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**BIOETHANOL FERMENTATION OF JERUSALEM ARTICHOKE USING MIXED
CULTURE OF *SACCHAROMYCES CEREVISIAE* AND *KLUYVEROMYCES
MARXIANUS***

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Short running title: Production of bioethanol

Effects of mixed cultures of *Saccharomyces cerevisiae* Levuline FB – higher ethanol tolerance – and different *Kluyveromyces* strains – higher inulinase activity – on production of ethanol from Jerusalem artichoke extract were investigated. Among investigated strains, combination of *S. cerevisiae* and *Kl. marxianus* strain Y00959 with simultaneous saccharification and fermentation resulted the best efficiency (76 %) of bioconversion. The optimal ratio of mixed cultures was determined to be 1:1 of *Kl. marxianus* and *S. cerevisiae*. Central Composite Design (CCD) was adapted to find optimum initial substrate concentration and inoculum size for maximal production of ethanol from Jerusalem artichoke juice. The optimum fermentation conditions were found to be 24 m/w% substrate concentration and 45 OD600*ml/100ml inoculum size of mixed culture. Use of these conditions, about 10.67 (v/v) % was produced at 148 h of alcoholic fermentation given. Results of this work provide benefits of mixed culture on production of bioethanol from Jerusalem artichoke.

Keywords: bioethanol, Jerusalem artichoke, *Kluyveromyces marxianus*, mixed culture, simultaneous saccharification and fermentation

Nowadays, the demand of energy of transport sector is extremely increasing. It is estimated that about 61,2 % of produced primary energy sources are used in this sector (INTERNATIONAL ENERGY AGENCY, 2009). Most motor vehicles are still functioned with fossil fuel that is

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responsible to greenhouse gas emissions causing the climate change (BALLESTEROS *et al.*, 2006).

The term biofuel is attributed to any alternative fuel that derives from organic material, such as energy crops (corn, wheat, sugar cane, sugar beet, cassava, among others), crop residues (e.g. rice straw, rice husk, corn stover, corn cobs) or waste biomass (for instance, food waste, livestock waste, paper waste, construction-derived wood residues and others) (NEVES *et al.*, 2007; HANCSÓK, 2006). Of all biofuels, bioethanol has been trusted as an alternate fuel for the future and is already produced on a fair scale (29000 millions of gallons in 2011) worldwide (RENEWABLE FUELS ASSOCIATION www.ethanolrfa.org, 2011). In recent years, the first generation bioethanol (starch- or sugar-based) came into an increasing conflict with food industry and society (TILLMAN *et al.* 2009). Moreover, the bioethanol industry has always searched for improving the performance and the economic viability of the global process (ASTUDILLO & ALZATE, 2011). To overcome these problems, searching for alternative raw materials as well as development of new, efficient fermentation technologies should be good direction. Jerusalem artichoke (*Helianthus tuberosus*) that can be easily grown in extreme climatic and soil conditions has been proposed to be a possible feedstock for ethanol production since many years (SZAMBELAN *et al.* 2004; NAKAMURA *et al.* 1996). Jerusalem artichoke is rich in carbohydrates (approx. 11-20 %). The main carbohydrate of this plant's tubers is the inulin (70-90 %), which consists of linear chains of D-fructose units in the $\beta(2-1)$ position (KAYS & NOTTINGHAM, 2008). The chain is terminated by a D-glucose residue linked to fructose (NINESS, 1999; CHI *et al.*, 2009). The degree of polymerization of inulin from Jerusalem artichoke tubers is between 5 and 12 (BÖHM *et al.*, 2005). The distillery yeast, *S. cerevisiae*, does not readily ferment high molecular weight β -fructosides such as inulin due to lack of inulinase activity, thus an enzymatic or acid hydrolysis is needed prior to fermentation (KIM & RHEE, 1990; BARTHOMEUF *et al.*, 1991; SACHS *et al.*, 1981). Microorganisms such as *Kl. fragilis*, *Kl. marxianus*, *Aspergillus niger* etc. were reported to have inulinase activity, thus the use of them should allow saccharification of inulin (BARTHOMEUF *et al.*, 1991; PEKIC *et al.*, 1985; ROSA *et al.*, 1986; PESSOA & VITOLO, 1999;

SZAMBELAN *et al.*, 2004; 2005; MAZUTTI *et al.*, 2006; BAJPAI & MARGARITIS, 1993). But, main draw back of the use of these microorganisms is poor in the alcohol tolerance. The advantages of fermentation by mixed culture over monoculture have been extensively reported (ROSA *et al.* 1986; SZAMBELAN *et al.*, 2004; 2005), thus with this technology efficiency of fermentation process should be increasable. In the JA tubers case, combination of *S. cerevisiae* and *Kluyveromyces* species may lead to do simultaneous saccharification and fermentation process that should be attractive and effectiveness technology for inulin-based production of ethanol. In this study, efficiency of alcohol fermentation using mixed culture on Jerusalem artichoke is focused.

