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Inulin-Containing Biomass for Ethanol Production

Carbohydrate Extraction and Ethanol Fermentation

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Abstract

The use of stalks instead of tubers as a source of carbohydrates for ethanol production has been investigated. The inulin present in the stalks of Jerusalem artichoke was extracted with water and the effect of solid–liquid ratio, temperature, and acid addition was studied and optimized in order to attain a high-fructose fermentable extract. The maximum extraction efficiency (corresponding to 35 g/L) of soluble sugars was obtained at 1/6 solid–liquid ratio.

Fermentations of hydrolyzed extracts by baker's yeast and direct fermentation by an inulinase activity yeast were also performed and the potential to use this feedstock for bioethanol production assessed. The results show that the carbohydrates derived from Jerusalem artichoke stalks can be converted efficiently to ethanol by acidic hydrolysis followed by fermentation with *Saccharomyces cerevisiae* or by direct fermentation of inulin using *Kluyveromyces marxianus* strains. In this last case about 30 h to complete fermentation was required in comparison with 8–9 h obtained in experiments with *S. cerevisiae* growth on acid extracted juices.

Index Entries: Ethanol; fermentation; Jerusalem artichoke; sugar extraction.

Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) has shown excellent potential as an alternative sugar crop *(1)*. Jerusalem artichoke is a perennial herbaceous plant belonging to the sunflower family, which is well adapted to a wide variety of climates *(2)*. It does not require soil fertility and develops underground stolons forming shaped tubers, which are similar to potatoes. Like sugar beet, Jerusalem artichoke produces sugars in the above ground and stores them in the roots and tubers. The tubers consist

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of 75–79% water, 2–3% proteins, and 15–16% carbohydrates, of which the D-fructose polymer inulin can constitute 80% or more.

This D-fructose polymer, which is initially in high levels in the stems, is transferred to tubers by the end of the growing cycle and the majority of the sugar produced in the leaves does not enter the tuber until the plant has nearly reached the end of its productive life *(3,4)*. Traditionally, Jerusalem artichoke has been grown for the tubers utilization and it is harvested in late autumn when the migration of carbohydrates from the aerial part of the plant to underground tubers has been completed. According to its high solubility in hot water, techniques similar to those based on sugars diffusion for sugar beet have been used to extract the inulin.

Over the past decades, Jerusalem artichoke has received interest for the production of fructose syrups *(5,6)* and ethanol *(7,8)* from tubers but the high cost of the harvesting and the infesting nature of tubers left in the soil that has been a limiting factor to the expansion of the crop. However, an alternative to harvest and handle the tuber exists. The possibility to harvest the above-ground biomass before tubers development and use the inulin containing stalks as feedstock for ethanol production is now envisaged as an interesting option. Thus, it could be possible to harvest the Jerusalem artichoke crop when the sugar content in the stalk reaches a maximum, thereby avoiding the harvesting of the tubers. In this case, the harvesting equipment and procedures are essentially the same as for harvesting sweet sorghum or corn for ensilage, reducing operation costs.

Although various strains of yeasts (*Kluyveromyces*, *Candida*, and *Schizosaccharomyces*) have been efficiently used for the direct fermentation of extracts from Jerusalem artichoke tubers *(9–11)*, the process is slow, taking typically 30–40 h. As a consequence, for a commercial operation a hydrolysis step before fermentation with conventional yeast is preferable.

In this work the use of stalks instead of tubers as a source of carbohydrates for ethanol production has been investigated. The effect of solid–liquid ratio, temperature, and acid addition on the water extraction of inulin from fresh Jerusalem artichoke stalks has been studied and optimized in order to attain a high fructose fermentable extract. Furthermore, fermentation of hydrolyzed extracts by baker's yeast *Saccharomyces cerevisiae* and direct fermentation by yeast *Kluyveromyces marxianus* CECT 10875 having inulinase activity has been performed and the potential to use this feedstock for bioethanol production assessed.

