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Full Length Research Paper

Process Optimization for Bioethanol Production Using Jerusalem Artichoke Powder and Fresh Tubers

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A process for ethanol production from sugars released from inulin by hydrolysis was developed with yeast *Saccharomyces cerevisiae.* The impact of different microorganisms and pH correction on the ethanol fermentation performance was studied. We have shown that a part of the potential inulin of the tuber of Jerusalem artichoke can be fermented without acidic or enzymatic pretreatment prior to fermentation, but this process turns out to be long and uncertain. *Saccharomyces cerevisiae* was found to efficiently ferment the inulin-containing carbohydrates in Jerusalem artichoke after acidic hydrolysis of inulin at a pH 3.0 at 120° C for 15 minutes. This procedure turns out to be optimal for yield of alcohol and speed of fermentation. The conversion efficiency of inulin-type sugars to ethanol was 99% of the theoretical ethanol yield.

Keywords: Bioethanol, Inulin, Jerusalem artichoke, *Saccharomyces cerevisiae*, Fermentation

INTRODUCTION

In fresh Jerusalem artichokes a small amount of free fermentable sugars is present, which is approximately 2,5% of the potential. The Jerusalem artichoke tubers contain reserves attributable to inulin; inulin is linear polymer of D-fructose joined β-(2 →1) linkages and termined with a D-glucose, which is linked to fructose by an α (1→2) bond. Inulin has a degree of polymerization (DP) in the range of 2-60.

When inulin is used for alcohol production a lower degree of polymerization is more effectively converted; the production of free reducing sugars from inulin involves hydrolysis.

Inulin hydrolysis appears to be the limiting factor in the fermentation rate of Jerusalem artichoke pulp and juice. Variations in residual sugar composition observed during fermentation observed using a flocculing strain of yeast *Saccharomyces diastaticus* (NCYC 625), for instance, were due to differences in the inulin polymer distribution of the tuber extracts. Hydrolysis due to enzymatic activity and fermentation yield decrease when the fructose/glucose ratio of the extract increase (Schorr-Galindo *et al.* 2000). *Saccharomyces diastaticus* NCYC 625 facilitates high overall productivity by converting monosaccharides and short-chain fructoligosaccharides efficiently into ethanol. This yeast has an α-glucosidase which is active on dextrins but not on inulin, nevertheless the fermentability of biomass from the Jerusalem artichoke was not complete and not too different from other yeasts.

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Vranesic D. et al. (2002) have investigated the effects of pH and fermentation temperature on the production of inulinase by *Kluyveromyces marxianus* var. *bulgaricus* (ATCC16045). The pH optimum for the performance of inulinase was between 3.4 and 3.6, the optimum temperature was 29-31° C.

Negro *et al.* (2006) have shown that the production of ethanol can also be done by using the stalks of Jerusalem artichoke, instead of the tubers, as a source of carbohydrates; the inulin present in the stalks was extracted with water and then subjected to acidic hydrolysis. The maximum extraction efficiency, corresponding to 35 g/L of soluble sugars, was obtained at 1/6 solid/liquid ratio. The acidic hydrolysis was followed by fermentation with *Saccharomyces cerevisiae*. The direct conversion (without hydrolysis step) of inulin into ethanol was also studied using a selected strain of *Kluyveromyces marxianus*; in this case the direct conversion of inulin in ethanol was slower (30 hours); the fermentation of simple sugars was made by *Saccharomyces cerevisiae* (8-9 hours).

Yuan et al. (2008) have used, for the production of ethanol from the Jerusalem artichoke, *Kluyveromyces marxianus* (ATCC8554) cultivated in a saline medium; the optimum temperature was 38° C for inulinase production and strain growth, while the most efficient fermentation resulted at 35° C. Aeration was not necessary for ethanol fermentation. Bekers et al. (2008) proposed the use of the bacterium *Zymomonas mobilis* as active inulin-hydrolyzing enzyme producer; the fermentation was carried out in combination with *Saccharomyces*.