1. Materials and methods

1.1 Substrate

Jerusalem artichoke concentrate was used in experiments. The concentrate was obtained from the Central Food Research Institute in Budapest (Hungary) and contained approximately 70 % of dry matter. Different media were prepared from the Jerusalem artichoke concentrate for alcoholic fermentation processes.

1.2 Microorganisms

The strains *S. cerevisiae* (dried Levuline FB) and different *Kl.* yeasts (*K. thermotolerans* Y007002, Y00715, Y00775, Y00798, Y00873, Y00963; *K. waltii* Y01184 and *K. marxianus* Y00959) were used. All *Kluyveromyces* strains were provided by the National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary) and maintained on malt agar slants (yeast extract 3 g/l; malt extract 3 g/l; peptone 5 g/l; glucose 10 g/l and agar 15 g/l). After 24 h incubation at 30 °C the agar slants were stored at 4 °C until use.

1.3 Inoculum preparation

For the inoculum preparation of *Kluyveromyces* strains two loopfull of cells were added to each 250 cm³ flasks containing 100 cm³ of sterile culture medium. The inoculum medium

contained yeast extract 10 g/l, glucose 20 g/l and bacterial peptone 20 g/l. The flasks were incubated (30 °C, 120 rpm) in a rotary shaker for 12 h.

1.4 Analytical methods

Different carbohydrates (glucose, fructose, sucrose, oligofructose, inulin) and ethanol were determined by HPLC using an Aminex-87H column or Aminex-42C (Bio-Rad, USA) coupled to RI and PDA detectors. Mobile phases were 5 mM sulphuric acid or water, respectively. The flow rate was 0.6 ml/min. Reducing sugars were determined by Somogyi-Nelson method (Nelson, 1944; Somogyi, 1945, 1951). Ethanol concentration was measured after end of fermentation by Anton Paar DMA 35N and Gibertini scale. Mash was distilled by Büchi Distillation Unit K-350 and ethyl-alcohol content was determined based on relative density. The fermentation yield was expressed as the percentage of theoretical yield, referring to the carbohydrates consumed during the fermentation and as grams of alcohol produced per 1 g of carbohydrate consumed.

1.5 Fermentation processes

The classical batch fermentations were carried out in 500 ml Erlenmeyer flask. All flasks contained 250 ml mash prepared from concentrated JA juice. The pH of mash was adjusted to <3.5 with phosphoric acid. Furthermore, chemical manure (UVAVITAL) was supplemented prior initiation of alcoholic fermentation process. Depending on purpose of individual experiment, the mash was inoculated with *Kluyveromyces* and *S. cerevisiae* yeasts or in mixed cultures. Fermentation was carried out at 30 °C for a week in an incubator. All fermentations were executed in a parallel experiment series. The sampling procedures were done daily in the course of the fermentation.

1.6 Statistical analysis

Result values are generally the mean of triplicate trials. Calculation of standard deviation, analysis of variances (ANOVA) and other statistical probe tests were performed using SPSS

software package for MS Windows version 12 (SPSS Inc., USA). Experimental design (design and analysis) was carried out based on Design and Analysis of Experiments (DOE) module in STATISTICA 9 (StatSoft Inc., Tulsa, USA).