Material and Methods

Material

Stalks from *Helianthus tuberosus* L. var. Violet de Rennes were kindly supplied by Agronomy School of Madrid. The harvest was performed at the end of September, before flowering. Fresh stalks, without leaves, were crushed with a Blixer 4 vv (Robot Cupe, UK) and frozen at –18°C for storage.

Carbohydrate Extraction Procedure

The aqueous extraction of free sugars, sucrose, and inulin from stalks was carried out at different temperatures and different solid–liquid ratios (1/6, 1/4, and 1/3 w/w).

In order to test the effect of water temperature on free sugars, sucrose, and inulin solubilization, 20 g of stalks, crushed into small pieces, were placed into 500-mL Erlenmeyer flasks containing 120 mL of deionized water at 60°C and 80°C and boiling water for 30 min. The extract then, was separated and the solids were resuspended with an equal amount of water for a second extraction. As very low sugar content (<2%) was achieved in the second extraction, just one-step extraction was used in further experiments.

After the extraction samples were taken, the liquid phase removed by filtration and an aliquot of this extract analyzed for glucose, fructose, and sucrose content. To determine inulin concentration, a part of the filtrate was treated with HCl 1 *N* at 60°C for 30 min for inulin hydrolysis and then analyzed for sugars content. The glucose and fructose concentrations released from inulin were calculated by the difference in both determinations. The inulin content, corrected for water loss during hydrolysis, was calculated as follows *(12)*:

$$\text{Inulin} = k\,(G_i + F_i)$$

where: $k = [180 + 162\,(n–1)]/180n$; n is the average polymerization degree $n = [(F_i/G_i) + 1)$; G_i the glucose release from fructans; and F_i the fructose release from fructans.

$$\text{Considering that: } G_i = G_t - G_l \text{ and } F_i = F_t - F_l$$

where: G_t is the total glucose after hydrolysis; G_l the free glucose before hydrolysis and glucose from sucrose; F_t the total fructose after hydrolysis; and F_l the free fructose before hydrolysis and fructose from sucrose.

In order to test the effect of solid content on sugars solubilization, experiments at different solid–liquid ratios (1/6, 1/4, and 1/3) were carried out. So, 20, 30, and 40 g of stalks, crushed into small pieces, were placed into 500-mL Erlenmeyer flasks containing 120 mL of deionized water and boiled for 30 min. Samples were withdrawn at 10, 20, and 30 min and analyzed as described earlier. In order to test the direct extraction of monosaccharides (glucose and fructose) from inulin contained in Jerusalem artichoke stalks, clorhydric acid at concentrations of 0.05, 0.1, and 0.5 *N* instead of water were used. Extractions were performed at 1/6 solid–liquid ratio and boiled for 30 min. Samples were taken at 10, 20, and 30 min and analyzed.

Microorganisms and Growth Conditions

Saccharomyces cerevisiae baker's yeasts and *Kluyveromyces marxianus* CECT 10875 (a microorganism capable of fermenting both monomers and

inulin) were used in fermentation experiments. Active cultures for inoculation were prepared by growing the organisms on a rotary shaker at 150 rpm for 16 h at 35°C in a growth medium containing 5 g/L yeast extract (Difco, East Molesley, UK), 5 g/L peptone (Oxoid, Hampshire, UK); 2 g/L NH_4Cl, 1 g/L KH_2PO_4, 0.3 g/L $MgSO_4 \cdot 7H_2O$, and 30 g/L glucose or inulin.

Fermentation Assays

Fermentation experiments were carried out in 100-mL Erlenmeyer flasks, each containing 50 mL of the Jerusalem artichoke stalk extract, and were incubated at 35°C and 150 rpm. Flasks were inoculated with 4% (v/v) yeast cultures and periodically analyzed for ethanol and sugars. Fermentations by *S. cerevisiae* were performed on acid-extracted juice. *K. marxianus* was grown on extracted juice without any hydrolysis step. Fermentation results of ethanol production and sugars consumption were reported as percentage of the theoretical yield.

Analytical Procedures

Compositional analysis of the substrate was performed according to the Laboratory Analytical Procedures developed by the National Renewable Energy Laboratory *(13)*.

