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ORIGINAL RESEARCH PAPER

COMPARATIVE STUDY OF DIFFERENT METHODS OF HYDROLYSIS AND FERMENTATION FOR BIOETHANOL OBTAINING FROM INULIN AND INULIN RICH FEEDSTOCK

Camelia (Bonciu) Neagu*, Gabriela Bahrim

"Dunărea de Jos" University of Galaţi, Food Science and Engineering Faculty, 111 Domnească Street, 800008 Galaţi, Romania

*Corresponding author: cbonciu@ugal.ro

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Abstract: Bioethanol serves as liquid fuel or gasoline enhancer in many countries in response to the progressive depletion of the world's energetic resources. Production of bioethanol from inulin rich raw materials has been a subject of great interest for many years due to the large amount of existing and not completely developed technologies. The aim of this work was to study three different methods for hydrolysis and fermentation of pure inulin and Jerusalem artichoke flour: separate hydrolysis by *A. niger* MIUG 1.15 strain as active producer of inulinase, in stationary phase and under agitation, followed by fructose fermentation and simultaneous hydrolysis and fermentation of inulin and Jerusalem artichoke flour respectively, in order to increase the yield of biotransformation of substrate into ethanol. The highest amount of ethanol was formed during simultaneous hydrolysis and fermentation, for both pure inulin and Jerusalem artichoke (*Helianthus tuberosus*) tubers used as raw materials, of 16.2 $g \cdot L^{-1}$ and 28.1 $g \cdot L^{-1}$ respectively.

Keywords: *bioethanol, Helianthus tuberosus, Jerusalem artichoke, separate hydrolysis and fermentation (SHF), simultaneous hydrolysis and fermentation (SSF)*

INTRODUCTION

As the world faces the progressive depletion of its energetic resources and rapid accumulation of atmospheric greenhouse gases, biofuels from renewable raw materials attracted scientists' attention. Ethanol is currently used as liquid non-pollutant fuel or as gasoline enhancer in many countries, such as USA, Brazil, China and India [1-3].
The most used raw materials for bioethanol production can be classified into three main types: sugars, starches and cellulose materials [4]. Inulin, consisting of linear chains of β 2,1 linked D-fructofuranose molecules, terminated with a glucose residue through a sucrose-type linkage, have recently received increased attention as a renewable raw material for fructose syrup and ethanol production [2]. Inulin is present as reserve carbohydrate in various plants; such are Jerusalem artichoke, dahlia and chicory [2]. Jerusalem artichoke is a low requirement crop with high sugar content (the dried material of the tubers contains over 70% inulin); 4000-6000 L of bioethanol per hectare can be obtained from Jerusalem artichoke [5-6]. Jerusalem artichoke can grow in non-fertile land and is resistant to plant diseases [7].
It has been shown that inulin-type fructans in Jerusalem artichoke (*Helianthus tuberosus*) can be converted to ethanol either by direct fermentation using *Kluyveromyces marxianus* or *Saccharomyces cerevisiae* yeast selected strains [7], or by inulin hydrolysis to fructose using inulinase producing microorganisms, followed by sugars conversion to ethanol using fermenting yeasts. In the ethanol production from inulin rich feedstock, different modes of hydrolysis and fermentation were developed, such are simultaneous hydrolysis and fermentation (SSF), separate hydrolysis and fermentation (SHF) [8-11]. Acid hydrolysis is not very often used due to the secondary products which are formed during this process and which are toxic for yeasts [12-14]. The use of enzymatic hydrolysis is presented as advantageous since it requires less energy and uses mild environmental conditions [14].
Inulinase producing microorganisms are the best hydrolysis agents because of their easy cultivation, high yields of enzymes and no toxic by-products formation [6]. It has been found that the microorganisms that can produce high amounts of inulinases include moulds belonging to *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. genus, bacteria from *Bacillus* spp., *Clostridium* spp., *Streptomyces* spp. and yeasts like *Pichia* spp., *Kluyveromyces* spp., *Saccharomyces* spp. [6, 11, 15-16].
Therefore, the development of new technologies for fuel ethanol production is a priority for many researchers. The main objective of this work was to compare different saccharification and fermentation techniques for bioethanol production from Jerusalem artichoke flour, at laboratory scale.

MATERIALS AND METHODS

Substrates

Pure non-hydrolyzed inulin and Jerusalem artichoke tubers were used in experiments. The pure inulin derived from chicory was purchased from Sigma Aldrich. The Jerusalem artichoke tubers were purchased from the local market, washed and milled

prior to experiment. The Jerusalem artichoke tubers had a medium water content of 29.9%.

