is believed to be one of the best biofuels alternatives if supported by national legal and strategic energy orientations.

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