The maximum amount of free sugars, sucrose, and inulin in the stalks were determined according to AOAC standard method *(14)* and used to calculate extraction efficiency.

Sugars were quantified by high-performance liquid chromatography (HPLC) in a 1081B Hewlett Packard (HP) apparatus with refractive index detector under the following conditions: column, Aminex HPX-87P (300 mm × 7.6 mm) (BioRad, Hercules, CA); temperature, 85°C; eluent, water at 0.6 mL/min.

Ethanol was measured by gas chromatography (GC), using a HP 5890 Series II apparatus (Palo Alto, CA), equipped with an Agilent 6890 automatic injector and a flame ionization detector (Palo Alto, CA). A column of Carbowax 20 *M* (2 m × 1/8 in.) using helium as carrier gas at 35 mL/min was used. The GC oven temperature was held at 85°C. The injector and detector temperature was maintained at 150°C.

Results and Discussion

Composition of Jerusalem Artichoke Stalks

Table 1 shows chemical composition of Jerusalem artichoke stalks. Stalks were made up of about 58.5% moisture, and the remaining 41.5% solids were made up of 58% organic and aqueous extractives, 17.2% of cellulose, 14.6% klason lignin, 6.5% hemicellulose (mainly xylans), and 3.7% total ash. The total water soluble sugars (both free glucose and fructose, sucrose, and inulin) content in Jerusalem artichoke stalks was 58.7% of dry

Table 1
Composition of Jerusalem Artichoke Stalks

Component	% Dry matter
Cellulose	17.2
Glucose	18.9 ± 0.16
Hemicellulose	6.5
Xylose	5.6 ± 0.55
Galactose	1.2 ± 0.01
Arabinose	0.6 ± 0.01
Klason lignin	14.6 ± 0.41
Extractives	58.0 ± 0.1
Total ash	3.7 ± 0.05
Total	100

Data are the mean value of three replications.

weight of which 13% were monosaccharides, 3% was sucrose and 84% was oligofructose consisted of polymers about 20 fructose units with one glucose unit at the end of the molecule.

It is important to note that the total potential sugar content (both structural and nonstructural carbohydrates) in stalks at this developmental stage of the plants is close to 80% of dry matter content.

Effect of Temperature, Solid–Liquid Ratio, and Acid on Water Extraction of Inulin

Different methods, such as grinding or cutting the tubers with sugar beet slicer, followed by water extraction (with both boiling or cold water) have been normally used for the extraction of inulin in Jerusalem artichoke (*15,16*). In this work, a similar method for sugar extraction was applied to Jerusalem artichoke stalks. The effect of different variables (water temperature, amount of water, and acid addition) on the extraction of inulin, sucrose, and simple sugars was studied.

To test the effect of water temperature on the extraction efficiency of carbohydrates (inulin, sucrose, and simple sugars) from Jerusalem artichoke stalks, water at 60°C and 80°C temperature and boiling water were employed (Fig. 1). Extraction efficiency was calculated as the ratio of the amount of sugars obtained to the theoretical maximum amount of sugars available (7.8, 1.4, and 49.5% dry-wt for free sugars, sucrose, and inulin, respectively). As it can be observed, water temperature had not effect on the extraction of free sugars and sucrose, obtaining 100% extraction efficiency for all tested temperatures. However, at 60 and 80°C water temperature only 80% of the potential inulin was recovered in the juice, whereas using boiling water inulin extraction of 100% of the theoretical was obtained. As a result, for the extraction of carbohydrates in further experiments boiling water were used.