2. Results and discussion

Jerusalem artichoke concentrated juice was processed by hot water extraction from plant tubes and concentrated to about 85 % by evaporation. This juice was found to contain 59.4 (w/w) % carbohydrates with higher degree of polymerisation ($DP \geq 7$ 7.3 %, DP_6 8.9 %, DP_5 9.8 %, DP_4 12.7 %, DP_3 13.4 %), 9.6 % sucrose, 1.28 % glucose and 3.6 % fructose (NGUYEN *et al.*, 2011). High amounts of oligosaccharides and inulin that are not readily fermented by *S. cerevisiae*, cause inefficiency of production of ethanol from Jerusalem artichoke. Fig. 1A shows that after 48 hours of fermentation, fermentable sugars such as glucose, fructose and sucrose was dropped drastically in the case of use of *S. cerevisiae* strain. This is well known that *S. cerevisiae* has high invertase activity (DEÁK, 1998), but lacks of inulinase activity, thus fermentation of inulin or inulo-oligosaccharides goes slowly. After 1 week of alcoholic fermentation, about 26 % of carbohydrates were be residue in mash (Fig. 1A). The yield of conversion (ethanol) was calculated to be between 60-65 %. In the case of fermentation of JA juice by *Kl. marxianus* strain, concentration of reducing sugars (glucose and fructose) in mash increased in the beginning stage. The maximum amounts of fructose and glucose were 5.61 g/100 ml and 0.73 g/100 ml, respectively, while carbohydrates with higher polymerization degree decreased from 10.5 g/100ml to 2.2 g/100ml after 24 hours of fermentation (Fig. 1B). This can be explained by inulinase activity of *Kluyveromyces* yeast (DEÁK, 1998; LANE & MORRISSEY, 2010). After 144 h of fermentation the ethanol concentration showed 6.54 v/v% in fermenting medium, which was sufficient to interfere the efficiency of *Kl. marxianus* yeast cells. The yield of ethanol was 65-75% comparing with theoretical yield. From this point of view, monocultural fermentation by both yeast, even different fermentation profiles were obtained, resulted about 60-75 % yield of bioconversion. This result is in agreement with the one reported by ROSA *et al.* (1986), but much

1 lower than one (80-84 %) by SZAMBELAN *et al.* (2004). Both groups applied *Kl. fragilis* for
2 fermentation of JA tubers. In the case of mixed cultures of *S. cerevisiae* and *Kl. marxianus*
3 combinations of both monocultural profiles were observed (Fig. 1C). Concentration of
4 carbohydrates with high polymerisation ($DP \geq 4$) was dropped drastically, meanwhile fructose
5 concentration increased to about 10 w/v % after 1 day of fermentation due to inulinase activity
6 from *Kl. marxianus*. Concentration of glucose and sucrose decreased slightly with the
7 fermentation time. After a week, amounts of residual carbohydrates were less than 1 w/v % and
8 about 9 v/v % of ethanol were detected in mash yielding 75-84 % of efficiency of bioconversion.
9 Overall, mixed cultural fermentation of JA juice should result higher efficiency of bioconversion
10 than using neither only *S. cerevisiae* nor *Kl. marxianus*.
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23 Screening combination of *S. cerevisiae* and different *Kluyveromyces* species/strains for mixed
24 cultural fermentation of JA juice was carried out. The most of mixed cultures (*Kl. thermotolerans*
25 and *Kl. waltii*) produced only 4.69-5.25 % (v/v) ethanol after 144 hours given 57-62 % of the
26 theoretical yield. Best combination should be mixture of *S. cerevisiae* and *Kl. marxianus* that 6.57
27 % (v/v) in mash with 76 % ethanol yield. ROSA *et al.* (1986) applied *Kl. fragilis* for ethanol
28 fermentation of JA tubers with about 80 % of efficiency. Effect of ratio of mixed cultures on the
29 ethanol yields was investigated. The native mashes of JA were initiated with mixture of pre-
30 inoculated *Kluyveromyces* and *Saccharomyces* yeasts in ratios of 1:0, 3:1, 1:1, 1:3 and 0:1 (based
31 on OD_{600nm}). Efficiencies of bioconversion were calculated when production of ethanol in
32 fermentation process came to stationary phase (Fig. 2). Maximum efficiency of bioconversion (79
33 %) was reached when using both yeast cultures in ratio one to one. It is worth to note that no
34 significant differences were obtained in either the case of ratio 3:1 not 1:3 of *Kl. marxianus* and
35 *S. cerevisiae*. Ethanol concentrations were lowest (5.81 and 6.42 %) when pure yeast culture was
36 applied (data are not shown). In these cases (*Kl. marxianus* and *S. cerevisiae*), **bioconversion**
37 **efficiencies** were only 71.7 % and 64.9 %, respectively. GUO *et al.* (2010) also found that ratio of
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1:1 in mixed cultures of *Kl. marxianus* and *S. cerevisiae* resulted best bioconversion efficiency when ethanol fermentation of whey.

