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Optimisation of ethanol fermentation of Jerusalem artichoke tuber juice using simple technology for a decentralised and sustainable ethanol production

Javier Matías [a,*], José María Encinar [b], Jerónimo González [a], Juan Félix González [b]

[a] Agricultural Research Centre of Extremadura "La Orden-Valdesequera Farm" ,CICYTEX , Guadajira, Badajoz, Spain
[b] University of Extremadura, Chemical Engineering School, Department of Chemical and Physical Chemistry, Badajoz, Spain

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ABSTRACT

Ethanol production from Jerusalem artichoke was optimised using simple technology according to tuber harvest date. The optimal treatment for winter juice was the addition of 0.25 mL L^{-1} of a commercial inulinase (17 U g^{-1}) and a juice heating at 52.5 °C for 60 min before the beginning of the fermentation. For autumn juice, the optimal treatment was a previous heating at 80 °C for 15 min followed by the addition of 0.75 mL L^{-1} of the inulinase at 60 °C kept for 120 min, prior to the fermentation. Ethanol yields of 0.458 and 0.454 g g^{-1} were obtained with autumn juice and winter ones, respectively. Fermentation was conducted at 30 °C by *Saccharomyces cerevisiae*. These results could be useful for a staggered and decentralised ethanol production from a low-requirement crop which does not interfere with the food chain.

Introduction

Nowadays, governments encourage the development and use of biofuels with the aim of reducing greenhouse gas (GHG) emissions and because of the need to find alternative sources of energy other than fossil fuels to increase security of supply. In this direction, the European Directive on biofuels (EU-Directive 2009/28/EC, 2009) established the goal of reaching a 10% share of renewable energy in the transport sector in Community by 2020 and introduced mandatory sustainability criteria which biofuels must meet under this Directive. Biofuels must deliver greenhouse gas savings of at least 35% compared to fossil fuels, rising to 50% in 2017 and to 60%, for biofuels from new plants, in 2018. According to this premise and taking into account the default values for GHG emission savings of some biofuels included in the annex V of this Directive, new sustainable crops and process are required to be investigated in order to achieve the European renewable targets.

Therefore, the use of biomass for energy is a great opportunity for agriculture in the twenty-first century, but it is required to choose the right crops, in the right place with the right techniques, with a different approach from traditional agri-food products (Fernández, 2006).

Sustainability of biofuels is increasingly taken into account. However, sustainable technologies to produce biofuels from different kinds of biomass resources are required (Coppola et al., 2009). Thus, an optimisation of the production process is needed to improve the sustainability of biofuels (Matías et al., 2011).

Ethanol is the most produced biofuel worldwide. The United States is the largest ethanol world producer, followed by Brazil. Corn accounts for more than half of global ethanol production, and sugar cane for more than one third (REN 21, 2010).

At present, most of the main raw materials for ethanol production are food and starchy grains (Li and Chan-Halbrendt, 2009). The conventional enzymatic saccharification of starch by amylases has many disadvantages and the process is complicated (Chi et al., 2009). In the process to convert starchy grains into ethanol, starch must be gelatinized and liquefied at high temperature before the saccharification and fermentation so, compared to inulin hydrolysis using inulinases, the process for hydrolysis of starch is more complex (Zhang et al., 2010). Ethanol can also be obtained from lignocellulosic biomass, but the development of cost-effective and sustainable technologies is required (Chi et al., 2011).

Decentralised bioenergy systems are receiving increasing attention due to the potential ability to support local development and to create local employment (Mangoyana and Smith, 2011). The decentralised production of biofuels has been proposed for several reasons, such as the possibility of small scale production, the fact that there is no need to use high technology or make large investments, and because small

* Corresponding author. Tel.: +34 924014046; fax: +34 924014001.
E-mail address: javier.matias@gobex.es (J. Matías).

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plants do not need highly specialised technical staff (Iglesias et al., 2012).

Jerusalem artichoke (*Helianthus tuberosus* L.) is a low-requirement crop which has been reported to have one of the highest carbohydrate yields. Nowadays it does not interfere with food chain. It is, then, a promising energy crop for sustainable ethanol production (Matías et al., 2011). The clone of JA called "Nahodka" has been reported to provide high sugar and biomass yield (Matías et al., 2013; Curt et al., 2006; González et al., 2004; Conde et al., 1991). The main storage carbohydrate of Jerusalem artichoke (JA) is inulin, which is a polydisperse β (2–1) fructan, mainly a mixture of two linear fructan oligosaccharides (FOS), one with a terminal sucrose (GFn) and the other with a fructopyranose (Fm) (Bruggink et al., 2005). In JA, inulin has an average degree of polymerisation (DP) of 8 to 10 (Vijn and Smeekens, 1999), although depolymerisation of inulin during ageing of tubers has been observed (Schorr-Galindo and Guiraud, 1997). This phenomenon should be taken into account for ethanol production from JA tubers, especially if harvest is staggered in order to optimise the production costs.