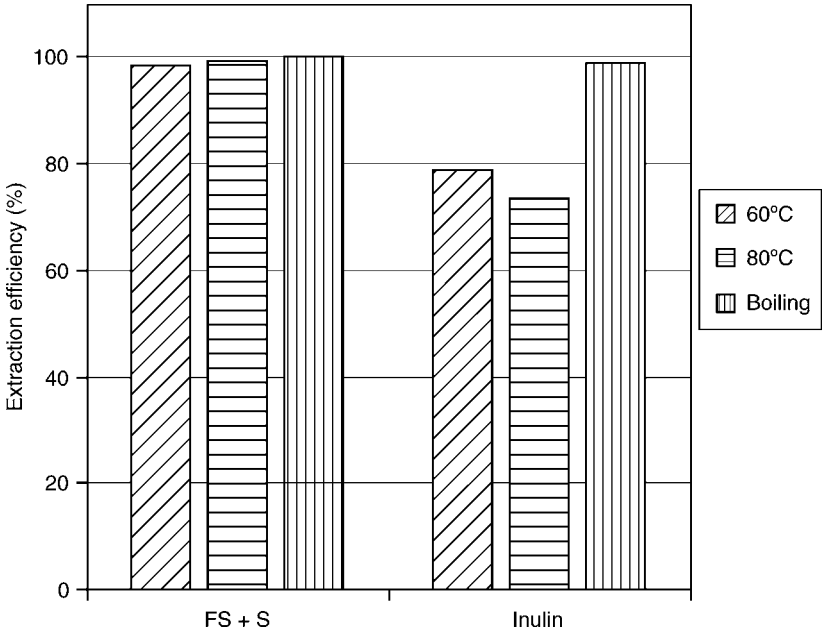

Fig. 1. Carbohydrate extraction efficiency of carbohydrates from Jerusalem artichoke stalks at different water temperatures. FS, free sugars; S, sucrose.

Figure 2A shows the sugar content in water extracts obtained from the extraction of Jerusalem artichoke stalks with boiling water at different solid–liquid ratio. Incubations longer than 10 min did not result in higher sugar concentration in the water extract. As expected, higher oligofructose concentration was obtained at higher substrate loading. Juices containing more than 56 g/L of total sugars were obtained at 1/3 solid–liquid ratio.

The effect of water amount on the extraction efficiency of soluble sugars was also analyzed (Fig. 2B). As can be observed, higher sugar extraction efficiencies were obtained at lower solid–liquid ratio. Free sugars and inulin efficiencies extraction about 90% were achieved at 1/3 and 1/4 solid – liquid ratio. The maximum extraction efficiency of 100% for free sugars and sucrose and 95% for inulin was attained at 1/6 solid–liquid ratio (corresponding of total carbohydrate concentration of 38.5 g/L); therefore these conditions were selected to further studies.

For ethanol production from carbohydrates (free sugars, sucrose, and inulin) contained in stalks of Jerusalem artichoke by *S. cerevisiae*, the inulin has to be first converted into simple sugars (glucose and fructose) by acidic or enzymatic hydrolysis. In order to examine the direct extraction of monosaccharides contained in stalks, boiling clorhydric acid at concentrations of 0.05, 0.1, and 0.5 *N* instead of water were used (Fig. 3). Carbohydrates were completely extracted and easily hydrolyzed in 0.05 *N* HCl within 10 min with no change thereafter. However, higher acid concentrations produced a negative effect on the sugar concentration owing to the