Optimal ratio of substrate to inoculum size was determined using experimental design namely Central Composite Design one of frequently used response surface methods (RSM). To standardize method of inoculation, new quantity unit namely Abs600nm.ml was introduced meaning that 1 ml of cell suspension with 1 Abs at 600 nm. This unit can be applied in measurement of inoculum size. In our experiments, two factors (substrate concentration and inoculum size) were selected as independent variables, while ethanol concentration and bioconversion efficiency were dependent variables. Numerous preliminary designs with different central points and steps were carried out and analyzed (data are not shown). The final design and results of experiments are summarized in Table 2. Highest efficiency (90.86 %) was calculated in the case of 13 (w/v) % substrate concentration and 52 Abs600nm.ml/100ml due to high number of cells supplied. In this case, high amounts of sugars were converted to ethanol in mash. Use of efficiency as dependent factor may not give real optimum, thus, only concentration of ethanol was evaluated. Regression analysis of experimental data was done by Design and Analysis of Experiments (DOE) in STATISTICA software and the following second-order regression model was obtained:

$$Y = -12.086 + 1.385 \cdot x_1 - 0.026 \cdot x_1^2 + 0.274 \cdot x_2 - 0.002 \cdot x_2^2 - 0.004 x_1 \cdot x_2 \quad /eq. 1/$$

Where X_1 is substrate concentration (w/v %), X_2 is inoculum size (Abs600nm.ml/100ml) and Y is ethanol concentration (v/v %).

The effect estimates and standard errors of each variable were checked statistically by t-test (**Table 3**). All effect estimates including interaction of both factors are statistically significant at 95 % or higher of probability level. Analysis of variance (ANOVA) was used to investigation the fit of model. The ANOVA of the regression model demonstrates that equation 1 was a highly significant model (**Table 2**). The F-value (77.519) of lack of fit of model with low p value (0.012763) indicates the significance of model at 95 % level. Moreover, both linear and quadratic

of factors (X_1 , X_2 , X_1^2 and X_2^2) as well as interaction ($X_1.X_2$) were significant with a probability of 95%. Three-dimension graph was produced based on equation 1 by STATISTICA software to demonstrate the changes of ethanol concentration depending on substrate concentration and inoculum size. Based on regression model the predicted maximum ethanol concentration (10.23 v/v %) should be obtained when substrate concentration and inoculum size were 24 (w/v) % and 45 Abs600nm.ml/100ml, respectively (**Fig. 3**). The ethanol production at optimal conditions was validated experimentally. Three parallel experiments were carried out using 24 (w/v)% substrate concentration and 45 Abs600nm.ml/100ml inoculum size. The ethanol concentration was 10.67 (v/v) % at 148 h of alcoholic fermentation that was very closed to predicted value (10.23 v/v % with 9.94-10.51 v/v % at 95 % confident interval). Slightly increase in ethanol concentration was also detected after 6th day of fermentation. Overall, it can be concluded that the regression equation (**Eq. 1**) was proven to be adequate model to describe change of ethanol during fermentation.

3. Conclusions

S. cerevisiae with high ethanol tolerance was beneficially combined with *Kl. marxianus* with high inulinase activity for production of bioethanol from Jerusalem artichoke extract. The time and the conversion efficiency of fermentation process with mixed culture can be shortened and enhanced, respectively, due to synergistic effects of simultaneous saccharification and fermentation. Moreover, RSM was proved to be very strong and efficient tools for optimisation of substrate concentration and inoculum size for maximising production of ethanol and conversion efficiency. The application of the mixed cultures inulin-based for production of ethanol seems to be very promising technology, thus with a wider investigation, applications of it in biotechnological processing are warranted.

