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Production of Bioethanol from agro-industrial wastes

Alma Rosa Domínguez-Bocanegra [a,*], Jorge Antonio Torres-Muñoz [b], Ricardo Aguilar López [a]

[a] Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional Cd, de México, 07360 México, Mexico
[b] Departamento de Control Automático, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional Cd, de México, 07360 México, Mexico

HIGHLIGHTS

- Bioethanol production in coconut milk, pineapple juice and tuna juice was validated.
- Maximal ethanol production of 22% v/v in coconut milk.
- Pineapple juice fermentation reached the maximal ethanol productivity 0.47 g/g.

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ABSTRACT

A suitable alternative to replace fossil fuels is the production of bioethanol from agro-industrial wastes. Coconut, pineapple and tuna are fruits that available almost along the year in Mexico but a high percentage of these fruits are wasted by producers. The aim of this study was to investigate the using of agro-industrial wastes natural carbon sources such as those present in coconut milk, pineapple juice and tuna juice, to promote the synthesis of bioethanol by yeast *Saccharomyces cerevisiae* CDBB 790. Cultures were grown in 500 mL Erlenmeyer flasks containing 350 mL of culture media (YM medium, coconut milk, pineapple juice or tuna juice) the yeast cells were inoculated with 35 mL YM medium in the exponential growth phase. Results show that the highest ethanol concentration obtained from was 20% v/v in coconut milk, 22% v/v in pineapple juice and 12% v/v in tuna juice. The consumption of sugars at 36 h was 88.62% in coconut milk, 93.75% in tuna juice, 90.62% in pineapple juice and 98.6% in YM medium.

1. Introduction

Bioethanol is an increasingly important alternative fuel for the replacement of gasoline, with a world production in 2009 of 19,535 millions of gallons and an estimate, only for USA in 2022, of 36,000 millions of gallons. It is thus expected that the production of bioethanol will keep on increasing in the next 10 years [1]. The ethanol obtained from biomass-based waste materials or renewable sources is called as bioethanol and can be used as a fuel, chemical feedstock, and a solvent in various industries. It has certain advantages as petroleum substitutes, viz., alcohol can be produced from a number of renewable resources, alcohol as fuel burns cleaner than petroleum which is environmentally more acceptable. It is biodegradable and thus, checks pollution. It is far less toxic than fossil fuels. It can easily be integrated to the existing transport fuel system, i.e., up to 5% bioethanol can be blended with conventional fuel without the need for modification [2,3]. Agricultural organic wastes are currently one of the major problems of agriculture from an environmental point of view so that the use of this biomass for generating energy it is vitally important. Production of bioethanol from agro-industrial wastes is a suitable alternative, given the need to replace fossil fuels [4].

Bioethanol is by far the most widely used bio-fuel for transportation worldwide, because it is a renewable, nontoxic, biodegradable resource and it is oxygenated, there by provides the potential to reduce particulate emissions in compression–ignition engines [5]. Second-generation biofuels (Biomass to liquid) are made from organic materials, such as straw, wood residues, agricultural residues, reclaimed wood, sawdust, and low value timber. Microorganisms are a key component of the technology used in different fermentation regimes, including ethanol. Diverse groups of microorganisms are capable of producing ethanol [6–8]. These include yeasts, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, bacteria *Zymomonas mobilis*, fungus *Fusariumoxys porum*, yeast like fungus *Pachysolen tannophylus*, and thermophilic bacteria. [9]. *S. cerevisiae* and *S. pombe* represent the organisms of choice for the industrial production of ethanol due to the following features: they are capable of fermenting a diverse range of sugars for production of ethanol under anaerobic. Contamination problem is under

* Corresponding author.
E-mail address: adomin@cinvestav.mx (A.R. Domínguez-Bocanegra).

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control as the fermentation process operates at low pH and high sugar concentration and are genetically stable and ferment 20–25% (w/v) [5]. Thus, second-generation bioethanol production is important as it allows improved CO_2 balance and make use of cheap, waste source which does not compete with human food products. In brief, the use of ethanol as a biofuel is gaining increasing popularity [6]. Although it is produced from several sources but the technologies using the waste material for its production is most attractive as it does not interfere with food particular substrates needed for the ever increasing world population. However, there are relevant obstacles such as production costs, technology and environmental problems that need to be overcome in the production of second-generation bioethanol [10–14]. The goal of this work was to investigate the use of raw agro-industrial wastes, namely coconut milk, pineapple juice and tuna juice, for bioethanol production by yeast *S. cerevisiae* CDBB 790.

2. Materials and methods

2.1. Microorganism

S. cerevisiae strain CDBB 790 was obtained from the Microbial Culture Collection of Mexican CINVESTAV-IPN.

2.2. Agro-industrial wastes

Raw residual coconut milk was obtained from a local candy industry located in Mexico City (Col. Vicentina 09340, Delegación Iztapala) and raw residual tuna juice was obtained from Pachuca, Mexico and raw residual pineapple juice was obtained from Central de Abastos, Mexico. It was sterilized by filtration and no nutrient was added. The chemical composition was utilized gas chromatograph Perkin Elmer Auto system.