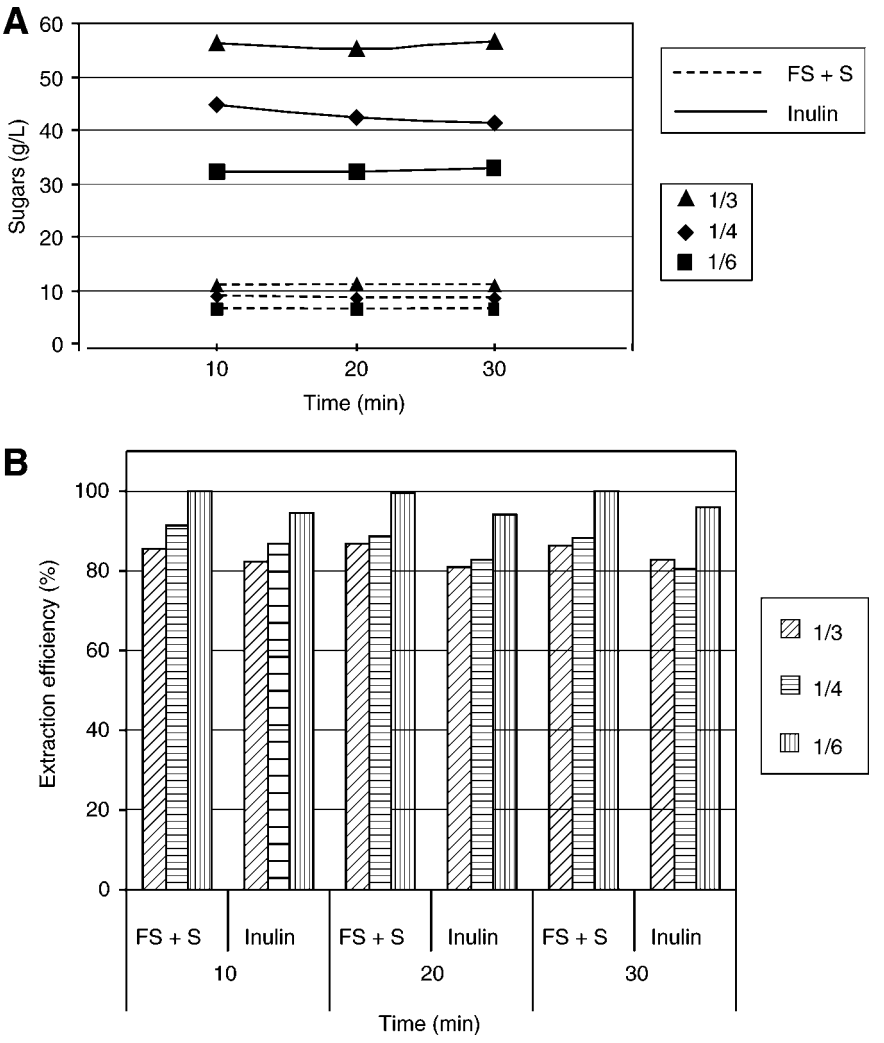

Fig. 2. Carbohydrate concentration **(A)** and extraction efficiency **(B)** in extracts obtained from the extraction of Jerusalem artichoke stalks using boiling water at different solid–liquid ratio (1/3, 1/4, and 1/6). FS, free sugars; S, sucrose.

degradation of fructose formed during depolymerization process. So, juices prepared by acid extraction using 0.05 *N* HCl were used for further fermentation experiments.

Ethanol Fermentation Assays

Sugars consumption and ethanol production results by *S. cerevisiae* of acid-extracted juices from Jerusalem artichoke stalks at different solid to liquid ratios are shown in Fig. 4A. Neither the rate of sugars utilization nor the final sugars concentration remaining in the media were affected by the initial sugar concentration in the range tested. Fermentation was completed

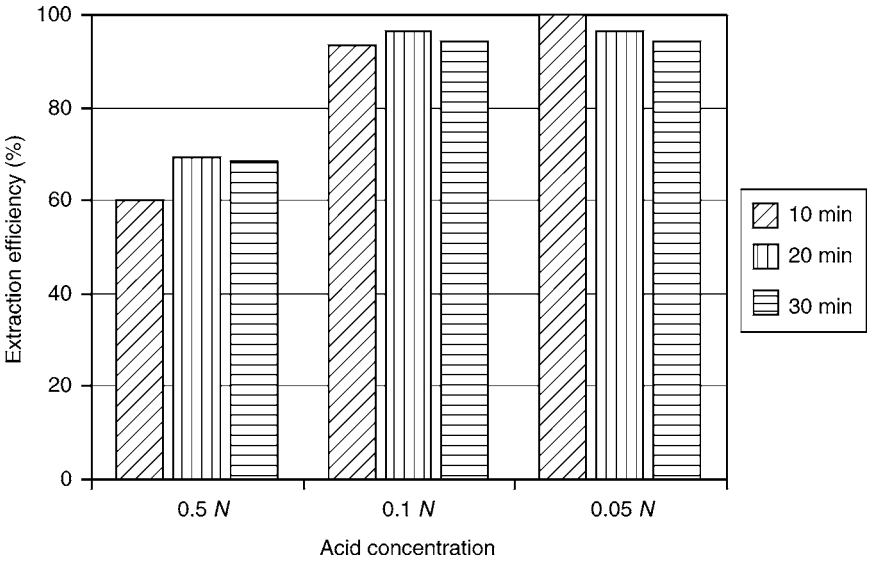

Fig. 3. Sugars extraction efficiency using boiling clorhydric acid at different concentrations.