*

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References

- ASTUDILLO, I.C.P. & ALZATE C.A.C. (2011): Importance of stability study of continuous systems for ethanol production, *J. Biotechnol.* 151, 43–55.
- BAJPAI, P. & MARGARITIS, A. (1983): Effect of sugar concentration in Jerusalem artichoke extraction *Kluyveromyces marxianus* growth and ethanol production. *Appl. Env. Microb.* 52, 723-725.
- BALLESTEROS, I., NEGRO, M.J., OLIVA, J.M., CABANAS, A., MANZANARES, P. & BALLESTEROS, M. (2006): Ethanol production from steam-explosion pretreated wheat straw. *Appl. Biochem. Biotechnol.* 130, 496-508.
- BARTHOMEUF, C., REGERAT, F. & POURRAT, H. (1991): High-yield ethanol production from Jerusalem artichoke tubers. *W. J. Microbiol. Biotechnol.* 7, 490-493
- BÖHM, A., KAISER, I., TREBSTEIN A. & HENLE, T. (2005): Heat-induced degradation of inulin. *Eur. Food Res. Technol.*, 221, 466-471
- CHI, Z., CHI, Z., ZHANG, T., LIU, G. & YUE, L. (2009): Inulinase- expressing microorganisms and applications of inulinases. *Appl. Env. Microb.* 82, 211–220.
- DEÁK, T. (1998): Yeast in nature and in industry, Mezőgazdasági Szaktudás Kiadó, Budapest, pp. 11-193.
- GUO, X., ZHOU, J. & XIAO, D. (2010): Improved ethanol production by mixed immobilized cells of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* from cheese whey powder solution fermentation. *Appl. Biochem. Biotechnol.* 160, 532-538
- HANCSÓK, J., KRÁR, M., HOLLÓ, A. & THERNESZ, A. (2006): Next-generation bio-engine fuels I. *J. Hungarian Chem.* 61, 260-264

- INTERNATIONAL ENERGY AGENCY (2009): Key world energy statistics
http://www.iea.org/textbase/nppdf/free/2009/key_stats_2009.pdf
- KAYS, S. J. & NOTTINGHAM, S. F. (2008): Biology and chemistry of Jerusalem artichoke: *Helianthus tuberosus* L.. Taylor & Francis Group, Boca Raton, London, New York, pp. 1-148.
- KIM, C.H. & RHEE, S.K. (1990): Ethanol production from Jerusalem artichoke by inulinase and *Zymomonas mobilis*. *Appl. Biochem. Biotechnol.* 23, 171-180.
- LANE, M. & MORRISSEY, J. P. (2010): *Kluyveromyces marxianus*: A yeast emerging from its sister's shadow. *Fungal Biol. Rev.* 24, 17-26.
- MAZUTTI M., BENDER J.P., TREICHEL H. & DI LUCCIO M. (2006) Optimization of inulinase production by solid-state fermentation using sugarcane bagasse as substrate. *Enzyme Microb. Technol.* 39, 56-59.
- NAKAMURA, T., OGATA, Y., HAMADA, S. & OHTA, K. (1996): Ethanol production from Jerusalem artichoke tubers by *Aspergillus niger* and *Saccharomyces cerevisiae*. *J. Ferment. Bioeng.* 81, 564-566.
- NELSON, N. (1944): A photometric adaptation for the Somogyi method for the determination of glucose. *J. Biol. Chem.* 153, 375-380.
- NEVES, M.A., KIMURA, T., SHIMIZU, N. & NAKAJIMA, M. (2007): State of the Art and Future Trends of Bioethanol Production. *Dynamic Biochem. Process Biotechnol. Mol. Biol.* ©2007 Global Science Books (<http://globalsciencebooks.info/>).
- NGUYEN, Q.D., REZESSY-SZABÓ, J.M., CZUKOR, B. & HOSCHKE, Á. (2011): Continuous production of oligofructose syrup from Jerusalem artichoke juice by immobilized endo-inulinase. *Process Biochem.* 46, 298–303.
- NINESS, K. R. (1999): Inulin and oligofructose: What are they? *J. Nutr.* 129, 1402-1406.
- PEKIC, B., SLAVICA, B., LEPOJEVIC, Z. & PETROVIC, S.M. (1985): Effect of pH on the acid hydrolysis of Jerusalem artichoke inulin. *Food Chem.* 17, 169-173.