Microorganisms and media

In the present study *Aspergillus niger* MIUG 1.15 strain, with proved inulinase activity, from MIUG collection was used for inulin hydrolysis. A newly isolated yeast strain, *Saccharomyces* spp., was used for hydrolysates fermentation to ethanol. The isolated yeast strain was selected after a prior testing of its ability to ferment fructose and glucose. The stock of pure culture was maintained at 4°C on slants based on malt extract agar and subcultured every two weeks.
As a model system, medium with pure inulin was used, with the following composition (g/100 g): inulin 8, $(NH_4)_2SO_4$ 0.15, $MgSO_4 \cdot 7H_2O$ 0.5, KCl 0.05 and K_2HPO_4 0.1. The medium was sterilized at 12°C for 15 minutes. The medium with *Jerusalem artichoke* consisted from 10% milled Jerusalem artichoke tubers in tap water. The medium was also sterilized prior to use.

Inoculums obtaining and dimensioning

The yeast and mould inocula were obtained by transferring the yeast cells or the spores of *A. niger* respectively from malt extract agar medium in sterile physiological serum. The inoculum was dimensioned by direct counting method using Thoma cytometer.

Hydrolysis and fermentation procedures

150 mL of the fermentative media prepared as above were inoculated as following:
1st sample was stationary hydrolyzed with *Aspergillus niger* MIUG 1.15 ($1.5 \cdot 10^7$ spores$\cdot$mL^{-1}), for 3 days at 37°C, followed by yeast inoculation ($6 \cdot 10^5$ cells$\cdot$mL^{-1}) and fermentation.
2nd sample was hydrolyzed with *Aspergillus niger* MIUG 1.15 ($1.5 \cdot 10^7$ spores$\cdot$mL^{-1}), for 3 days at 37°C and 150 rpm on an orbital shaker, followed by yeast inoculation ($6 \cdot 10^5$ cells$\cdot$mL^{-1}) and then fermentation.
3rd sample was simultaneous hydrolyzed and fermented; the sample was inoculated in the same time with *Aspergillus niger* spores and yeast cells in the same concentrations as above.
The samples were kept at 25°C for fermentation and weighted daily to determine the CO_2 liberation during fermentation.

Determination of ethanol yield

200 grams of the fermented medium were mixed with 100 grams of water and distilled. The ethanol content of the distillate was measured by using the picnometer. The ethanol content was expressed in g$\cdot$L^{-1}.

RESULTS AND DISCUSSION

Simultaneous and separate hydrolysis and fermentation tests were performed on pure inulin and Jerusalem artichoke as substrate in order to establish the best method for inulin bioconversion into ethanol. The dynamics of fermentation for both pure inulin and Jerusalem artichoke substrates are shown in Figures 1 and 2.

As it can be seen in Figures 1 and 2, the medium with 10% Jerusalem artichoke ferments faster than the model system (96 hours of fermentation until constant weight, instead of 168 hours of fermentation of the model system) due probably to the natural nitrogen and salts contained in the Jerusalem artichoke tubers that are available for the microorganisms. Jerusalem artichoke has good potential for alcohol production if proper microorganisms are used for hydrolysis and fermentation [9].

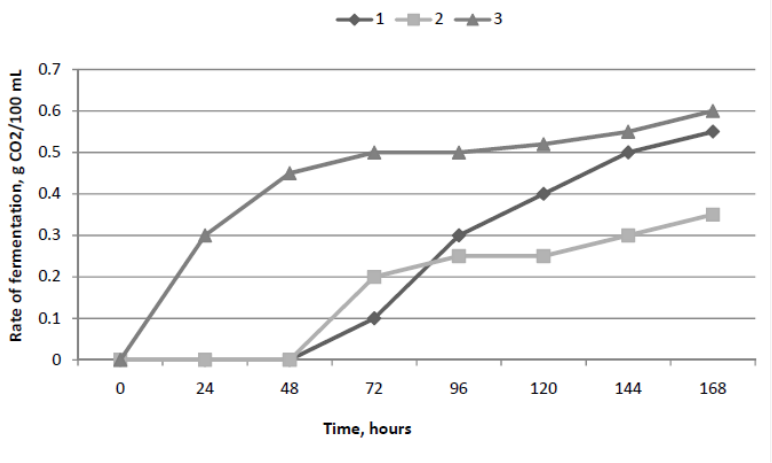

Figure 1. *Fermentation dynamics of the fermentative substrate containing 8 % pure inulin (1 – SHF with stationary hydrolysis, 2 – SHF with hydrolysis under agitation at 150 rpm, 3 – SSF)*