in about 8–9 h from duration of inoculation. Using initial sugar concentrations of 38.3, 58, and 71.8 g/L (corresponding to 1/6, 1/4, and 1/3 solid–liquid ratio), final ethanol concentrations of 18, 27, and 32.5 g/L, respectively were obtained.

The direct conversion (without hydrolysis step) of inulin into ethanol using a selected *K. marxianus* strain growing on juices from Jerusalem artichoke stalks extracted at different solid–liquid ratio was also studied. (Fig. 4B). Direct conversion of inulin to ethanol by yeasts, which possesses the inulinase enzyme activity, was slower than fermentation of simple sugars by *S. cerevisiae*. Juices containing inulin could be completely fermented in 30 h regardless of initial carbohydrate concentration, although slight amounts of residual inulin were found at the end of fermentations at the highest initial sugar concentration.

As it can be seen, the rate of sugars utilization was not affected by initial inulin concentration. On the other hand, a delay in ethanol production was observed in comparison with inulin consumption. Thus, although inulin content in the media was negligible after 15 h from the onset of fermentation, the maximum ethanol concentration was found at 30 h. These results suggest that the microorganism has to hydrolyze the inulin first to monomeric sugars and subsequently convert them into ethanol. It should be noted that juices containing inulin used as fermentation broth, were prepared at a low inoculum loading and without any external assimilable nitrogenous sources. Long fermentation times required for the direct inulin conversion into ethanol could be reduced increasing cell population in the media. Thus, the use of higher inoculum size and

