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Industrial optimization of acetone-butanol fermentation: a study of the utilization of Jerusalem artichokes

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Summary. Acetone-butanol fermentation of the Jerusalem artichoke has been studied as a case for systematic investigation of the industrial optimization of both strain selection and fermentation operation. Hydrolysis of the inulinic oligofructans of the substrate was found necessary for optimal performance but could be achieved with a selected strain using a moderate amount of inulinase added at the beginning of the fermentation. Apart from ammonia, no nutritional supplementation of the medium was found necessary. The marked influence of pH in the fermentation performance prompted a detailed search for a method of controlling pH during fermentation. With an optimized procedure, solvent production of 23—24 g/l were obtained in 36 h. Detailed fermentation balances are presented. An industrial process for ABE production from Jerusalem artichoke or sugar beet has been defined and tested in the pilot plant.

Introduction

Butanol-acetone fermentation (ABE fermentation) has been industrially used in the past with two major substrates: grain and molasses (Gabriel 1928; Spivey 1978). Recently, the interest of this fermentation for the valorization of biomass has led to the evaluation of other potential feedstocks such as whey (Maddox 1980) and pentose sugars from lignocellulosic materials (Volesky and Szczesny 1983; Marchal et al. 1984). The Jerusalem artichoke is another possible carbohydrate source, and this plant has often been suggested to have important agricultural potential (Fleming and Groot Wassink 1979; Stauffer et al. 1981; Chabbert et al. 1983; Kosaric et al. 1984).

Although the carbohydrates stored in the tubers are mainly made up of short oligomeric fructans of inulinic structure (Fleming and Groot Wassink 1979; Guiraud et al. 1983; Heyraud et al. 1984) ABE fermentation of this substrate has been shown to require prior acid hydrolysis (Thaysen and Green 1927; Wenland 1937; Asai et al. 1941) as well as supplementation with corn or soy meals (Wendland et al. 1941; Reynolds and Werkman 1934).

As part of our research programme on the production of substitute fuels by ABE fermentation of biomass, we studied the utilization of Jerusalem artichokes as a case for a systematic investigation of the industrial optimization possibilities regarding both strain selection and fermentation operation. The results obtained on laboratory scale are presented here.

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Materials and methods

Microorganisms. Clostridium acetobutylicum NCIB 8052 (ATCC 824) and two selected strains, IFP 902 and IFP 904 were used. *C. acetobutylicum* IFP 904 was a butanol-resistant mutant derived from a strain isolated from Jerusalem artichoke tubers (Hermann et al. 1982). These strains were maintained at 4° C in anaerobic conditions.

Fermentation media. Jerusalem artichoke juices were prepared by pressing crushed tubers of the variety "Violet de Rennes" with a cider press. Diffusion juices were also prepared at the pilot plant using a technique adapted from that used in the sugar beet industry. These juices contained fructose and glucose in an approximate ratio of 4/1, as inulinic oligofructans and polyfructans. When necessary, these juices were totally hydrolysed. Enzymatic hydrolysis was continued, unless otherwise stated, for 5 h at pH 4.65 and 50° C with 600 units (u) per litre of fungic inulinase (β-1,2-fructan fructanohydrolase, E.C. 3.2.1.7.) kindly supplied by Novo Industries (inulinase Novo-

zym SP 230, 5000 u·ml^{-1}). One inulase unit corresponds to the production of 1 μmole fructose (or glucose) from a 2.5% (w/v) dalhia inulin solution in optimal conditions (pH 5, 50° C). In some cases, acid hydrolysis was performed using sulphuric acid at pH 2 and 100° C for 45 min. Average total sugar concentrations of the juices (expressed as indicated below in "Analytical Methods") were 150 g·l^{-1} for press juices and 110 g·l^{-1} for diffusion juices.

The maintenance medium contained non hydrolysed Jerusalem artichoke diluted to a final sugar concentration of 30 g·l^{-1} supplemented with 2.5 ml·l^{-1} of acetic acid and neutralized to pH 6.8 with ammonia. Two types of fermentation media were used, both based on hydrolysed or non-hydrolysed Jerusalem artichoke juice. The first, referred to as acetate medium, was prepared in the same way as the maintenance medium except that the final sugar concentration was adjusted to 65—70 g·l^{-1}. The second medium, known as carbonate medium, contained Jerusalem artichoke juice at the same final sugar concentration (65—70 g·l^{-1}) and was supplemented with 3 g·l^{-1} $(NH_4)_2SO_4$ and 5 g·l^{-1} $CaCO_3$.