Saber et al. (2009) have selected strains of *Aspergillus* from rotting tubers of the Jerusalem artichoke grown in soils containing inulin, in order to identify the best inulinase activity for treatment of parts of different plants which contain inulin. The most appropriate strain was *Aspergillus Tamarii* AR-IN9; its maximum inulinase activity was recorded at pH 5.2. The enzyme retained about 80% of its activity for 24 hours at pH values between 4.4 and 7.2 and 75% at 50° C for 90 minutes. The hydrolytic activity was 71.64% for the Jerusalem artichoke, 67.55% for dahlia and 55.11% for chicory roots.

Zhang et al. (2010) have proposed the hydrolysis of inulin by the use of exogenus inulinase from *Pichia pastoris* with recombinant DNA (X-33/pPICZaA-INU1); 98,8% of sugars were converted into alcohol in 120 hours and after *Saccharomyces* inoculation. Lim el al. (2011) have fermented Jerusalem artichokes biomass without enzymatic pretreatment or chemical hydrolysis before fermentation, using a strain of *Saccharomyces cerevisiae* (KCCM50549) proved to be suitable among the many ones tested. The conversion of sugars from inulin was 70% compared to the theoretical quantity; the pH of the medium was adjusted at 5.00.

Yu et al. (2011) have employed *Saccharomyces cerevisiae* in which the gene for the extracellular production of inulin has been introduced; the gene was extracted from *Kluyveromyces cicerisporus* to hydrolyse inulin from the Jerusalem artichoke and to extract fructose.

Yuan et al. (2012) have described a new fermentation process for Jerusalem artichokes biomass using a yeast of strain *Kluyveromyces marxianus* (Y179) with enhanced inulinase activity in comparison with *Kluyveromyces marxianus* (ATCC8554); the effects of inoculum age, aeration, nutrients integration and pectinase were evaluated. The incubation time of inoculum had little impact on the inulinase activity; the aeration facilitated the fermentation process but lowered the alcohol yield, while it increased the risk of contamination; the use of pectinase reduced the viscosity of the fermentation broth and improved the fermentative efficiency slightly, but the incidence of the cost of the enzyme must be carefully assessed; also the addition of K_2HPO_4 implemented the ethanol production. Under the operating conditions described by the authors, after 48 hours of fermentation, *Kluyveromyces marxianus* (Y179) had transformed about 68% of the potential into alcohol and *Kluyveromyces marxianus* (ATCC8554) had transformed about 63.7%.

The main aim of our work was to ascertain what the real alcohol yield from Jerusalem artichokes biomass was and find the best fermentation conditions in relation to the prefermentative treatment of hydrolysable sugars in order to make them fermentable by *Saccharomyces* alone or in association with some other species of yeasts.

MATERIALS AND METHODS

Jerusalem artichoke

Dry powder, obtained by drying the Jerusalem artichoke tubers at 50° C, was used; the power was homogenized with shredder knives Retsch SM 100 Confort. The samples were analyzed for the quantitative determination of free and complex sugars (inulin). For fermentation tests 20 g of dried material were used; they were brought to 200 mL total volume with deionized water, with and without pH adjustment. The chemical analysis showed that the dry powder contained 64.16 g% of total sugars (glucose + fructose), of which 2.42%, are free (glucose, fructose, sucrose); the glucose/fructose ratio is equal to 0.1044 and the glucose is 9.45% of hydrolysable inuline. It was not necessary to perform testing of fermentation using other parts of the plant (leaves and stems) because their fermentable component, after hydrolysis, is perfectly identical to that of the tubers; therefore, the whole plant should be treated with the same procedures. The fresh

biomass was obtained trough trituration using shredder knives; tubers were first cut into small pieces at about 1 to 4 grams of weight, then 100 g of them were added with 130 mL of deionized water. The dry substance, calculated by drying in an oven at 105° C until constant weight after cutting tubers into slices of 2 mm thickness, which corresponded to to 27.97% of total weight.

