Renewable Energy from Agricultural Waste

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This paper has aimed at evaluating the concentration of bioethanol obtained using sunflower stem as natural support, molasses as carbon source and Saccharomyces cerevisiae yeast in a continuous flow reactor. The natural support was tested to investigate the immobilization/growth of S. cerevisiae yeast. The concentration of bioethanol produced by fermentation was analyzed by gas chromatography using two methods: aqueous solutions and extraction in organic phase. The CO_2 flow obtained during the fermentation process was considered to estimate when the yeast was deactivated. The laboratory experiments have highlighted that the use of plant-based wastes to bioconversion in ethanol could be a non-pollutant and sustainable alternative.

Key words: bioeconomy, bioethanol, vegetable wastes, renewable energy

The subject is topical and enrolls in the development strategy of Romania for 2016-2035, which addresses, inter alia, the bioenergy sub-sector (biogas, biomass, biofuel) and biotechnologies of environment. These approaches could be feasible by measures and provisions to manage the vegetal products and wastes [1,2]. Until 2030, about 220 million tons of cellulosic material (residues from farming, forestry or town wastes) could be used to produce ethanol, resulting in about 300,000 of new jobs in Europe, mainly in countries with hi-tech, as direct result [3,4].

At present, many biomass sources have been studied to obtain bioethanol. The presence of ethanol in fuels oxygenates the fuel and reduces air pollution. Conversion of biomass into ethanol varies considerably depending on the nature of feedstock [5-7]. The first-generation bioethanol involves feedstock rich in sucrose and starch [8].

Suitable technologies to convert lignocellulosic materials to second generation ethanol are based on biochemical or thermochemical conversion. Biochemical conversion in the process of ethanol manufacturing from lignocellulosic biomass generally follows the following steps: shredding → pretreatment → hydrolysis → fermentation [9-11].

The most common microorganism used in ethanol fermentation is *Saccharomyces cerevisiae* yeast [12]. The immobilization of yeast cells on a support is a technology commonly applied in fermentation process. The benefits of immobilized cells over free cells include higher substrate conversion, shorter reaction time, easier separation from the reaction medium, less inhibition by products, higher cell density per reactor volume and cell replication control [13]. There are many types of supporting materials that have been used for immobilization/growth of yeast cells, such as calcium alginate [14], k-carrageenan [15], marrow stem sunflower [16], sugarcane bagasse [17], orange peel [18], maize stems [19,20], sweet sorghum stalks [21], sorghum bagasse [22], zeolite [23], polyacrylamide hydrogels [24, 25] and different polymeric supports [26].

Bioethanol by itself or by blending with gasoline has been identified as the most widely used biofuel worldwide [27].

In this paper we have studied the use of agricultural waste (sunflower stems) as a support for the immobilization of yeast *Saccharomyces cerevisiae* in the fermentation of molasses to bioethanol.

Experimental part

The experimental study involved several stages, namely: selection of raw materials, preparation of the support, immobilization/growth of yeast onto the substrate, production of bioethanol and determination of its concentration by gas chromatography (GC).

Materials

Sunflower stems, belonging to five hybrids purchased from NARDI Fundulea, peeled and chopped as cubes (about 0.5 cm size), were selected as natural support. Sunflower stem is a lignocellulosic material containing *cca.* 42% cellulose, 30% hemicellulose, 13% lignin [28]. Molasses obtained in the process of sugar manufacturing from sugar beet was the carbon source and *Saccharomyces cerevisiae* was the yeast used to produce bioethanol.

Experimental equipment and procedures

Immobilization/growth of yeast onto the vegetal support

Immobilization/growth of *Saccharomyces cerevisae* yeast onto the sunflower stem support was conducted by alcoholic fermentation of sugar from molasses according to the following steps: (i) for each hybrid species, a sample of stem cubes and distilled water was introduced into a Berzelius flask, heated to reflux for 30 min and then cooled; (ii) a molasses solution (120 g/L molasses, 5 g/L $(NH_4)_2SO_4$, 3 g/L KH_2PO_4, 5 g/L $MgSO_4$. $7H_2O$, 5 g/L citric acid, 7 g/L Na_2HPO_4) was heated to reflux in a round-bottom flask for 30 min and further cooled; (iii) the yeast (2% or 4% from the mass of each hybrid vegetal material) and an amount of 25 mL of cooled molasses solution were added over the sample of vegetal support. In order to a better fixing on the support, the aqueous suspensions of sunflower stem support, molasses and yeast were left for 96 h in a stove at a constant temperature of 32.5°C. The yeast and molasses solution were refreshed every 24 h. Six cubes of sunflower stems were extracted from each sample every 24 h and weighed, left to drain for 15 min on filter paper in order to remove excess water, and then weighed.