Fermentation conditions. The cultures were started without heat shock. A tube containing 10 ml of inoculum on maintenance medium was transferred into a flask containing 200 ml of prereduced culture medium which was then kept overnight in an anaerobic jar. The culture was performed in a 6-l fermentor (Biolaffite, France) with a working volume of 4 liters. After sterilization, the fermentor headspace was flushed with nitrogen until the culture was started. During the fermentation the pH could be controlled according to various profiles by a Apple II microcomputer.

Analytical methods. Solvents, acids and sugars were determined on centrifuged culture samples. Gas chromatography was used for the determination of solvents according to the method of Barber et al. (1979) and of acids according to that of Marchal et al. (1984). Sugars were determined enzymatically as glucose using the method of Bergmeyer et al. (1974) and fructose with that of Bernt and Bergmeyer (1974) using the enzyme kit supplied by Boehringer-Mannheim. Total hydrolysis of the inulinic structures of the sugars prior to these determinations was performed when necessary for 30 min at 50° C and pH 4.65, with 7000 u Novo inulinase per g of sugars. Total sugar concentrations in Jerusalem artichoke juices or culture media are expressed as the sum of the fructose plus glucose determinations in these conditions.

Analyses of gases were performed in line of the fermentation using a volumetric counter (1 dm^3 Flonic gas counter) fitted with a recording device and two gas chromatographs (Model 30, Girdel France) equipped with 2-m Porapak Q columns, operated at 50° C with thermal conductivity detectors. Helium and argon were the carrier gases for the determination of CO_2 and H_2, respectively. The equipment for gas analysis was connected to an Apple II microcomputer. CO_2 and H_2 analyses were automatically performed for every liter of gas produced.

Results

Fermentation of chemically hydrolysed Jerusalem artichoke juice

Preliminary experiments were conducted with the reference strain *C. acetobutylicum* NCIB 8052 on chemically hydrolysed Jerusalem artichoke juice supplemented with calcium carbonate to limit acidification of the medium. In these conditions however, rapid acidification of the medium took place and, when metabolic activity ceased, the products obtained, were mainly acetic and butyric acids.

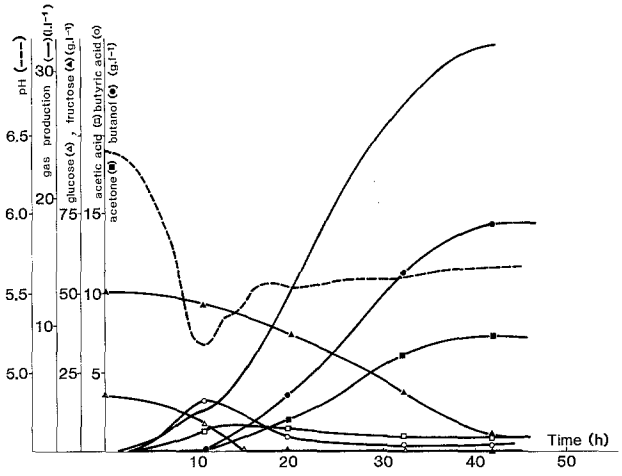

Fig. 1. ABE fermentation of Jerusalem artichoke press juice hydrolysed with acid. Conditions: strain 904 on carbonate medium. ----: pH; —: total gas volume; ●—●: butanol; ■—■: acetone; ○—○: butyric acid; □—□: acetic acid; △—△: glucose; ▲—▲: fructose

Table 1. ABE fermentation on carbonate and acetate media with strains 902 and 904. Acid-hydrolysed Jerusalem artichoke diffusion juice was used

	Strain 902		Strain 904	
Medium Performance[a]	Carbonate	Acetate	Carbonate	Acetate
Acetone (g·l^{-1})	7.2	7.6	7.2	7.1
Butanol (g·l^{-1})	13.5	13.1	13.8	13.8
Final acetic acid (g·l^{-1})	0.8	0.8	0.6	1.3
Final butyric acid (g·l^{-1})	0.4	0.2	0.7	0.3
Total gas (l·l^{-1})	31.0	28.0	31.5	29.0
Consumed sugars (g·l^{-1})	63	58	62	58.5
Fermentation time (h)	42	40	41	40
Solvent yield on sugars (% w/w))	32.8	35.7	33.8	35.7

[a] Mean of at least six fermentations

A number of strains capable of producing solvents in these fermentation conditions was obtained by systematic screening among collection and newly isolated strains (Hermann et al. 1982). Two of them, strain IFP 902 and 904 were selected for further study. As shown in Fig. 1 a normal fermentation pattern was obtained with strain 904 with production of acetic and butyric acids during the first 12 h and solvent production with reutilization of the acids after the "pH breakpoint". Similar results, summarized in Table 1, were obtained with strain 902. Supplementation of the medium with phosphate, magnesium, iron or yeast extract did not markedly improve the fermentation performances. Only small amounts of ethanol (<0.3 g·l^{-1}) were produced under all conditions tested and are not included in the results presented.