The dilutions were made to obtain 6% of alcohol in volume, which would also correspond to an optimum solid/liquid ratio to obtain a manageable biomass for the fermentation process. It obviously takes into consideration micro-fermentation; at industrial level, you have to evaluate the viscosity of the medium in relation to the technology employed.

Yeast strains and fermentation

Microorganisms used: *Saccharomyces cerevisiae* physiological races *cerevisiae* (AT1), *uvarum* (S6U), *globosus* (SG3), *Schizosaccharomyces japonicus* (Schiz .3), *Saccharomycodes ludwigii* (Codes 1), *Kluyveromyces marxianus* var. *bulgaricus* (KLM) (ATCC catalog No.16045). The physiological race *uvarum*, being characterized by the metabolic capacity to ferment raffinose being equipped with α-galactosidase, could be useful if sugars are present with these links. *Saccharomyces cerevisiae* r.f. *globosus* is characterized by having no hydrolytic activity of α and β type, then it ferments only in the presence of simple sugars. All the microorganisms are from our collection, except KLM. The fermentations were conducted at 28° C and followed by noting the weight loss at regular intervals. At the end of fermentation, the fermented product was centrifuged; the supernatant was analyzed to determine the alcohol content by distillation of 100 mL supernatant and its indirect measurement with hydrostatic balance.

RESULTS

The preliminary tests of fermentability (Kreger-van Rij, 1984) have been carried out using standard inulin from dahlia tubers of SIGMA-ALDRICH company; the results showed that the inulin was hydrolysed into fermentable sugars at pH 3.0, at sterilization temperature of 120° C for 15 minutes.

Ethanol fermentation of Jerusalem artichoke powder suspension by different microorganisms was studied with and without pH correction (Materials and Methods); the results are reported in Table 1.

The strains of *Aspergillus niger* of our collection have shown limited and extremely slow hydrolytic activity, whereby this fungus is not useful for industrial practices for which the rapidity is a priority.

Under standard conditions *Kluyveromyces marxianus* var. *bulgaricus* started fermentation of inulin 8 days after inoculation; it has also been documented that *Kluyveromyces marxianus* cannot tolerate high concentration of ethanol in medium compared to *Saccharomyces cerevisiae* and produce much less ethanol than *Saccharomyces cerevisiae*; this confirmed that the use of *Kluyveromyces marxianus* var. *bulgaricus* alone or in combination with *Saccharomyces cerevisiae* isn't an interesting prospect for ethanol production coming from inulin-containing materials in the fermentation industry.

From Table 1 data it is evident that adjusting the pH to 3.0 and sterilizing the medium (15' to 120°C), *Saccharomyces cerevisiae* fermented the full potential sugar and the inulin was hydrolyzed into simple sugars; this procedure turns out to be the optimal for yield of alcohol and speed of fermentation (29 hours in tested conditions), that represents a fundamental parameter for technological economy. We adjusted the pH to 3.0 by adding approximately 8 L of concentrated sulfuric acid per ton of dry biomass or about 2,25 L of concentrated sulfuric acid per ton of fresh biomass.

It is important highlight that, in any case, a total solubilization of inulin is obtained by boiling for an hour; furthermore, when using enzymatic hydrolysis of biomass, the percentage of fructose varies in relation to the polymerization degree of inulin. Sterilization, in addition, guarantees the total hygiene of all technological treatment phases, avoiding fermentative anomalies that would cause serious difficulties in process management. In addition, glycerin can be recovered in a quantity equal to 2.2% of dry weight.

The hydrolysis of inulin with the same treatment was confirmed by using *Saccharomyces globosus* (SG3); but this yeast can only ferment glucose and fructose because it is unable to ferment complex sugars.

In conclusion the use of no-*Saccharomyces* yeasts has no practical, technological and economic reason. At the end of the technological process, the no-hydrolyzable fraction (about 30-35% of dry weight) can be easily used as a fertilizer or alternatively as fodder, because it is a product rich in sulfates (if sulfuric acid for adjusting pH) and in yeast with high nutritional value, in fact it is estimated that every kilogram of biomass contains 1.6 grams of dry yeast.

