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EFFECTS OF ACETIC AND BUTYRIC ACIDS ON SOLVENTS PRODUCTION BY CLOSTRIDIUM ACETOBUTYLICUM

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SUMMARY

The fermentation of glucose by Clostridium acetobutylicum on a synthetic medium is carried out with a conversion of carbon source into solvents of 32 %. The ratio of butanol, acetone and ethanol products is approximately 0.6 - 1.9 - 6. The synthetic medium supplemented with acetic acid at a concentration of 2 g/l increases acetone formation and the ratio of products is approximately 0.5 - 3 - 6 with a conversion of glucose into solvents of 34 %. The addition of 2 g/l butyric acid permits obtaining a distribution of 0.8 - 2.4 - 6, with a conversion of 35 %.

INTRODUCTION

The solvent production by Clostridium acetobutylicum has been studied extensively, but generally studies on this sugar fermentation have always been made on complex or unknown media (Davies and Stephenson, 1941 ; Beesch, 1952 ; Taha et al., 1973 ; Prescott and Dunn, 1959).

Recently, work in this laboratory demonstrated that the glucose conversion into solvents could be obtained on a synthetic medium with a yield of 32 % (Monot et al., 1982). Such a medium is a valuable tool for studying the fermentation mechanism.

It is well known that during the batch-wise fermentation of glucose by Clostridium acetobutylicum, two phases can be distinguished (Peterson and Fred, 1932) ; the first fermentation phase is an acid phase with production of acetic and butyric acids ; during the second phase, solvents are produced concomitant with the conversion of substantial amounts of acetate and butyrate into acetone and butanol and further consumption of the carbon source. Here we describe the effect of the initial concentrations of acetate and butyrate on the final production of acetone and butanol in batch cultures of Clostridium acetobutylicum that was grown on a defined synthetic medium.

MATERIALS AND METHODS

- BACTERIA and CULTURE MAINTENANCE : Clostridium acetobutylicum ATCC 824 was maintained by growth on Reinforced Clostridia Media (Oxoid) at 35°C for 5 days followed by storage at 4°C. For inoculum preparation, this stored, culture was transferred to Reinforced Clostridia Media. After heat shocking at 80°C for 45 min the culture was incubated at 35°C under anaerobic conditions.

- MEDIA AND TEST CONDITIONS : The synthetic medium used had the following composition : glucose : 60 g/l ; KH_2PO_4 : 0.5 g/l ; K_2HPO_4, $3H_2O$: 0.5 g/l ; $MgSO_4$, $7H_2O$: 0.2 g/l ; $MnSO_4$, $1\ H_2O$: 0.01 g/l ; $FeSO_4$, $7H_2O$: 0.01 g/l ;

NaCl : 0.01 g/l ; NH_4Cl : 1.53 g/l ; p-amino-benzoïc acid : 1 mg/l ; biotine : 0.01 mg/l.

Experiments were carried out in a 2 l BIOLAFFITTE fermentor. The total volume of culture was 1.5 l ; the volume of the inoculum formed 10 % of the total volume.

The pH of the culture was maintained above 4.8 by means of an automatic titrator using two normal NaOH as the titrant.

Preliminary transfers were made after 24 hours of culture. The temperature, inside the fermentor, was adjusted and maintained at 32°C.

- METHODS OF ANALYSIS : Cell growth was estimated by the optical density at 600 nm.

The concentration of residual glucose was determined according to the method of Miller et al. (1960).

The solvent (ethanol, acetone, butanol) and acid (acetic and butyric) concentrations were determined by injecting acidified and centrifuged samples into a CARLO-ERBA fractovap G I chromatograph equipped with a flame ionization detector. The glass column was 2 m in length and packed with PORAPACK Q (100 - 120 mesh). The products analyses were carried out under the following conditions : column temperature, 190°C ; injector temperature, 210°C. The carrier gas is N_2.

The percentages of gas were estimated by injecting samples into an INTERSMATT IGC 120 MB chromatograph equipped with a katharometer detector. The glass column was 2 m in length and packed with CARBOSIEVE B (100 - 120 mesh). The gas analyses were carried out under the following conditions : column temperature, 98°C ; injector temperature, 132°C ; the carrier gas is Argon.

RESULTS

Doi and Sugama (1960) have shown that ammonium acetate, as nitrogen source, permitted good fermentation of glucose ; but these authors could not substitute ammonium chloride for ammonium acetate as nitrogen source.

We have shown a glucose conversion into solvents with a yield of 32 % on a synthetic medium and with ammonium acetate as nitrogen source. Unfortunately, to study the effects of acetic acid on solvents production by Clostridium acetobutylicum, ammonium chloride is preferable to ammonium acetate as nitrogen source to avoid the presence of acetic acid in the medium.