Development work conducted in fermentation volumes of 2000 l showed the disadvantages of using a medium with a high insoluble mineral load (calcium sulphate and calcium carbonate) to the process, especially at the sterilization and distillation stages. Calcium carbonate was then replaced by acetate. As shown in Table 1, similar solvent concentrations were obtained in the conditions used, although the yields on sugars were higher in the case of acetate medium because of partial utilization of acetate for solvent production.

Fermentation of enzymatically hydrolysed Jerusalem artichoke juice

Enzymatic hydrolysis of the juice by a commercial inulinase preparation was studied in order to replace chemical hydrolysis and further reduce the solid mineral load of the broth. Hydrolysis kinetics of Jerusalem artichoke juice is shown in Fig. 2. The fermentation performances in calcium carbonate or acetate media obtained with juice (60 g·l^{-1} total sugars) previously hydrolysed with inulinase as described in "Materials and methods", were similar to those obtained with acid-hydrolysed juice for both strains 902 and 904.

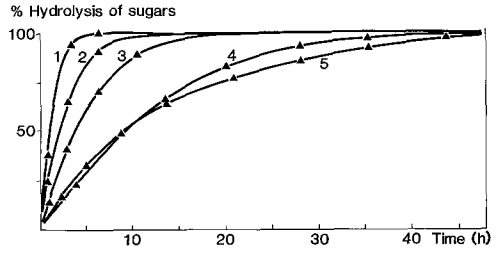

Fig. 2. Enzymatic hydrolysis of Jerusalem artichoke press juice. Novo inulinase (5000 u/ml enzyme) was used on Jerusalem artichoke at 50°C and pH 4.5. The total sugar concentrations of the juices and the amounts of enzymes per litre of juice were, respectively: *1:* 61 g·l^{-1}, 600 u·l^{-1}; *2:* 61 g·l^{-1}, 400 u·l^{-1}; *3:* 61 g·l^{-1}, 200 u·l^{-1}; *4:* 123 g·l^{-1}, 200 u·l^{-1}; *5:* 185 g·l^{-1}, 200 u·l^{-1}

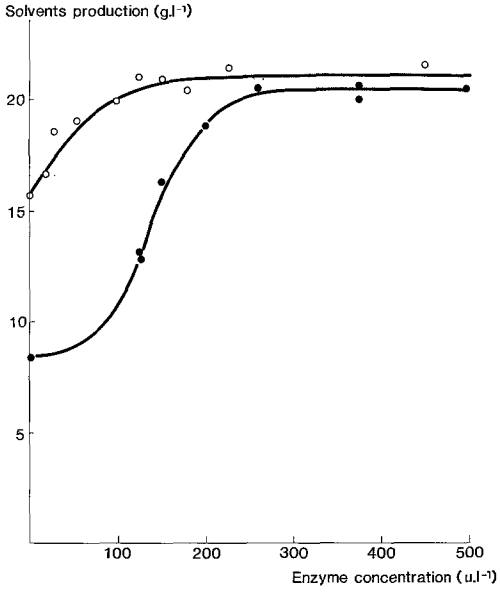

Fig. 3. Effect of the amount of inulinase added on final ABE production in simultaneous hydrolysis and fermentation. The acetate medium with non hydrolysed Jerusalem artichoke diffusion juice was used. O—O: with strain 904; ●—●: with strain 902

In order to simplify the process, conditions allowing simultaneous enzymatic hydrolysis and fermentation were defined, using the acetate medium. The minimal amount of enzyme that allows a performance approaching that of fermentation on previously hydrolysed medium was found about 125 u·ml^{-1} in the case of strain 904 and 250 u·ml^{-1} in the case of strain 902 (Fig. 3). As shown in Fig. 4, the concentrations of free fructose and glucose remained low in these conditions during the whole fermentation.