When we fermented fresh biomass, obtained by trituration of freshly harvested tubers, not subjected to heating, at physiological pH (Table 2), we obtained a partial fermentation of sugars (100 being the fermentable sugars after hydrolysis at pH 3.0) which varies from 1.16% for *Saccharomyces globosus* (it ferments only simple sugars such as glucose and fructose) to 63.5% for *Saccharomyces cerevisiae* as a function of either mechanical treatment of tubers and their physiological state. Since that *Saccharomyces globosus* ferments only

Table 1. Ethanol fermentation from Jerusalem artichoke powder using different microorganisms (average of three repetions). Statistical analysys: test ANOVA, HSD of Tukey α=0.05. Inoculum 50 Mc/mL; fermentation temperature: 28°C.

	AT1	S6u	Schiz.3	Codes1	KLM	AT1+KLM 50:50	S6u+KLM 50:50	SG3
pH 3, sterilization for 15' at 120°C								
% sugars into alcohol*** (conversion ratio 0,615 vol.)	99,46^{e} ±0,115	97,73^{a} +,30	95,20^{d} ±0,26	92,40^{c} ±0,36				98,00^{a} ±0,30
Fermentation time (hours)	29	29	35	40	45			32
Glycerol g%g dry matter***	2,23^{b,c} ±0,06	1,58^{b} ±0,076	2,20^{b,c} ±0,01	2,51^{c} ±0,38	1,496^{a} ±0,04			2,03^{b} ± 0,06
Acetic acid mg%g dry matter^{ns}	139 ±0,6	122 ±0,4	29 ±0,5	80 ±0,5	186 ±10			100 ±1,5
physiological pH, no sterilization								
% sugars into alcohol*** (conversion ratio 0,615 vol.)	63,67^{d,e} ±0,61	61,56^{c,d} ±1,42	60,43^{b,c} ±1,05	59,36^{b,c} ±0,75	58,36^{b} ±0,86	64,16^{e} ±0,80	63,65^{d,e} ±1,03	1,46^{a} ±0,01
Fermentation time (hours)	70	75	80	80	90	75	70	5
Glycerol g%g dry matter***	1,52^{b} ±0,076	1,58^{b} ±0,076	1,25^{b} ±0,132	1,1^{b} ±0,264	1,01^{b} ±0,246	1,7^{b} ±0,655	1,43^{b} ±0,45	0^{a}
Acetic acid mg%g dry matter***	110^{c} ±20	86^{c} ±15	20^{a,b} ±10	50^{b} ±10	90^{c} ±10	33^{a,b} ±15	16^{a,b} ±11	

Values designed by the same letter do not differ significantly (p≤.0.05)

simple sugars and that β-fruttofuranosidase preferentially hydrolyzes the terminal bond glucose-fructose of the fructose chains, it was deduced that β-fruttofuranosidase acts also on terminations with only fructose in relation to the spatial arrangement and to the degree of polymerization. The fermentation times are long (70 hours under experimental conditions) and this fact depends on both the difficulty of inulin solubilization at cold both the degree of biomass homogenization, as you can seen from Figure 1.

The partial fermentability of Jerusalem artichoke fructans makes this class of compounds different from those of other plants. In fact the degree of polymerization of fructans can vary from 3 to 10, while the fructans with a higher molecular weight are represented by inulin with degree of polymerization around 60 fructose units.

Table 2. Ethanol fermentation from fresh Jerusalem artichoke tubers biomass (100g added with 130mL deionized water). Tubers dry weight: 27,97%. *Saccharomyces cerevisiae* AT1 inoculum: 50Mc/mL. *Saccharomyces globosus* SG3 inoculum: 50Mc/mL.