Figure 1 shows that a culture on a synthetic medium with NH_4Cl and with the pH of the culture maintained above 4.8 permits obtaining results analogous to fermentations in the presence of ammonium acetate.

Figure 1 A shows that 65 g of glucose are fermented, the growth is comparable with what we found for a growth on $CH_3 COONH_4$ in the medium. The fermentation started at pH 5.1 and was allowed to fall to pH 4.8 due to organic acid production. The pH was then controlled by the addition of NaOH on a demand basis. Correlated with the formation of solvents (fig. 1B) the pH increases to pH 5.4.

On figure 1B, we observe a fermentation with an acid phase and a solvent phase ; the distribution of gas is in favor of CO_2. This is a characteristic profile of an acetone-butanol fermentation by Clostridium acetobutylicum. Under these experimental conditions, we have studied the action of an addition of acetic or butyric acid, on the development of the fermentation.

The effects of acetic and butyric acids on solvents production are summarized in table 1.

The fermentation, shown on figure 1, is used as reference for this study.

The addition of 2 g/l acetic acid improves the acetone production ; with glucose as substrate the acetone yield is 22.5 % of total solvents, using both glucose and acetic acid the acetone yield is increased to 31.6 %. Some acetic acid is consumed and the yield of solvents derived directly from glucose is 34.1 %. The addition of a higher concentration of acetic acid gives lower results.

On the basis of the reference fermentation, the addition of 2 g/l butyric acid increases the production of all solvents. The glucose, conversion in solvents reaches 35 % but more butyric acid is used than acetic acid (2 g/l butyric acid but only 0.9 g/l acetic acid). The butyric acid becomes inhibitory for a concentration of 4 g/l acid.

The addition of acetic and butyric acids, at a suitable concentration (2 g/l), gives good results : the effects of each of these acids are present in this fermentation ; we obtain 24.7 g/l solvents with a ratio Butanol/Acetone of 2.

DISCUSSION

The acetone-butanol fermentations begin by a production of acetic and butyric acids ; there is a simultaneous increase in the number of cells. The utilization of the synthetic medium (Monot et al., 1982) has permitted us to investigate the role played by these acids in the final production of solvents.

The acetic acid increases the biosynthesis of acetone, since the distribution of solvents goes from 0.6 - 1.9 - 6, for ethanol, acetone and butanol respectively with glucose as substrate, to 0.5 - 3 - 6 with glucose and acetic acid.

The butyric acid increases the quantity of all solvents with a ratio of 0.8 - 2.4 - 6.

The addition of the two acids gives a good yield of solvents as percentage of fermented glucose (34.7 %) with a distribution similar to that obtained with addition of acetic acid (0.8 - 2.9 - 6).

It is well known that 15 g butanol is very toxic to the cells ; acetone is the least toxic substance to the clostridia ; Costa (9) specifies that concentrations of 29 g/l acetone do not cause any inhibition of growth. The decrease of the butanol/acetone ratio is then a possible way of improving the yields of solvents in acetone-butanol fermentations. Our work provest that the quantity of acids added plays a role in the evolution of these ratios.

The acid action can be explained by the fact that our fermentation pH is fixed at 4.8 : this is close to the pKa of the acids (acetic acid : pKa 4.76 ; butyric acid : pKa 4.82), thus the acids enter into the cells by passive diffusion (Kell et al., 1981).

The orientation of the metabolic pathways by the concentration of acids can to explained if we refer to the work carried out on the NADH-ferredoxin oxidoreductase (Martin et al., 1982) of which the activity is dependent on the concentration of acetyl CoA and thus of acetic acid.