- PESSOA, A. & VITOLO, M. (1999): Inulinase from *Kluyveromyces marxianus*: culture medium composition and enzyme extraction. *Braz. J. Chem. Eng.* 16, 3. DOI: 10.1590/S0104-66321999000300003
- ROSA, M.F., VIERA, A.M. & BARTOLOMEN, M.L. (1986): Production of high concentration of ethanol from mash. juice and pulp of Jerusalem artichoke tubers by *Kluyveromyces fragilis*. *Enzyme Microb. Technol.* 8, 673–676.
- SACHS, R.M., LOW, C.B., VASAVADA, A., SULLY, M.J., WILLIAMS, L.A. & ZIOBRO, G.C. (1981): Fuel alcohol from Jerusalem artichoke. *Californ. Agr.* 29, 4-6.
- SOMOGYI, M. (1945): A new reagent for the determination of sugars. *J. Biol. Chem.*, 160. 61-68.
- SOMOGYI, M. (1951): Notes on sugar determination. *J. Biol. Chem.* 195, 19-23.
- SZAMBELAN, K., NOWAK, J. & CZARNECKI, Z. (2004): Use of *Zymomonas mobilis* and *Saccharomyces cerevisiae* mixed with *Kluyveromyces fragilis* for improved ethanol production from Jerusalem artichoke tubers. *Biotechnol. Lett.* 26, 845-848.
- SZAMBELAN, K., NOWAK, J. & JELEN, H. (2005): The composition of Jerusalem artichoke (*Helianthus tuberosus* L.) spirits obtained from fermentation with bacteria and yeasts. *E. Life Sci.* 5, 68-71.
- TILMAN, D., SOCOLOW, R., FOLEY, J.A., HILL, J., LARSON, E., LYND, L., PACALA, S., REILLY, J., SEARCHINGER, T., SOMERVILLE, C. & WILLIAMS, R. (2009): Beneficial biofuels-the food, energy, and environment trilemma. *Science* 325, 270-271

Table 2 Experimental design and results

Runs	Substrate concentration (w/v %)	Inoculum size (Abs600nm.ml/100ml)	Ethanol concentration (v/v %)	Conversion efficiency (%)
1	27	52	10.6	60.98
2	25	65	9.4	60.68
3	25	39	10.0	63.19
4	20	70	9.5	72.29
5	20	52	10.3	79.22
6	20	52	10.2	78.11
7	20	52	10.2	80.05
8	20	34	10.2	78.39
9	15	65	8.4	86.20
10	15	39	8.1	83.82
11	13	52	7.9	90.86

Table 3 Analysis of experimental data

	SS	Df	MS	F	p	Effect	Std. Err.	t
X ₁	5.634141	1	5.634141	1690.242	0.000591	10.24476	0.033322	307.4432
X ₁ ²	2.314103	1	2.314103	694.231	0.001437	1.68687	0.041030	41.1126
X ₂	0.205627	1	0.205627	61.688	0.015827	-1.29561	0.049172	-26.3483
X ₂ ²	0.661273	1	0.661273	198.382	0.005003	-0.32402	0.041254	-7.8542
X1.X2	0.202500	1	0.202500	60.750	0.016065	-0.70504	0.050057	-14.0848
Lack of Fit	0.775195	3	0.258398	77.519	0.012763			
Pure Error	0.006667	2	0.003333					
Total SS	9.301818	10						

X₁ is substrate concentration (w/v %), X₂ is inoculum size (Abs600nm.ml/100ml), **R²: 0.91595**

