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Effect of Sugar Concentration in Jerusalem Artichoke Extract on *Kluyveromyces marxianus* Growth and Ethanol Production

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The effect of inulin sugars concentration on the growth and ethanol production by *Kluyveromyces marxianus* UCD (FST) 55-82 was studied. A maximum ethanol concentration of 102 g/liter was obtained from 250 g of sugars per liter initial concentration. The maximum specific growth rate varied from 0.44 h^{-1} at 50 g of sugar per liter to 0.13 h^{-1} at 300 g of sugar per liter, whereas the ethanol yield remained almost constant at 0.45 g of ethanol per g of sugars utilized.

The continuing escalation of prices for nonrenewable oil resources has stimulated worldwide interest in the utilization of fermentation ethanol as a potential liquid fuel (6, 11, 16). A systematic perusal of the literature has shown that different types of carbohydrate materials have been used to produce ethanol (9, 13, 16). However, very little published information is available on the use of Jerusalem artichoke (*Helianthus tuberosus*) as a carbohydrate resource for the production of ethanol (3, 12, 25, 26). The Jerusalem artichoke is a native plant of North America which is presently grown in very small quantities in various parts of the world. The plant produces tubers in the ground in addition to the stem and branches. The tubers contain inulin, which is a polymer of about 2 to 35 fructose units with one glucose unit at the end of the molecule (4, 14). The main advantages of Jersusalem artichoke over traditional carbohydrate crops include the following: (i) minimal fertilizer requirements, (ii) very good growth on secondary poor quality land without competition for the good quality fertile land required to grow traditional crops, (iii) it possesses high tolerance to frost and plant diseases which normally attack traditional crops, and (iv) the carbohydrate yields per acre per year are among the highest reported, ranging from 5,930 to 14,580 kg of sugars per hectare per year, depending on soil conditions and the strain used (5, 7). The Jerusalem artichoke tuber is composed of about 80% moisture, and the remaining 20% is solids made up of about 85% inulin-type sugars and about 5% ash, and the rest is protein and cellulosic material (10).

Previous work by Margaritis et al. (21) showed that *Kluyveromyces marxianus* UCD (FST) 55-82 was the best ethanol producer of eight yeasts screened for their ability to ferment Jerusalem artichoke extract. In this paper, we report results on the kinetics of batch growth and ethanol production by *K. marxianus* at different sugar concentrations present in the Jerusalem artichoke extract. This paper is part of a continuing research activity in our laboratory aimed at the efficient and economical utilization of Jerusalem artichoke to produce fuel ethanol (17–21).

The inulin sugars were extracted from the tubers of Jerusalem artichoke as described by Margaritis et al. (21) and then concentrated by evaporation as needed. The sugar-containing extract was then autoclaved at 120°C for 20 min and was stored at 0°C for further use.

K. marxianus UCD (FST) 55-82 was maintained on yeast extract glucose agar slants by subculturing at weekly intervals. Media consisted of different sugar concentrations of Jerusalem artichoke juice, ranging from 5 to 30% sugars supplemented with 0.05% Tween 80, 0.01% oleic acid, and 0.01% corn steep liquor. The pH of the media was adjusted to 4.6 with 1 N HCl. For the preparation of inoculum, 10 ml of the stock culture obtained from a washed slant was transferred to a 250-ml Erlenmeyer flask which contained 50 ml of the artichoke juice. This culture was incubated in a New Brunswick rotary shaker at 35°C and 140 rpm for about 24 h. After incubation, this inoculum was transferred to a 1-liter Bellco jar fermentor containing 500 ml of the medium. All the fermentations were performed at 35°C.

Biomass concentration was determined by the dry weight method and ethanol concentration by gas chromatography as described by Margaritis et al. (21). The concentrations of the sugars were determined by the anthrone reagent method (24) as total fructose equivalents by using standard fructose solutions.

The biomass, sugars, and ethanol concentra-

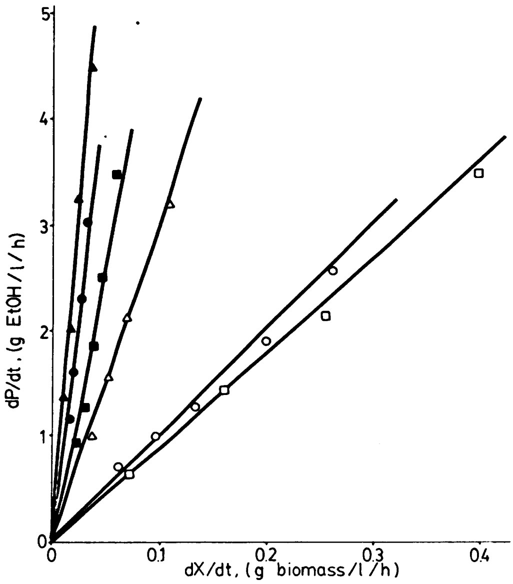

FIG. 1. Growth-associated plot for *K. marxianus* at different sugar concentrations. Initial sugar concentrations: ▲, 30%; ●, 25%; ■, 20%; △, 15%; ○, 10%; □, 5%.