In the calcium carbonate medium the optimal enzyme concentrations were a little higher in the case of strain 904 but lower in the case of strain 902.

Simultaneous hydrolysis and fermentation with controlled pH

In order to avoid addition of acetic acid to the broth, fermentation tests with controlled pH were carried out. In a first approach, the pH was kept

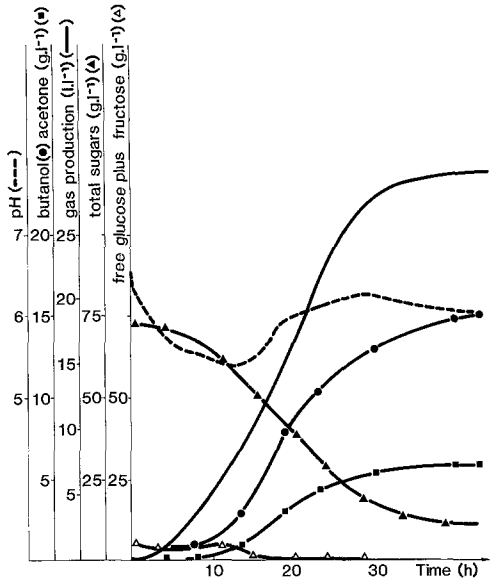

Fig. 4. Kinetics of simultaneous hydrolysis and ABE fermentation with an optimal amount of inulase added. Strain 904 was used on acetate medium with non hydrolysed Jerusalem artichoke press juice. Inulinase concentration: 125 u/l of culture. ----: pH; —: total gas volume; ▲—▲: total sugars; △—△: free glucose plus fructose; ●—●: butanol; ■—■: acetone

constant during the phase of acid production (acidogenesis). In this case, as shown in Table 2, lower pH values favoured solvent production but increased total fermentation time, whereas acid production was predominant at higher pH values. A compromise between these conflicting pH requirements was sought by testing various pH profiles allowing satisfactory growth during acidogenesis, while maintaining the conditions for satisfactory solvent production.

The first type of pH profile obtained was a programmed linear pH decrease, aimed at limiting acidification during acidogenesis by calculated addition of ammonia. Various pH gradients were tested. In this case, the programme of ammonia addition to produce the pH profile to be tested was started only after the pH had decreased to 6.1, a value adopted after optimization. Ammonia addition stopped as soon as the rate of acidification became less than that dictated by the programme; from this point on, the pH was thus uncontrolled and increased during solventogenesis.

Table 2. Simultaneous hydrolysis and fermentation of Jerusalem artichoke Juice with strain IFP 904 with pH regulation at various values (4 N ammonia was used for pH regulation)

Fermentation performance	pH value		
	5.25	5.50	6.30
Sugars consumed ($g \cdot l^{-1}$)	60	64.95	54.50
Acetone ($g \cdot l^{-1}$)	7.0	7.3	3.7
Butanol ($g \cdot l^{-1}$)	13.5	14.75	13.2
Acetate ($g \cdot l^{-1}$)	0.4	1.0	4.5
Butyrate ($g \cdot l^{-1}$)	0.3	1.2	2.0
Total gas ($l \cdot l^{-1}$)	27.1	33.4	24
Fermentation time (h)	48	33	34