List of figures

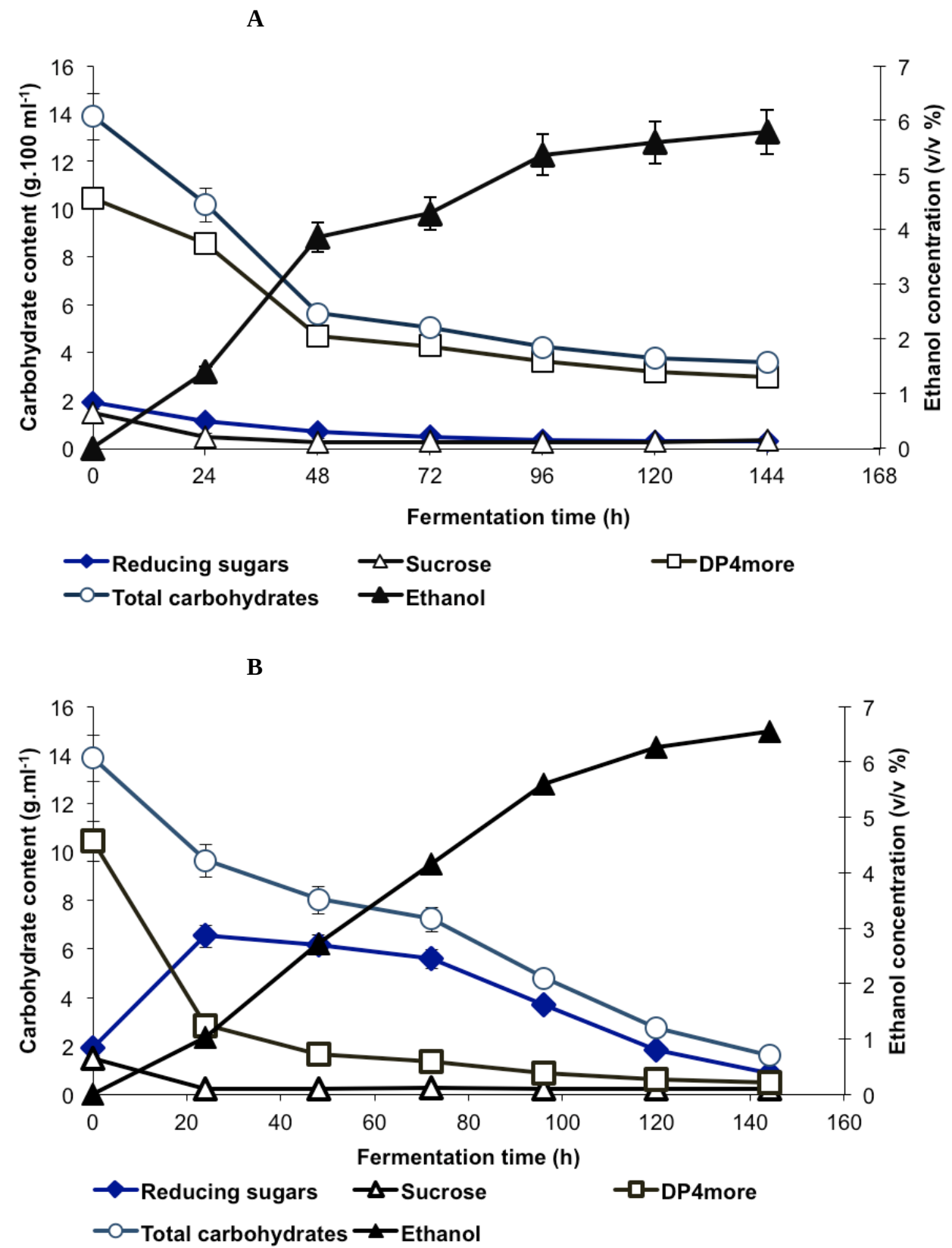
Figure 1 Fermentation profiles of JA juice by different mono- and mixcultures

A: monoculture *Saccharomyces cerevisiae*. B: monoculture *Kluyveromyces marxianus*. C: mixed cultures *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*

Figure 2 Effects of ratio of mixed cultures on efficiency of bioconversion of ethanol fermentation

Figure 3 Effects of substrate concentration and inoculum size on JA based production of ethanol by co-cultural fermentation