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Production of ethanol by continuous fermentation

The schema of the experimental setup used to produce ethanol is shown in figure 1. The process was performed for four days into the continuous flow bioreactor (1). Molasses solution from the tank (2), whose temperature was controlled by the thermostat (3), was fed by the dosing pump (4) into the bioreactor (1) containing the yeast immobilized onto sunflower stem support. The carbon dioxide produced by fermentation process was evacuated into the atmosphere and its volumetric flow rate was measured by the flowmeter (5). The ethanol solution was collected in the product tank (6).

The following process parameters were directly measured: feed volumetric flow rate of molasses solution (D_s), volume of molasses solution in the bioreactor (V_s), and volumetric flow rate of CO_2 (D_{CO_2}). Ethanol yield was determined by chromatographic analysis.

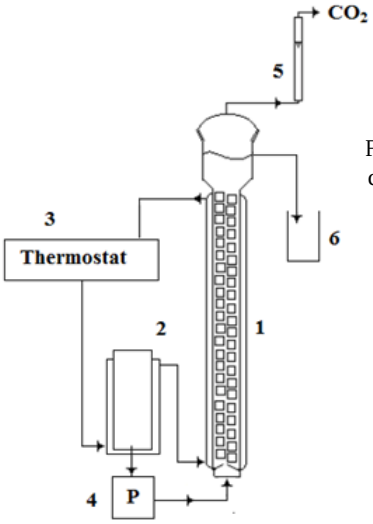

Fig. 1. Laboratory setup: (1) continuous flow bioreactor; (2) molasses tank; (3) thermostat; (4) dosing pump; (5) flowmeter; (6) product tank

Determination of ethanol yield

Two ways were considered to determine the ethanol yield obtained by the continuous fermentation process: chromatographic analysis of the aqueous solution and extraction in organic solvent followed by chromatographic analysis of the extract. In both cases, the calibration curve was carried out with solutions of known concentrations of ethanol. Yeast mass percentage in the immobilization stage (2 or 4% from the mass of vegetal support) and feed volumetric flow rate of molasses solution (D_s=60-230 mL/h) have depended on the method selected to estimate the ethanol yield.

Chromatographic analysis of aqueous ethanol solution

A solution of 50 g/L ethanol in water was prepared and diluted 4 times. A solution of 1% isopropanol in water was used as internal standard. Samples of aqueous ethanol solution obtained every 24 h were filtrated, mixed with the internal standard (1:1) and further analyzed using a Buck Scientific gas chromatograph on a Resteck MXT-1 60m×0.53mm×5µm chromatographic column.

Characteristic factors of immobilization and continuous fermentation processes were as follows: 2% yeast mass percentage in the immobilization stage and D_s=60-230 mL/h. The feed volumetric flow rate of molasses solution (D_s) was kept at 230 mL/h in the first day and it was reduced in the following three days (table 1).

Ethanol extraction in organic solvent and chromatographic analysis

A stock solution of 20% ethanol in butanol and a standard solution of 1% methanol in butanol were prepared. The extraction was performed under magnetic stirring. Samples were taken from the top layer with a microliter syringe.

Measurements were carried out using the same gas chromatograph as used before. The injected samples were mixed 1:1 between stock solution and standard solution.

Immobilization and continuous fermentation factors were as follows: 4% yeast mass percentage in the immobilization stage and D_s=60 mL/h.

Results and discussions

This research aimed at evaluating the growth of yeasts onto the natural support and the yield of ethanol produced by continuous fermentation.

Immobilization/growth of yeast onto the vegetal support

By weighing 6 sunflower stem cubes from each hybrid every 24 h, it was observed that the yeast cells have grown onto all five hybrids (fig. 2). Only the hybrid 1 was selected as natural support for further continuous fermentation experiments.