Inulin cannot be directly fermented by classic fermentation yeasts, so either a hydrolysis followed by fermentation with classical yeasts or the use of yeasts with inulinase activity is required to produce ethanol from inulin sources. In the case of JA, acid hydrolysis was the main method used at first (Kays and Nottingham, 2007). Nevertheless, for a sustainable and decentralised production, this method has several disadvantages because it uses large amounts of both acid (toxic reagents) and energy (Guiraud et al., 1982). Enzymatic hydrolysis of inulin has also been used later (Ricca et al., 2009; Rocha et al., 2006; Szambelan and Nowak, 2006; Szambelan et al., 2004, 2005; Nakamura et al., 1996), but the aim of most of these researches was the syrup production. Szambelan and Nowak (2006), studied the enzymatic hydrolysis of JA tubers for further ethanol production, but only two doses of inulinases from *Aspergillus niger* were studied and the average degree of polymerisation of inulin was not taken into account. Inulinases can be produced by a series of microorganisms, including fungi, yeasts, and bacteria. There are two different subclasses of inulinase, endo- and exo-inulinase: exoinulinase (EC 3.2.1.80) hydrolyses the terminal fructose from the inulin chain, whereas endoinulinase (EC 3.2.1.7) reduces the long chain of inulin into smaller oligosaccharides (Basso et al., 2010). Ethanol from Jerusalem artichoke can also be produced by simultaneous enzymatic saccharification and fermentation (SSF) (Zhang et al., 2010; Szambelan and Nowak, 2006; Xiang-Yang and Wei-Guo, 2005; Nakamura et al., 1996; Ohta et al., 1993). However, in previous works, high enzyme concentrations and non-commercial microorganisms were required to obtain relatively high ethanol yields. On the other hand, yeasts with inulinase activity, like *Kluyveromyces marxianus*, had also been studied. These kinds of microorganisms can produce both active inulinase and ethanol. Nevertheless, compared to *Saccharomyces cerevisiae*, *K. marxianus* cannot tolerate a high concentration of ethanol in the medium and produces less ethanol, so that it has not been used for ethanol production from inulin-containing materials in the fermentation industry so far (Zhang et al., 2010). Furthermore, *K. marxianus* requires more stringent anaerobic conditions than *S. cerevisiae*, which means a more complex industrial installation (Guiraud et al., 1982).

The aim of this work has been to optimise ethanol fermentation of juice from JA tubers using commercial reagents and simple technology in order to favour decentralised industrial production.

Material and methods

Jerusalem artichoke tubers

Tubers used in the experiments were harvested from field trials conducted in experimental plots of Agricultural Research Centre of Extremadura, located in the Guadiana River Basin. The Nahodka clone of Jerusalem artichoke was used. Two harvest dates were carried out during the same season: one in autumn (December 2, 2010) and one late in winter (March 1, 2011).

Extraction of the juice of Jerusalem artichoke tubers

Just after harvest, juice was extracted from tubers by liquefying, using a blender (Model F2000, Frucosol). Previously, tubers were washed with water. Then, juice was immediately stored at −25 °C for further use.

Microorganism

S. cerevisiae was used in the fermentation trials. It was purchased from Laffort (Actiflore Cerevisiae), which contains about 20,000 million of live yeast cells per gram of dry yeast.

Experiments

Different experiments were carried out in order to optimise the ethanol fermentation yield of juice of Jerusalem artichoke tubers by a juice treatment followed by a simultaneous saccharification and ethanol fermentation. Three variables (heat treatment of juice prior to fermentation, dose of inulinase and moment of enzyme addition) were adjusted successively through different series of experiments.

Juice treatment

Juice heat treatment and partial enzymatic hydrolysis of the inulin were performed prior to the beginning of the fermentation. 500 mL Erlenmeyer flasks filled with 200 mL of JA juice were employed. Water baths were used to maintain the temperature. Different heat treatments and doses of enzyme were studied. The enzyme used was a commercial (Sigma-Aldrich) liquid mixture (density = 1.12 g mL^{-1}) of exo- and endo-inulinases, obtained from *A. niger*; the declared activity was 17 U g^{-1}.

Simultaneous saccharification and ethanol fermentation of Jerusalem artichoke juice

Later, the saccharification and ethanol fermentation of the JA juice from previous step were performed simultaneously in batch mode at 30 °C in partially anaerobic conditions, in the same Erlenmeyer flask of 500 mL. Inoculation was done with 3 g dry wt of the yeast described above per litre of juice. Yeast had been previously activated in a liquid medium (13 mL distilled water and 0.5 g sucrose per gram dry wt.) in a shake flask at 40 °C for 20 min. During the fermentation, samples (3 mL) were periodically withdrawn, cooled for 10 min at 3 °C and centrifuged at 4000 rpm. The supernatants were filtered through a 0.2 μm polyethersulfone syringe filter (Albet LabScience) and analysed for ethanol and sugars. The ethanol yield was determined as grams of ethanol produced per gram of total sugars. During the fermentation the medium pH was regularly measured using a digital pH metre (Model Basic 20, Crison). Juice was not previously sterilised due to the high cost of this operation.

Table 1
Sugar content and composition in the juice of Jerusalem artichoke tubers according to harvest date.

Harvest date	Free sugars (g L^{-1})	Total sugars (g L^{-1})	Inulin (% s/total sugars)	DP
Autumn	14.2	212.3	84.5	10.8
Winter	22.7	215.2	82.1	4.6