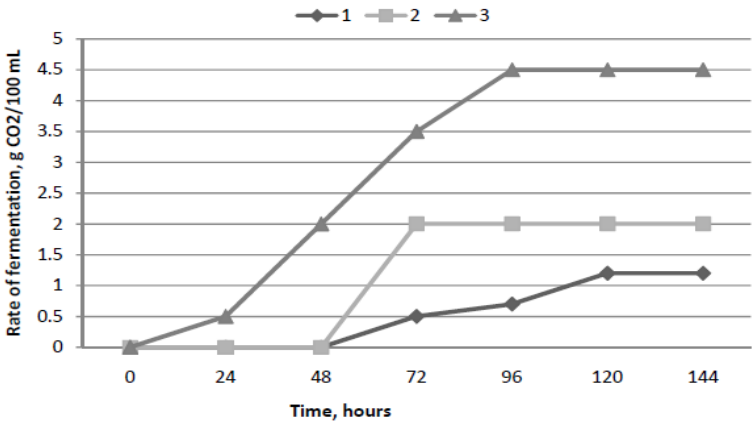

Figure 2. *Fermentation dynamics of the mash with Jerusalem artichoke used as substrate (1 – SHF with stationary hydrolysis, 2 – SHF with hydrolysis under agitation at 150 rpm, 3 – SSF)*

Also, higher ethanol concentrations were obtained after Jerusalem artichoke medium fermentation, as it can be seen in Figure 3.

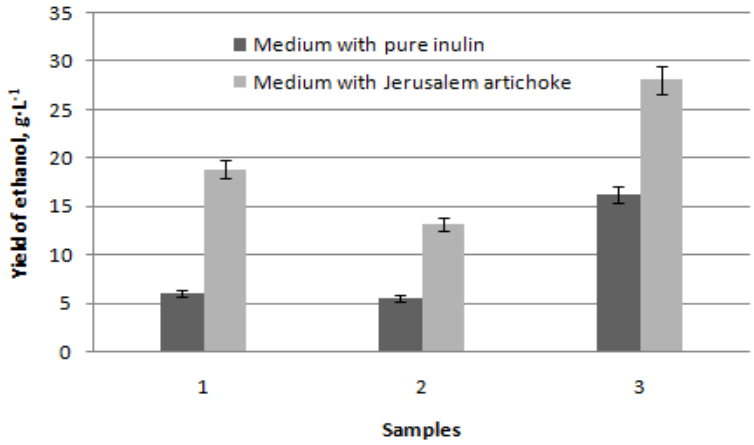

Figure 3. *Ethanol yield obtained in fermentative media with pure inulin and Jerusalem artichoke after hydrolysis and fermentation (1 – SHF with stationary hydrolysis, 2 – SHF with hydrolysis under agitation at 150 rpm, 3 – SSF)*

Also, as it can be seen in Figure 3, the SSF system gave the highest ethanol concentration in the fermentative media (28.1 $g \cdot L^{-1}$), either on pure inulin and Jerusalem artichoke used as substrates. The ethanol concentrations obtained in the present study are similar to those obtained by Kim *et al.* [11] and Lim *et al.* [7] but lower than those obtained by Ge and Zhang [9], Nakamura *et al.* [17], Ohta *et al.* [10]. Ohta *et al.* [10] obtained over 15% vol (120 $g \cdot L^{-1}$) of ethanol by simultaneous hydrolysis and fermentation procedure using an *A. niger* strain hyper-producer of inulinase and also Nakamura *et al.* obtained over 10% vol (80 $g \cdot L^{-1}$) ethanol in the medium prepared from Jerusalem artichoke tubers, after 120 hours of simultaneous saccharification and fermentation using the same strains of *A. niger* and *Saccharomyces cerevisiae* [17]. Ohta *et al.* [10] and Nakamura *et al.* [17] recommend the SSF process for bioethanol production from Jerusalem artichoke.

CONCLUSIONS

To establish the most appropriate method of inulin saccharification and hydrolysates fermentation of inulin rich feedstock, SSF and SHF techniques were used for ethanol production. *Aspergillus niger* MIUG 1.15 strain was used as inulinase producer and *Saccharomyces* spp. yeast selected strain fermented the inulin hydrolysates. The present study showed that SSF system is more advantageous than separate hydrolysis and fermentation system. Also, use of Jerusalem artichoke tubers instead of pure inulin as substrate gives higher ethanol yields. The tubers of Jerusalem artichoke (containing amino acids, proteins, vitamins and metal ions beside carbohydrates) have been proved to be an appropriate raw material for bioethanol production.

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