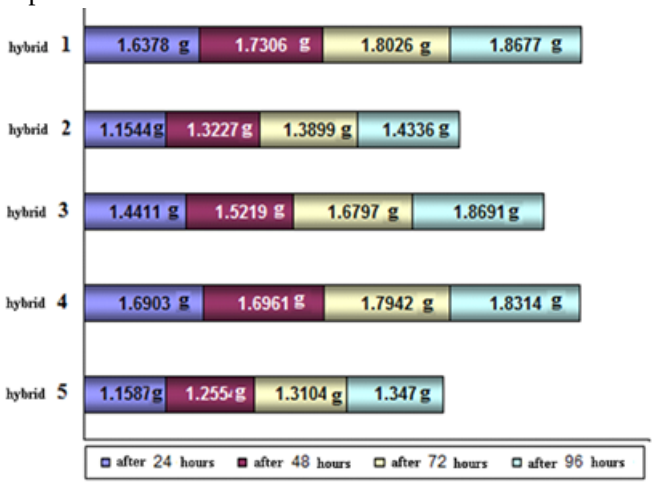

Fig. 2. Weighing results for 6 sunflower stem cubes

SEM images of hybrid 1 are shown in figure 3. The fresh structure has uniform pores with a mean pore diameter of 100 µm (fig. 3a), whereas, after 96 hr of incubation, there are yeast cells onto some joints of walls of the porous structure (fig. 3b, c). The immobilization of *Saccharomyces cerevisiae* yeast on sunflower stems is effective due to a very porous internal structure of the stem.

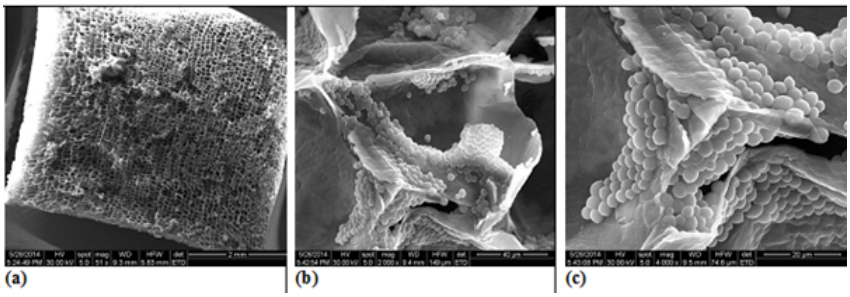

Fig. 3. SEM images of hybrid 1: (a) fresh; (b), (c) after 96 hr of incubation

Day	D_s (mL/hr)	D_{CO_2} (mL/hr)	V_s (mL)	t_s (min)	$Q_{molasses}$ (g/hr)	Q_{CO_2} (g/hr)
1	230	95-250	52	13.6	27.6	0.34
2	130	137-164	56	25.9	15.6	0.30
3	60	235-340	46	46.0	7.2	0.56
4	60	225-360	46	46.0	7.2	0.57

Table 1
MEASURED AND CALCULATED PARAMETERS OF CONTINUOUS FERMENTATION PROCESS

Time (hr)	Sample	Component	Retention time	Peak area	Peak height
48	1	ethanol	3.983	123.790	37.487
		standard	4.600	193.446	42.499
	2	ethanol	4.033	132.152	39.306
		standard	4.650	173.374	38.332

Table 2
EXPERIMENTAL DATA COLLECTED FROM THE CHROMATOGRAPHIC PEAKS

Determination of ethanol yield

In order to determine the concentrations of ethanol obtained, not only the analysis method was different, but also the amount of yeast and feed flow rate of molasses solution.

Chromatographic analysis of aqueous ethanol solution

Characteristic measured (D_s, D_{CO2}, V_s) and calculated parameters (t_s, $Q_{molasses}$, Q_{CO_2}) of continuous fermentation process leading to the aqueous ethanol solution which was analyzed by GC are summarized in table 1.

