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EFFECT OF ACID OR ENZYMATIC HYDROLYSIS ON ETHANOL PRODUCTION BY ZYMOMONAS MOBILIS GROWING ON JERUSALEM ARTICHOKE JUICE

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SUMMARY

Ethanol production from the inulin of Jerusalem artichoke by Z.mobilis was studied in batch and continuous fermentations. Both acid or enzymatic hydrolysis were used. In continuous cultures enzymatic hydrolysis showed better results. Ethanol productivities of 17.7 and 29.0 g/l.h were obtained at output concentrations ca 35 g/l (% of conversion 99 and 83 ; ethanol yield 0.45 g/g). The hydrolysed juice could be used without any nutrient addition.

INTRODUCTION

Among possible carbohydrate substrates for ethanol production, the advantages of the Jerusalem artichoke have been already pointed out (Fleming, 1979 ; Margaritis, 1983). The sugar content of the tubers represents 80 % of the dry matter and is constituted exclusively by inulin, a fructose polymer containing a terminal glucose. The direct conversion of inulin to ethanol has been studied using several yeast strains (Guiraud, 1981a ; Duvnjak, 1981 ; Margaritis, 1981,1982a,1982b,1983 ; Bajpai, 1982 ; Joshi, 1984). However the performances of these fermentations are lower than the ones reported for the bacteria Zymomonas mobilis grown on a glucose medium (Rogers, 1979, 1982) or, as shown in our laboratory, on a fructose medium (Toran-Diaz, 1983a, 1983b, 1984 ; Jain, 1985a, 1985b). Z.mobilis is able to utilize only glucose, fructose or sucrose as carbon source and an hydrolysis step is necessary for the fermentation of inulin .Here we present results on the comparison of acid and enzymatic hydrolysis of inulin from Jerusalem artichoke and subsequent fermentation to ethanol with Z.mobilis in batch and continuous cultures.

MATERIALS AND METHODS

The strains used in this study are Z.mobilis ZM4 (ATCC 31821) and a flocculent one (ZM4F) isolated in our laboratory (Toran-Diaz, 1984). The two strains were maintained on agar slants as previously described (Toran-Diaz, 1983a). The tubers of Jerusalem artichoke were sliced with a blade homogenizer. Inulin was extracted by two successive steepings (each for 1 hour at 70°C) with 30 % v/v added water, each followed by vacuum filtration. For acid hydrolysis, the pH was adjusted to 2.0 with phosphoric acid and incubation was at 80°C for 1-3 hours. After hydrolysis the pH was adjusted to 5.0 by addition of ammonia and the precipitate discarded by centrifugation. Sterilisation was done at 110°C during 30 minutes.

For enzymatic hydrolysis the juice was first adjusted to pH 5.0 and then sterilized (110°C, 30 minutes). A commercial preparation of inulinase (NOVO Enzymes) from Aspergillus ficuum (Zittan, 1981) was used after filtration on a Millipore filter 0.45 µm. The enzyme was added (35 units per g of total sugar) and hydrolysis was at 50°C for 24 or 48 hours.

Ethanol production was studied with a culture medium containing hydrolyzed Jerusalem artichoke juice without any other nutrients added. Batch cultures were done in erlenmeyers and continuous cultures were operated in a fermentor of total volume : 110 ml. An internal settler (Toran-Diaz, 1983b, 1984) allowed a high cell density in the reactor when the flocculent strain was used. Temperature was 30°C and pH was controlled at 5.0 by addition of 5 N KOH.

Biomass, ethanol and reducing sugars concentrations were estimated by dry cell weight determination, GLC and the dinitrosalicylic acid method as previously described (Toran-Diaz, 1983a). Total sugars were assayed by the anthrone method

(Sattler,1948). The characterization of sugars after hydrolysis was done by TLC on silica gel (Randerath, 1966). The elution solvent contained 2 butanol, acetic acid and water : 3/1/1 (v/v/v). Sugars were revealed by spraying a solution (0,5 %) of naphtoresorcin in ethanol.