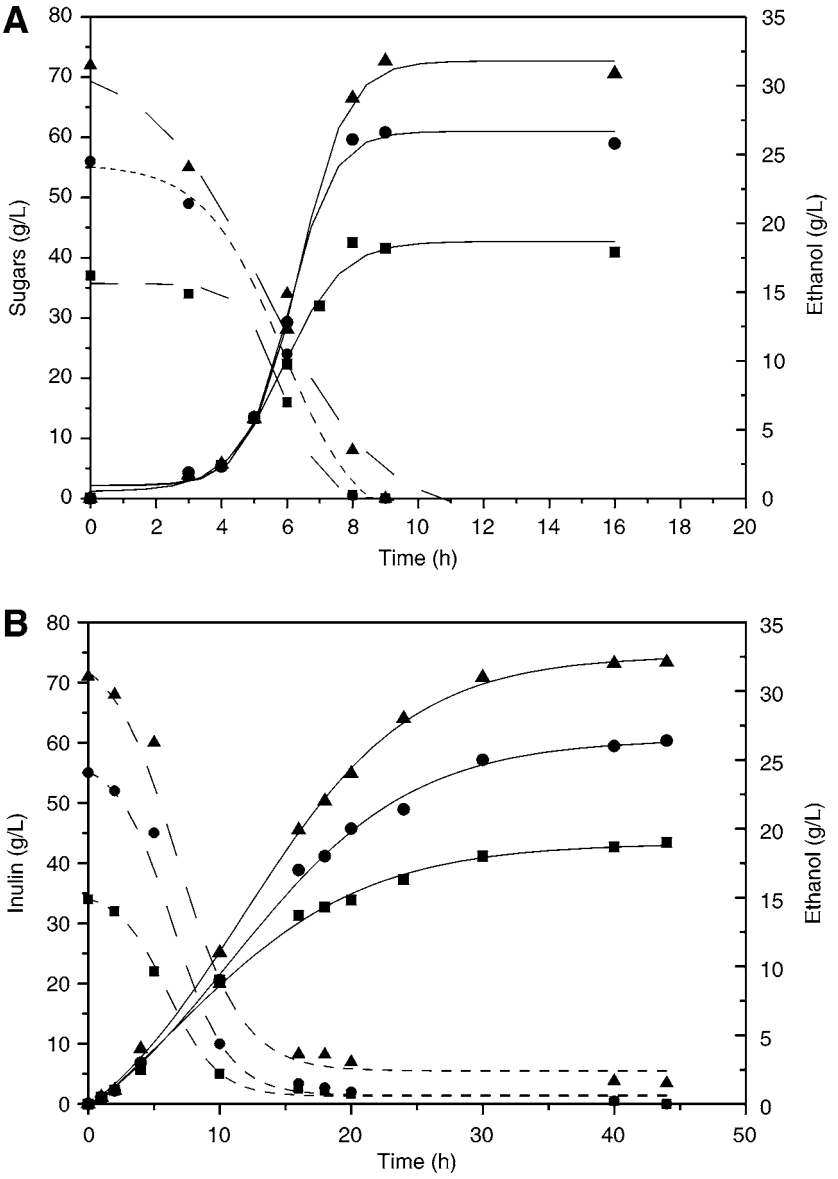

Fig. 4. Sugars utilization and ethanol production of juices from Jerusalem artichoke stalks extracted at different solid–liquid ratios (▲, 1/3; •, 1/4; and ■, 1/6). **(A)** Free sugars fermentation by *S. cerevisiae* on acid-extracted juice; **(B)** direct inulin fermentation by *K. marxianus* on water-extracted juice.

nutrient supplementation of the media should be studied. Further investigation will be carried out in our laboratory.

Data in Fig. 4 were used to calculate ethanol yield and percentage of substrate utilization (Fig. 5). Percentages of substrate utilization in direct inulin fermentation were slightly affected by initial sugar concentration, ranging from 96% to 100%. Ethanol yield was found to decrease with

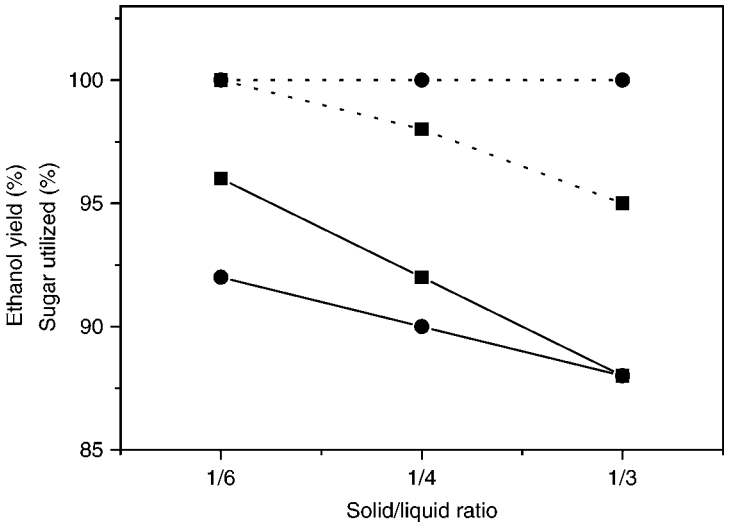

Fig. 5. Total sugar utilization and ethanol yield (expressed as percentage of the theoretical) of free sugars fermentation by *S. cerevisiae* (•) and direct inulin fermentation by *K. marxianus* (■) on juices obtained from Jerusalem artichoke stalks extracted at different solid–liquid ratios (1/3, 1/4, and 1/6).

increasing initial sugars content. Lower ethanol yields, between 88% and 92%, were obtained in fructose fermentations by *S. cerevisiae* in comparison to those obtained by *K. marxianus* growing on inulin. It has been stated that *S. cerevisiae* is glucophilic yeast, being less efficient in the utilization of fructose *(17,18)*. Ethanol yields obtained in this work were similar to those obtained by Bajpai and Margaritis *(19)* working with another strain of *K. marxianus* using inulin from an extract of Jerusalem artichoke tubers.