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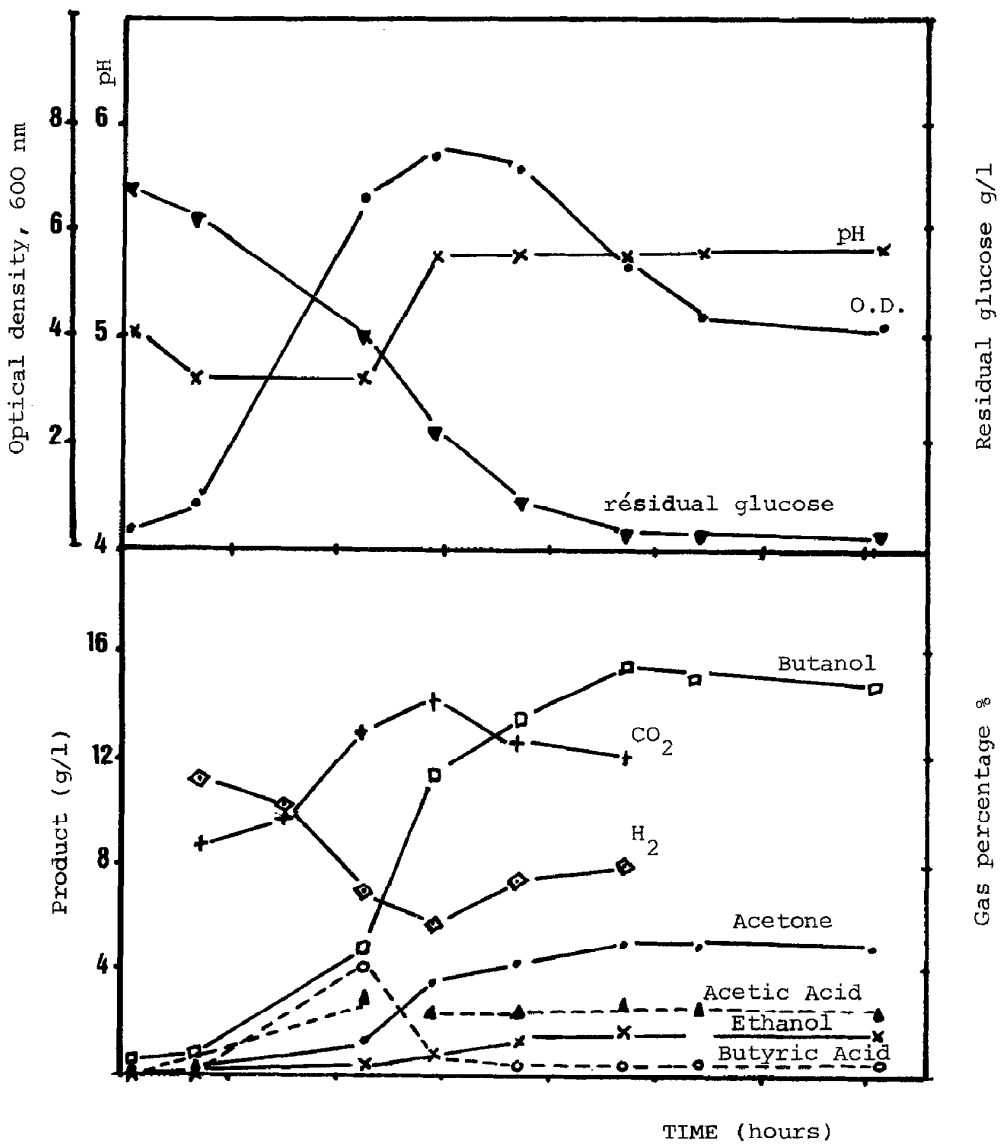

Figure I : Course of butanol fermentation using synthetic medium at pH above 4.8

ACIDS ADDED TO THE CULTURE MEDIUM	CONCENTRATION OF ACIDS ADDED (G/L)	CONCENTRATION OF ACIDS USED (G/L)	CONCENTRATION OF GLUCOSE FERMENTED (G/L)	CONCENTRATION OF TOTAL SOLVENTS (G/L)	DISTRIBUTION OF TOTAL SOLVENTS (G/L)			RATIO = BUTANOL / ACETONE	YIELDS OF SOLVENTS AS % GLUCOSE FERMENTED **
					BUTANOL	ACETONE	ETHANOL		
REFERENCE	-	-	65.8	21.3	15	4.8	1.5	3	32.4
ACETIC ACID	2	0.9	66.6	23.4	14.7	7.4	1.3	2	34.1
	2 + 5 *	-	46.3	12.4	7.6	4.2	0.6	-	-
	2 + 2 *	0.4	67.6	22.5	13.2	6.9	2.4	1.9	32.7
BUTYRIC ACID	2	2	62.7	23.4	15.3	6.1	2	2.5	35.1
	2 + 5 *	-	21.8	5.2	4	1	0.2	-	-
	2 + 2 *	0.9	57.6	20.7	14.5	5.3	0.9	2.8	34.9
ACETIC ACID + BUTYRIC ACID	2 + 2	0.2 + 2	66.6	24.7	15.2	7.4	2.1	2	34.7

TABLE 1 : DISTRIBUTION OF SOLVENTS AND YIELDS OF SOLVENTS AS % GLUCOSE FERMENTED AT THE END OF FERMENTATION.
2 G/L ACIDS WERE ADDED JUST AFTER THE INOCULATION AND 2* OR 5* G/L ACIDS WERE ADDED AFTER 24 HOURS OF CULTURE.

** SOME ACIDS WERE USED TO PRODUCE SOLVENTS, BY CLOSTRIDIUM ACETOBUTYLICUM, AND WERE DEDUCED TO OBTAIN SOLVENTS ISSUED FROM GLUCOSE FERMENTED.

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