The hydraulic residence time, t_s (min), was determined by eq. (1), where V_s (mL) is the volume of molasses solution in the bioreactor and D_s (mL/h) the volumetric flow rate of molasses solution.

$$t_s = \frac{V_s}{D_s} \quad (1)$$

The mass flow rates for the reactant and for the products, Q (g/hr), were calculated with eqs. (2) and (3), where $c_{molasses}$=120 g/L is the mass concentration of molasses in the feed solution, D_{CO2} (mL/h) the mean volumetric flow rate of CO_2, M_{CO2}=44 g/mole the molar mass of CO_2 and V_m=22.4 L/mole the molar volume.

$$Q_{molasses} = \frac{D_s c_{molasses}}{1000} \quad (2)$$

$$Q_{CO_2} = \frac{D_{CO_2}}{1000}\frac{M_{CO_2}}{V_m} \quad (3)$$

Chromatographic peaks of ethanol and internal standard after 48 h are shown in table 2. The fact that the process is not finished yet can be observed in the peak areas that are small and ethanol concentration the same.

Ethanol yields corresponding to hybrid 1 are marked in figure 4. A mean ethanol concentration of 1.42% was obtained by this method.

Ethanol extraction in organic solvent and chromatographic analysis

The experimental data used for establishing the calibration curve are presented in table 3. Table 4 contains characteristic measured (D_s, D_{CO2}, V_s) and calculated parameters (t_s, $Q_{molasses}$, Q_{CO2}) of continuous fermentation process. The experimental results obtained at the end of the research using hybrid 1 through extraction in organic phase are presented in figure 5. A mean ethanol concentration of 1.76% was obtained by this method.

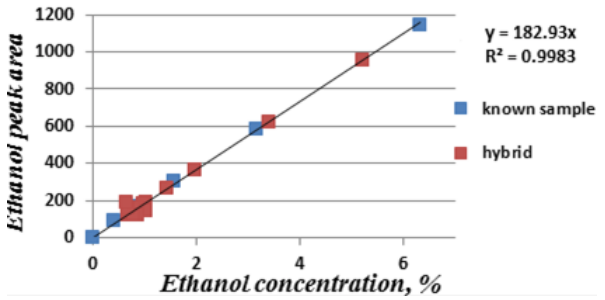

Fig. 4. Ethanol concentration depending on peak area for hybrid 1

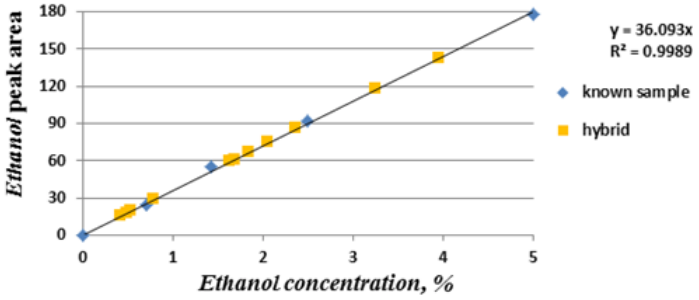

Fig. 5. Ethanol concentration depending on peak area for hybrid 1

Component	Peak area	Concentration (%)	Equation check	Error (%)
Ethanol	178.598	5.00	180.47	1.8
	92.236	2.50	90.23	2.0
	55.135	1.43	51.61	3.5
	24.616	0.71	25.63	1.4

Table 3
EXPERIMENTAL DATA FOR THE CALIBRATION CURVE

Day	D_s (mL/hr)	D_{CO_2} (mL/hr)	V_s (mL)	t_s (min)	$Q_{molasses}$ (g/hr)	Q_{CO_2} (g/hr)
1	60	45	51	51.0	7.2	0.09
2	60	90-180	51	51.0	7.2	0.27
3	60	180-257	52	52.0	7.2	0.43
4	60	180-257	49	49.0	7.2	0.43

Table 4
MEASURED AND CALCULATED PARAMETERS OF CONTINUOUS FERMENTATION PROCESS

Conclusions

The bioethanol is a fuel produced from various vegetable materials and its use could reduce the emissions of CO_2 (20%-100%).

The bioeconomy, based on bio-products from renewable sources, promotes biorefineries as engines of the new economy. The bioethanol could be feasible not only as fuel but as reducing wastes quantity and space available for their storage as well as preserving the natural resources.

This study aimed at evaluating the growth of *Saccharomyces cerevisiae* yeast onto a natural support (sunflower stems) and determining the ethanol yield produced by fermentation process. A mean ethanol concentration of 1.6% was obtained under conditions considered in the experimental study. The research led to knowledge regarding the selection and useful processing of any wastes, with repercussions regarding an increase in production in competitive places and a decrease in economic and environmental risks.

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