RESULTS AND DISCUSSION

The hydrolysis of inulin can be based on chemical hydrolysis by mineral or organic acids or on enzymatic hydrolysis. Initially these methods were proposed for the preparation of high fructose syrups (Guiraud, 1981b). Alternatively, these syrups could be the basis for the preparation of culture media containing highly assimilable sugars (glucose and fructose) for ethanol production using Z.mobilis.

1) Kinetics of hydrolysis of Jerusalem artichoke juice

The kinetics of acid (phosphoric acid) and enzymatic hydrolysis of inulin from Jerusalem artichoke is shown in Fig. 1. The acid hydrolysis was faster, with 73 % hydrolysis in one hour compared to 19 % for the enzymatic one. After seven hours the degrees of hydrolysis were respectively 90 and 82 %. A complete degradation was attained in 48 hours for the enzymatic hydrolysis. By TLC the presence of sucrose and oligofructosides containing a reducing end was detected. After three hours of acid hydrolysis a compound with a migration faster than carbohydrates was detected, possibly of furfuralic nature, formed from fructose by the acidic medium, or a difructose anhydride (Zittan, 1981).

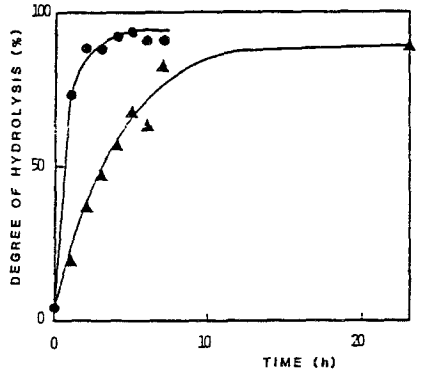

Fig.1 : Kinetic of hydrolysis of Jerusalem artichoke Juice. Acid hydrolysis (●),enzymatic hydrolysis (▲). Initial sugar concentration 130 g/l.

From these data it was concluded that the enzymatic hydrolysis presents advantages for the preparation of high fructose syrups compared to the acid hydrolysis : particularly absence of secondary reactions (Guiraud, 1981b). If the use of the hydrolyzed inulin is fermentation, only the comparison of the fermentation performances can give valuable informations.

2) Medium optimization from Jerusalem artichoke juice

The hydrolyzed juice (acid hydrolysis) was first fermented in erlenmeyers with Z. mobilis strain ZM4 with several nutrients addition .None of the additions : potassium phosphate (2 g/l), ammonium sulfate (1g/l), calcium pantothenate (5 mg/l), magnesium sulfate (0.5 g/l), yeast extract (0.05g/l)showed an effect on the growth rate (0.08-0.10 h^{-1}) or on the final ethanol concentration (35-39 g/l). Since Z.mobilis is auxotroph for calcium panthothenate (Belaich, 1965) it means that the juice probably contained this vitamin. Then, it can be concluded that the hydrolyzed Jerusalem artichoke juice can be used for ethanol production without any nutrient addition which is economically important. In fact, all the known media for this bacteria are supplemented with high amounts of yeast extract (Rogers, 1979,1982 ; Kosaric, 1982) or calcium pantothenate (Belaich, 1965 ; Toran-Diaz, 1983a).

3) Batch fermentation

Z.mobilis strain ZM4 was used to ferment Jerusalem artichoke juice hydrolyzed by different methods in fermentor with pH control. The results are shown in Table I. When the acid hydrolysis was used, an increase from one to three hours

of the hydrolysis time resulted in an inhibition of growth (growth rate, biomass yield, final biomass concentration and specific substrate uptake rate) and ethanol production (ethanol productivity and final ethanol concentration). These inhibitions were probably the result of by product formation during acid hydrolysis.