Figure 1



C

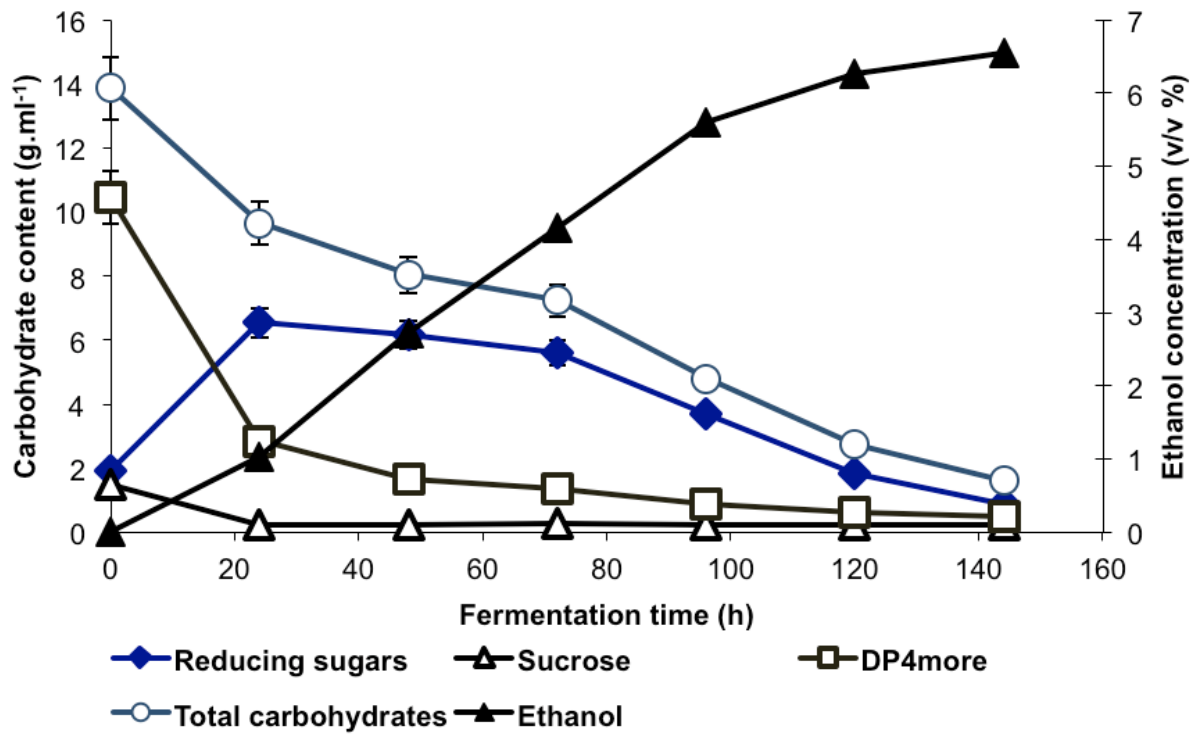


Figure 2

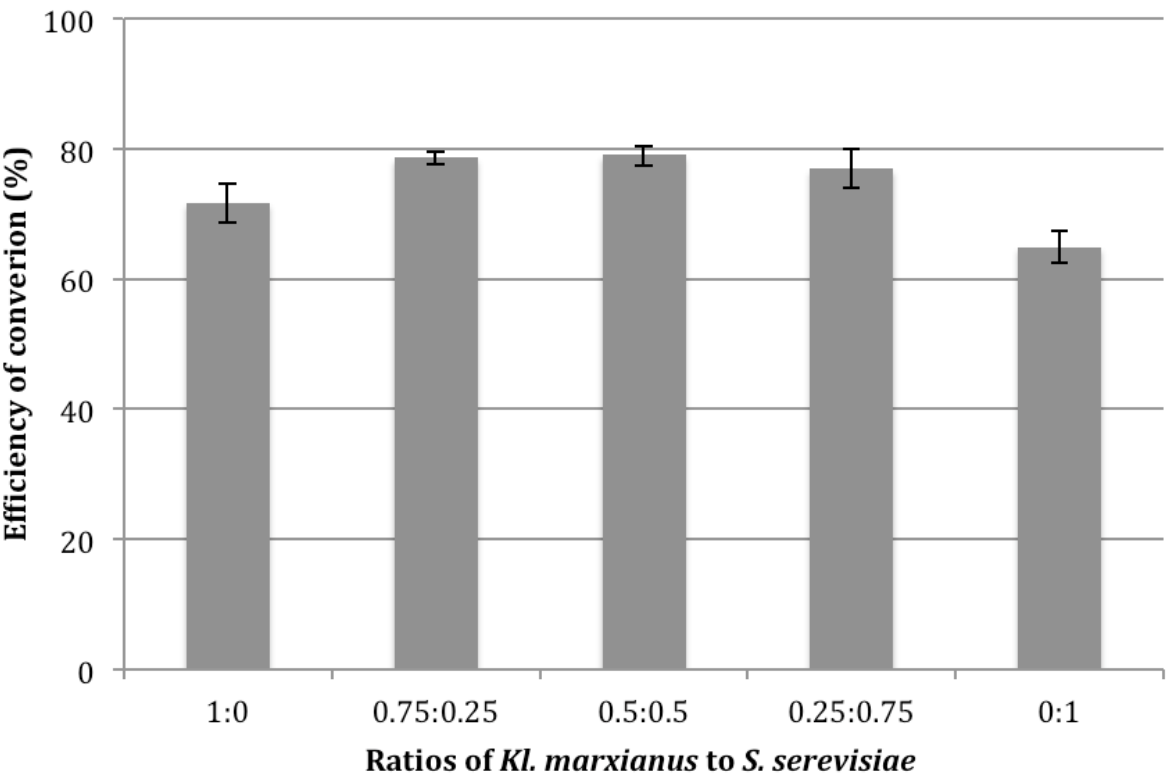


Figure 3