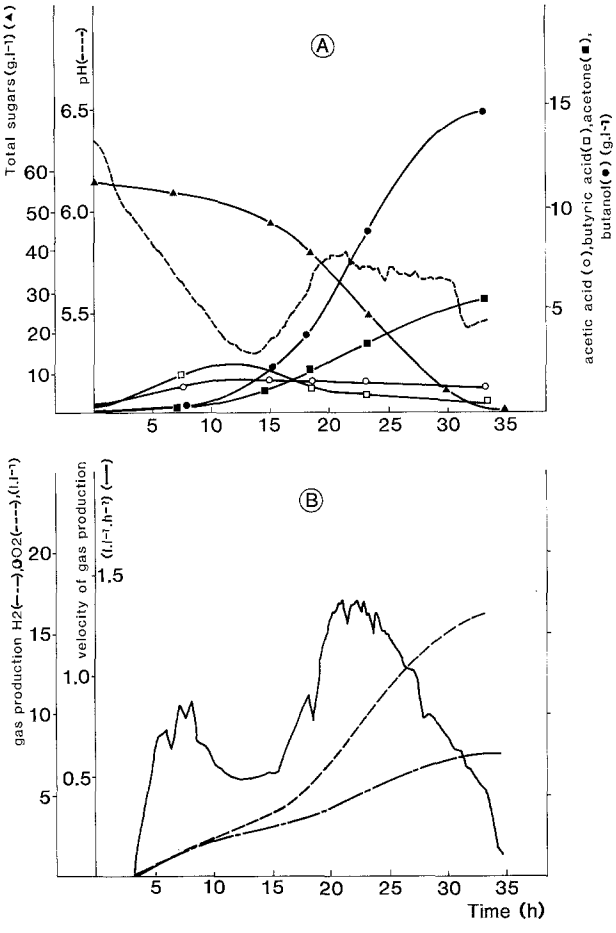

Fig. 5. Time course of ABE fermentation using an optimized linear decrease pH profile. The simultaneous hydrolysis and fermentation procedure was used on unbuffered medium containing non hydrolysed Jerusalem artichoke press juice. Strain 904 was used. Ammonia was added as described in "Materials and methods" to follow the pH profile selected. **A:** ----: pH; ▲—▲: total sugars; ●—●: butanol; ■—■: acetone; □—□: butyric acid; ○—○: acetic acid; **B:** ----: CO_2 volume produced; —·—: H_2 volume produced; ——: velocity of total gas production

The results obtained in optimized conditions with strain 904 are presented in Fig. 5; they show that the fermentation performance approached that obtained on acetate medium without pH control.

Comparable results were obtained with this procedure in the case of strain 902. The carbon balances obtained in these experiments are presented in Table 3. The fermentation on acetate medium differed in the somewhat lower solvent yield, the reason for which has already been mentioned.

A second type of pH profile which gave excellent results is illustrated in Fig. 6. In this case, the pH of the unbuffered broth was initially allowed to decrease by self-acidification, to a value of about 5. At this point, stepwise, addition of ammonia was used to bring back the pH to 6.5—6.7 and then again left free of control. As shown in Fig. 6, the results were clearly better than with all the other procedures, since solvent concentrations of 23—24 $g \cdot l^{-1}$ were obtained with strain 904. Excellent results were also obtained with strain 902.

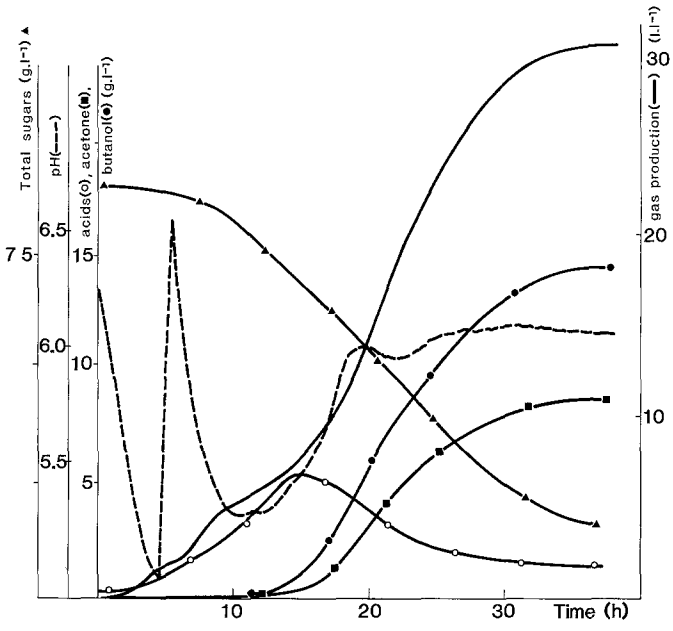

Fig. 6. Time course of ABE fermentation with a one-step pH readjustement profile. The conditions were as in Fig. 5 except that ammonia was added in one step as described in Materials and methods. ----: pH; —: total gas volume; ▲—▲: total sugars; ○—○: total acids; ●—●: butanol; ■—■: acetone

Discussion

The importance of pH in the performance of the ABE fermentation has been known for a long time, the standard industrial practice being to let the pH vary during the fermentation from a defined starting value after adjusting, when necessary, the buffering capacity of the medium. Acetate and calcium carbonate have been used for this purpose (Hastings 1971). Recent studies carried out at constant pH have shown that a pH value optimal for solvent production could be defined in these conditions, acid production being favoured at higher pH values (Bahl et al. 1982; Monot et al. 1983, 1984).