TABLE I Comparison of batch fermentations for ethanol production with acid or enzymatic hydrolysis of Jerusalem artichoke juice

Hydrolysis	Acid 1h	Acid 3h	Enzymatic 48 h
Initial sugar concentration (g/l)	100	100	100
Growth rate (h^{-1})	0.19	0.10	0.20
Biomass yield (g/g)	0.019	0.013	0.040
Ethanol yield (g/g)	0.44	0.51	0.45
Specific ethanol productivity (g/g.h)	4.8	3.9	2.5
Specific substrate uptake rate (g/g.h)	10.2	7.9	5.0
Final biomass concentration (g/l)	1.5	0.8	1.3
Final ethanol concentration (g/l)	41.2	31.4	38.0

When the enzymatic hydrolysis (48 hours) was used the growth rate, ethanol yield and final biomass and ethanol concentrations were similar to the ones found for an acid hydrolysis of one hour. However the specific substrate uptake rate and ethanol productivity were lower.

In these conditions, acid and enzymatic hydrolysis did not influence greatly the kinetic and yield parameters of ethanol production. The best results were observed for an acid hydrolysis of 1 hour or an enzymatic hydrolysis of 48 hours. The highest specific ethanol productivity found on hydrolyzed Jerusalem artichoke juice was 4.8 g/g.h. Higher values were reported on media containing glucose (Rogers, 1982) or fructose (Toran-Diaz, 1983b) which demonstrates that further improvements are possible. In contrast, the yeast Kluyveromyces marxianus showed a lower value of 1.7 g/g.h when cultivated on Jerusalem artichoke juice (Duvnjak, 1981).

4) Continuous fermentations

Table II shows the results of two separate experiments of continuous culture on Jerusalem artichoke juice which was acid or enzymatically hydrolyzed. In both cases the strain was ZM4F and the experimental conditions used have been

TABLE II Effect of the method of hydrolysis on ethanol production by Z.mobilis ZM4F in continuous culture

	Acid hydrolysis 1 h			Enzymatic hydrolysis 48 h	
Dilution rate (h^{-1})	0.1	0.21	0.5	0.53	0.82
Substrate concentration (g/l)	90	78	78	75	95
Ethanol concentration (g/l)	41.3	35.5	32.6	33.4	35.4
Effluent sugar (g/l)	10.8	10.9	10.9	0.7	16.1
Substrate conversion (%)	88	86	86	99	83
Ethanol yield (g/g)	0.51	0.51	0.49	0.45	0.45
Ethanol productivity (g/l.h)	4.1	7.5	16.2	17.7	29.0

previously described (Toran-Diaz, 1984). In all the experiments the sugars contained in the juice (at concentrations ranging from 75 to 95 g/l)were converted to ethanol with high yield (0.45 to 0.51). However the acid hydrolysis showed a lower sugar conversion. For instance at a dilution rate of 0.5 h^{-1} it was 86 % for the acid hydrolysis and 99 % for the enzymatic hydrolysis. Reducing the dilution rate to 0.21 or 0.10 h^{-1} did not increase significantly the conversion of substrate. Most probably some compounds were present in the acid hydrolyzed juice which cannot be utilized by Z.mobilis for growth. This is not the case for the enzymatic hydrolysis where all the sugars were transformed in ethanol. In the best condition, a volumetric productivity of 17.7 g/l.h was observed (99 % conversion, ethanol yield

96 % of theoretical). At a higher dilution rate (0.83 h^{-1})the productivity was further increased (29 g/l.h) but the percentage of conversion decreased to 83 %. Foaming problems in the fermentor were observed at high dilution rates which, because of the fermentor geometry, resulted in losses of biomass and lower performances.

By comparison the ethanol productivity obtained with the yeast Kluyveromyces marxianus (Margaritis, 1982b) was lower,showing clearly the advantage of using Z.mobilis. It should be reminded that much higher productivities were observed for the bacteria grown on media containing glucose (Lee, 1982) or fructose (Toran-Diaz, 1984). Then, progress can be done in fermentation of Jerusalem artichoke juice to approach the high values observed on defined media.

CONCLUSION

The results reported showed that Z.mobilis is able to ferment Jerusalem artichoke juice after hydrolysis without any addition of nutrient. Acid hydrolysis was faster than enzymatic hydrolysis but by products were formed which inhibited growth of the bacteria and resulted in low percentage of conversion of sugar in continuous culture. Then it appeared to us that the enzymatic hydrolysis should be retained. Further work on the continuous fermentation is in progress to increase the performance of the process.

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