2.3. Yeast inoculum preparation

The yeast inoculum was grown aseptically in 500 mL Erlenmeyer flasks containing 250 mL of YM medium (10 g/L glucose, 5 g/L peptone, 3 g/L malt extract, 3 g/L yeast extract), at a constant temperature of 30 °C and stirrer speed of 150 rpm, for 48 h. Fresh medium was then inoculated with the seed culture at 10% volume. The yeast was grown under the same culture conditions.

2.4. Fermentation kinetics

Cultures were grown aseptically in a 500 mL Erlenmeyer flasks containing 350 mL coconut milk or pineapple juice or tuna juice or YM medium and inoculated with 35 mL YM medium in the exponential growth phase and incubated using a gyrating shaker (Gyrating water batch shaker model G76; New Brunswick Scientific, Edison, N.J.) at 150 rpm and 28 °C, for 5 days. Kinetic experiments were carried out in triplicate.

2.5. Analytical methods

2.5.1. Dry weight determination

Culture samples of 2 ml taken from the cultured medium were centrifuged for 5 min at 3500*g* at room temperature in a model 5415 centrifuge (Brickman Instruments, NY). The cell pellet was washed twice with 2 ml of distilled water and filtered through a 0.45-μm Millipore pre-weight filter. The filters containing the biomass were dried at 60 °C for 24 h.

2.5.2. Growth determination

Yeast cell growth was measured daily by cell microscopic counting using an improved Neubauer haemocytometer (Proper Manufacturing Co. Inc., Long Island City, NY) and Absorbance was taken as in [15].

2.5.3. Sugar determination

The 3,5-dinitrosalicylic acid (DNS) method of Miller [16] was used to determine residual reducing sugars in the culture media. A 1-ml sample was centrifuged at 3500*g* for 5 min, after which 1 ml of DNS reagent was added to the supernatant. The tubes were covered, heated to boiling point for 5 min and then immediately placed in an ice-bath for rapid cooling. Then, 8 ml of distilled water was added. The tubes were shaken using a vortex (model G-560; Scientific, NY) for 5 min. A spectrophotometer, set at a wave-length of 575 nm, was employed for optical density measurement and the data were calibrated with a suitable standard reference curve to determine the glucose concentration of the samples.

2.5.4. Percentage of ethanol

The ethanol content in the samples was measured with a gas chromatograph Perkin Elmer Autosystem, with a column of HPLC grade ethanol Zebron FFAP-30 m mark 0–25 min, detector temperature 250 °C, injector temperature 30 °C column temperature 60 °C for 9 min–10°C/min 200°C–20 min.

2.5.5. Statistical analyses

All experiments were performed in triplicate. A tri-factorial analysis of variance was applied to cell density values of *S. cerevisiae* and post hoc comparisons were carried out through the Newman–Keuls test ($P = 0.05$); [17]. A similar statistical analysis was made for bioethanol content; and differences among applied treatments were determined by means of the post hoc Newman–Keuls test ($P = 0.05$).

3. Results and discussion

3.1. Chemical composition wastes

The chemical composition of YM medium, coconut milk, pineapple juice and tuna juice is shown in Table 1. Total carbohydrates represent 13–16% of dry mass, including mainly sucrose, which is a fermentable disaccharide for *S. cerevisiae*. Indeed, raw juice purity ranges between 85% and 90% which means that there are about 85–90% of sugars and 10–15% of non-sugars in dry matter. Coconut milk, pineapple juice and tuna juice, contain significant amounts of protein 0.33 g/L, 0.5 g/L and 0.5 g/L, respectively and 0.2 g/L lipid fat for pineapple juice. Such properties, together with a relatively low price, make of these raw juices very profitable and convenient materials for ethanol production.

Table 1
Chemical composition YM medium, coconut milk, pineapple juice and tuna juice.

Component	YM medium	Coconut milk	Pineapple juice	Tuna juice
Carbohydrates total	–	16 g/L	13.3 g/L	16 g/L
Glucose	10 g/L	–	–	–
Yeast extract	3 g/L	–	–	
Peptone	5 g/L	–	–	–
Malt extract	3 g/L	–	–	–
Sodium	–	0.25 g/L	0.1 g/L	–
Magnesium	–	0.1 g/L	–	–
Potassium	–	2.94 g/L	11.3 g/L	0.34 g/L
Chlorite	–	1 g/L	–	–
Protein	–	0.33 g/L	0.5 g/L	0.5 g/L
Phosphorus	–	11.3 mg/L	7 mg/L	28 mg/L
Lipid fat	–	–	0.2 g/L	–
Distilled water	1 L			