Yeast	Test	Alcohol g%g fresh weight	Alcohol %vol	Alcohol g%g dry weight dissolved	% sugars
AT1	A	19,26	5,15	68,85	100
AT1	B	12,23	3,27	43,72	63,5
AT1	C	8,92	2,38	31,89	46,31
AT1	D	8,60	2,30	30,75	44,65
SG3	D	0,224	0,06	0,8	1,16

A = pH3, autoclaved at 120°C for 15', trituration for 5'

B = unmodified, at start budding of tubers; trituration for 5'

C = unmodofied, trituration for 15'

D = unmodified, trituration for 5'

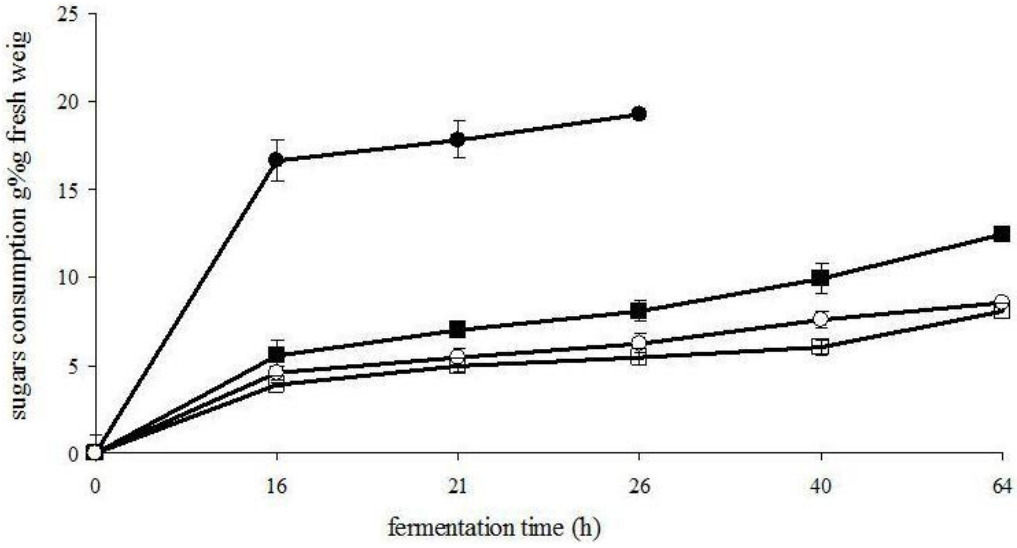

Figure 1. Fermentation performance: inoculum *Saccharomyces cerevisiae* (AT1) 50Mc/mL, 100g fresh tubers added with 130 mL deionized water (average values, n = 3, ± SD).(●) pH3, autoclaved at 120°C for 15', trituration for 5', (■) unmodified, at start budding of tubers; trituration for 5', (○) unmodofied, trituration for 15', (□) unmodified, trituration for 5'.

The process for ethanol fermentation from Jerusalem artichoke tuber includes saccharification of the inulin via acid or enzymatic hydrolysis, followed by fermentation with bacterium *Zymomonas mobilis* (Allais *et al.* 1987), yeast *Saccharomyces cerevisiae* (Ziobro and Williams 1983). As an alternative, both hydrolysis and fermentation steps have been combined by using immobilized *Aspergillus ficuum*-derived inulinase and the yeast organism (Kim and Rhee 1990) or direct conversion to ethanol utilizing a microorganism (e.g. *Kluyveromices marxianus*) that is capable of both hydrolysis and fermentation (Margaritis and Bajpai, 1982a, 1982b). However the ethanol production from Jerusalem artichoke tuber have resulted not practical from the viewpoint of industrial application, because pretreatment increases production cost and the maximum yield is low with direct fermentation.

DISCUSSION

From our results, the best technological and manageable solution for both short time and purity of fermentation appears to be the procedure of pretreatment of biomass with chemical hydrolysis of inulin at a pH 3.0 at 120° C for 15 minutes. In such a case the total solubilization of the potential sugar occurs as well as its transformation into simple sugars which are totally fermentable; the fermentation takes place in a short time with obvious economic advantage due to limited time engagement of equipment and easier management of fermentations; moreover, it is not absolutely necessary to add the fermentation medium with nutrients.

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