tion were plotted as a function of fermentation time at the following initial sugar concentrations: 5, 10, 15, 20, 25, and 30%. The kinetic data for biomass and ethanol during exponential growth phase were computer fitted (correlation coefficient, 0.992 or higher) and the derivatives dP/dt and dx/dt were calculated at specified times. These data were correlated according to a simple growth-associated model (1) shown by:

$$dP/dt = \alpha \, dx/dt$$

where dP/dt is the rate of ethanol production (g of ethanol per liter per h), dx/dt is the rate of biomass production (g dry weight of cells per liter per h), and α is the growth-associated constant (dimensionless).

Figure 1 shows the growth-associated plot of the data according to the equation. Table 1 is a summary of the important kinetic parameters of *K. marxianus* grown in Jerusalem artichoke extract at different initial sugar concentrations.

Figure 2 shows the effect of initial sugar concentration on ethanol yield, percent sugar utilization, and biomass yield. At initial sugar concentrations up to 25%, ethanol yield coefficients (g of ethanol per g of sugar utilized) were almost identical, whereas above an initial sugar concentration of 25% it began to decline slightly. Rogers et al. (22) in their kinetic studies of ethanol production by *Zymomonas mobilis* at high glucose concentration reported that ethanol yield remained almost unaffected by initial glucose concentrations up to approximately 20% and declined beyond that. About 92, 89, and 87% of the sugars were utilized in the media with initial sugar concentrations of 20, 25, and 30%, respectively. The cell growth yield (g dry weight of cells per g of sugar utilized) was found to decline sharply with the increase of the initial

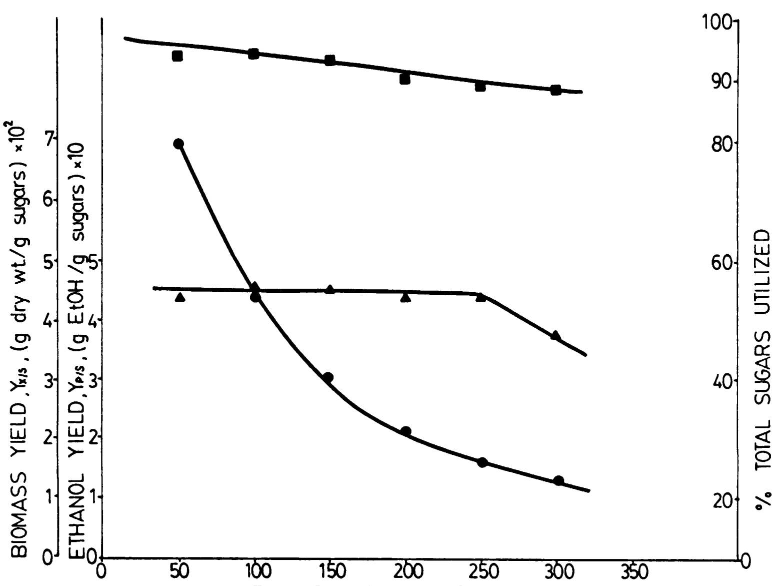

FIG. 2. Biomass yield, ethanol yield, and sugars utilized as a function of initial sugars concentration. Symbols: ■, percent sugars; ▲, ethanol yield; ●, biomass yield.

TABLE 1. Kinetic parameters of *K. marxianus* grown in Jerusalem artichoke extract at different sugar concentrations

Initial sugar concn, S (g/liter)	Kinetic parameter			
	Maximum ethanol concn, P (g/liter)	Maximum biomass concn, x (g/liter)	Maximum specific growth rate, μ_{max} (h^{-1})	Growth-associated constant, α
50	21	3.2	0.44	9.2
100	45	4.2	0.40	10
150	64	4.5	0.28	30
200	78	4.2	0.20	51.7
250	102	3.8	0.15	85
300	98	3.6	0.13	130

sugar concentration. Rogers et al. (22) and Lee et al. (15) have also reported diminishing growth yields with increasing initial glucose concentrations for *Z. mobilis* in batch and continuous culture. Belaich et al. (2) reported similar results. These changes are likely to result from the changes in the consumption of energy for cell maintenance, as recently discussed by Stouthamer and Bettehausen (23) or due to the alteration of cell permeability and influence on cell functional activity by high substrate concentration (8).

A systematic perusal of the literature has shown that there are no reported data on how the inulin sugars concentration affects the kinetics of growth and ethanol production by *K. marxianus*. The new information provided in this paper is essential in the design and economic evaluation of batch or continuous fermentation processes for the production of ethanol from Jerusalem artichoke extract with different inulin sugars concentrations.

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