Results reported here show that a similar situation holds for various *C. acetobutylicum* strains, although the optimal pH value may be different: 5.5 for strains IFP 902 and 904 instead of 4.3—4.5 for strain ATCC 824 (Monot et al. 1983, 1984). The lower pH optimum of strain ATCC 824 (NCIB 8052) may be a reason for the preferential acid production of this strain from Jerusalem artichoke on calcium carbonate medium.

As conditions for good solvent production appear to involve both lower pH values (which are not optimal for growth) and the presence of butyric acid (Bahl et al. 1982; Monot et al. 1984) an attractive pH profile for ABE fermentation was to hold the pH during part of the growth stage at a favorable pH in the range of 6 to 6.5, then let it drop by self-acidification to obtain the conditions for optimal solvent production. With such a two-step pH profile, however, the results were less satisfactory than with the linear pH decrease described above. The best results were, however, obtained using the single pH readjustement profile. The reason for this improved performance is not

Table 3. Fermentation balance with linear pH decrease using strain 904. Simultaneous hydrolysis and fermentation was used

Substrate or products	Average value[a]
Total sugars ($g \cdot l^{-1}$)	58.4
Acetone ($g \cdot l^{-1}$)	6.20
Butanol ($g \cdot l^{-1}$)	13.60
CO_2 ($g \cdot l^{-1}$)	29.9
H_2 ($g \cdot l^{-1}$)	0.64
Acetate ($g \cdot l^{-1}$)	1.1
Butyrate ($g \cdot l^{-1}$)	0.6
Carbon balance (%)[b]	92.3

[a] Mean value of 4 fermentations

[b] The balance does not include the bacterial mass produced. The mean solvent yield with respect to sugars was 33.9% on a mass basis

clear but a somewhat related procedure has been mentioned as advantageous in older literature (Hastings 1971). The performance reported here is exceptionally high for ABE fermentation. Furthermore, the procedure requires only the addition of ammonia, which is also necessary as a nitrogen source and constitutes the only nutritional supplementation of the medium. Finally, ammonia consumption is minimal in this case: 0.60 g NH_3 per litre of culture medium compared to 1 g NH_3 per litre in the case of the fermentation without pH control on acetate buffer and 1.2 g NH_3 per litre for the fermentation, regulated at a constant pH of 5.5.

Finally, this pH profile was originally devised to obviate acetate supplementation of the medium. However, as already mentioned, because of partial conversion of acetate to solvents, higher solvent to sugar yields were obtained with acetate buffer. Preferential conversion of acetate to acetone has been reported (Martin et al. 1983) but too little acetate conversion took place in the experiments reported above to confirm this point. In other experiments, however, pH regulation with acetic acid during the phase of solvent production was found to be a good way to increase acetate utilization by providing a constant non-inhibitory concentration of the acid. In such an experimentation on acetate medium at pH 5.45, increased acetone production was indeed observed since final solvent concentrations of 11.8 $g \cdot l^{-1}$ butanol and 8.6 $g \cdot l^{-1}$ acetone were obtained.

Another point illustrated by the present study is the value of automatic data recording of gas production for establishment of fermentation balances and for following physiological changes during fermentation. In the absence of pH control, or in pH-driven fermentation (Fig. 5) two peaks in the rate of gas evolution were always observed, corresponding to maximal activities of acidogenesis and solventogenesis. The minimum rate of gas evolution observed after the first peak was found to be characteristic of the physiological change from acid to solvent production; this was shown also by changes in the percentages of H_2 and CO_2, the relative proportion of H_2 being higher during the phase of acid production.

As shown above, hydrolysis of the Jerusalem artichoke sugars is necessary for optimal fermentation performance. Strain 904 is a stable butanol-resistant mutant with an improved capacity for solvent production derived from a strain isolated from Jerusalem artichoke tubers (Hermann et al. 1982). With this strain the amount of enzyme required is lower than with strain 902. This indicates a limited inulolytic capacity of strain 904 and suggests that better inulolytic strains might be obtained, capable of optimal fermentation performances on non-hydrolysed Jerusalem artichoke juices without inulinase addition.

On the whole, the results presented above illustrate the importance of strain selection and optimization of fermentation conditions for optimal ABE production on a particular substrate. Further work at the pilot plant level allowed the determination of satisfactory conditions for a process of ABE fermentation from Jerusalem artichoke involving juice production by diffusion using an adaptation of the technique used in the sugar beet industry, with sterilization of the juice, fermentation and continuous distillation of the beers. This process which presents the important advantage over classical industrial ABE fermentation of minimizing energy consumption, has also been successfully tested for the ABE fermentation of sugar beet.

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