Conclusions

Results show that the carbohydrates derived from Jerusalem artichoke stalks can be converted efficiently to ethanol by acidic hydrolysis followed by fermentation with *S. cerevisiae* or by direct fermentation of inulin using *K. marxianus* strains, although in this last case about 30 h to complete fermentation were required in comparison with 8–9 h for experiments with *S. cerevisiae* growth on acid extracted juices. Studies to decrease fermentation time are being carried out.

On the basis of 4.5 t/ha produced annually from Jerusalem artichoke stalks reported in the literature *(20)* and the ethanol yield of 0.49 obtained in our study for *K. marxianus*, it is feasible to produce 2.2 t of ethanol/ha/yr. Moreover, the solid left after extraction of soluble sugars contains about 24% of structural carbohydrates (cellulose and hemicellulose) on dry-weight basis of stalks. They could also be converted into ethanol, which would increase significantly ethanol production from Jerusalem artichoke stalks. Work is underway in our laboratory for the enzymatic hydrolysis and fermentation of cellulose component of Jerusalem artichoke stalks.

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References

1 Gosse, G. (1988), In: *Topinambour (Jerusalem artichoke),* EUR 11855, G. Grassi and G. Gosse, eds., Commission of the European Communities, pp. 3–14.
2 Kosarik, N., Consentino, G. P., and Wieczorek, A. (1984), *Biomass* **5,** 1–36.
3 Incoll, L. D. and Neales, T. F. (1970), *J. Exp. Bot.* **21(67),** 469–476.
4 Ballesteros, M. (1989), PhD thesis, UAM University of Madrid, Spain.
5 Chubey, B. B. and Dorrel, D. G. (1974), *Can. Inst. Food Sci. Tech.* **7,** 98–100.
6 Kriestan, M. P. J. (1978), *Biotech. Bioeng.* **3,** 447–4503.
7 Margaritis, A., Bajpai, P., and Cannell, E. (1981), *Biotech. Lett.* **3,** 595–599.
8 Szambelan, K., Nowak, J., and Chrapkowska, K. J. (2004), *Acta Sci. Pol. Technol. Aliment.* **3(1),** 45–53.
9 Guiraud, J. P., Daurelles, J., and Galzy, P. (1981), *Biotech. Bioeng.* **23,** 1401–1420.
10 Margaritis, A. and Bajpai, P. (1981), *Biotech. Lett.* **3,** 679–685.
11 Poncet, S., Jacob, F. H., Berton M. C., and Couble, A. (1985), *Ann. Inst. Pasteur. Microbiol.* **136B(1),** 99–109.
12 Prosky, L. and Hoebregs, H. (1999), *J. Nutr.* **129,** 1418S–1423S.
13 National Renewable Energy Laboratory (NREL). Chemical Analysis and Testing Laboratory Analytical Procedures: LAP-001 to LAP-005, LAP-010 and LAP-017. NREL, Golden, CO. www. ott.doe.gov/biofuels/analytical_methods.html.
14 Hoebregs, H. (1997), *J. AOAC Int.* **80,** 1029–1037.
15 Byun, S. M. and Nahm, B. H. (1948), *J. Food Sci.* **43,** 1871–1879.
16 Margaritis, A., Bajpai, P., and Bajpai, P. K. (1983), *Develop. Ind. Microbiol.* **24,** 321–327.
17 Berthels, N. J., Cordero Otero, R. R., Bauer, F. F., Thevelein, J. M., and Pretorius, I. (2004), *FEMS Yeast Res.* **4,** 683–689.
18 Wang, D., Xu, Y., Hu, J., and Zhao, G. (2004), *J. Inst. Brew.* **110(4),** 340–346.
19 Bajpai, P. and Margaritis, A. (1982), *App. Environ. Microbiol.* **44(6),** 1325–1329.
20 Caserta, G. and Cervigni, T. (1991), *Biores. Technol.* **35,** 247–250.