Biotechnology Letters Vol 7 No 8 607-610 (1985)

INFLUENCE OF TEMPERATURE ON SOLVENTS PRODUCTION FROM WHEY

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SUMMARY

The influence of temperature on solvent production from whey was investigated by using strains of Clostridium acetobutylicum and butylicum. Higher yields of solvents were observed at 37°C or at 30°C depending on the strain used.

INTRODUCTION

The acetone butanol fermentation has recently attracted a particular interest owing to the increasing values of oil products.

As the economic feasibility of the molasses based fermentation process is decreasing because of the cost of raw material which represents 60% of the total cost of final products (Lenz and Moreira, 1980) other cheaper substrates are desirable. For this reason other raw materials have been recommended, like whey (Maddox, 1980), bagasse and rice straw (Soni et al. 1982) or apple pomace (Voget et al., 1985).

Cheese whey is an interesting substrate owing to its disposal problem, lactose content, and availability in many countries. As whey generally requires the addition of growth factors sources for increasing yields, yeast extract is commonly added (Maddox, 1980).

The temperature used for the acetone butanol fermentation is 30°C or 37°C, but it seems that the influence of this variable on the yields has not been investigated with different strains and substrates. As some observations made on the course of our experiments on butanol production from whey suggested that temperature may play a role in the process we decided to investigate its influence on the yields of solvents at 30°C and at 37°C employing whey media and using two strains of Clostridium acetobutylicum, and one of Clostridium butylicum.

MATERIALS AND METHODS

Microorganisms and inocula

Two strains of Clostridium acetobutylicum namely ATCC 4259 and NRRL B 596 and one of Clostridium butylicum, NRRL B 592, were used. The strains were kept in a tioglycolate medium (OXOID) at 4°C.

A spore suspension, used as inocula, was preparated as follows: 50 ml of potato medium (Walton and Martin, 1979) was inoculated with 2 ml of tioglycolate culture stock, heat shocked at 80°C for 20 min. and then incubated at 30°C for 7 days. All the strains sporulated well with this procedure and a spore count ranging between 2×10^8 to 6×10^8 spores/ml was obtained. The cultures which gave more than 2×10^8 spores/ml were diluted with potato

medium in order to have the same value. Prior to use all the strains were shocked at 80°C for 20 min.

Media

The media used for acetone-butanol production were as follows: 1, Unsupplemented whey, and 2, Whey supplemented with yeast extract, 5 g/l (w/v). The whey employed was one sample of powder sweet whey supplied by a cheese factory located near Brandsen, Bs.As., Argentina, which was rehydrated with distilled water in order to obtain a solution containing 4% (w/v) of lactose. In some cases fresh sweet whey was also tested but as no differences were found with powder whey in connexion with butanol-acetone yields, the latter was preferred.

The pH of the two media were adjusted to 6.8 with NaOH prior to sterilization which was carried out in an autoclave at 121°C for 20 min. The pH observed after sterilization ranged between 5.9 and 6.1 for both media.

Fermentation procedure

The fermentation experiments were carried out in small tubes (25 mm x 200 mm) containing 50 ml of the corresponding whey medium. After the sterilization 2.5 ml of spore suspension obtained in the potato medium were inoculated. The tubes were sparged with N_2 and stopped with a rubber stopper to which a double U shape tube containing sterile vaseline was adapted. This device avoids the entrance of oxygen and allow the exit of fermentation gases. The tubes were incubated at 30°C or at 37°C during 4 days, at which time gas production ceased.

Analysis

Butanol and acetone were determined at the end of the experiments in centrifuged samples, by gas chromatography as previously described (Voget et al., 1985). Lactose was evaluated by the method of Hyvarinen and Nikkila (1962) and spore count by means of a Petroff-Hausser chamber.

RESULTS AND DISCUSSION

Table I shows the results of solvents production by the three strains in whey media at 30°C and 37°C.

It is evident that for a particular strain the yields of solvents obtained in the whey media tested depend on the temperature used. Strain 4259 produced higher yields of butanol at 37°C either with unsupplemented or supplemented whey. As was to be expected the highest yields corresponded to the whey medium which contained yeast extract as growth factors source.

Strain B 592 on the contrary, produced higher yields at 30°C than at 37°C. The reason for this result may be related to differences in the growth requirements with temperature and its association with solvents production. Although nothing is known on this subject in connexion with Clostridium acetobutylicum strains, the influence of temperature on the vitamin requirements of some microorganisms like Pasteurella pestis or some strains

of yeasts, has been reported (Pirt, 1975). The maximum yields of the strain B 592, either at 30°C or at 37°C corresponded to whey supplemented with yeast extract.

Finally, strain 596 presented the most intriguing results, because with the supplemented whey medium gave lower yields than those obtained with the unsupplemented medium. One possible explanation for this behaviour may be due to the presence of some inhibitor in the yeast extract which could affect the growth of this strain. In this respect it is interesting to point out that for some strains of Rhizobium a similar effect was reported by using media supplemented with a concentration of yeast extract exceeding 4 g/l (Sherwood, 1972).

From the industrial point of view it is important to consider the process conversion yield of the products from the carbon and energy source. As has been reported (George & Chen, 1983) the yield of solvents by industrial strains of Clostridium acetobutylicum is about 30, calculated as the solvents produced versus the sugar metabolized (w/w). It can be seen that in those cases where higher concentration of solvents were attained the values of solvents yield observed for the strains used are around 30 depending on the temperature and media employed.

Although these are preliminary results, and further research is needed it is clear that temperature, and possibly also the concentration of growth factors source, may affect butanol-acetone production from whey.

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Acknowledgement

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and by the Secretaría de Ciencia y Tecnología (SECYT) of Argentina. The technical assistance of Mrs. S. Canneva and J. Argemí is gratefully acknowledged. We wish also to thank the Northern Regional Research Laboratory (Peoria, Illinois, U.S.A.) for the strains NRRL B 596 and B 592.

Table I. Influence of temperature on acetone-butanol fermentation of different strains grown in whey media.

Strains	4259				592				596			
Temperature (°C)	30		37		30		37		30		37	
Media	1	2	1	2	1	2	1	2	1	2	1	2
Butanol (g/l)	2.1	2.2	6.2	7.8	2.1	5.5	1.4	4.5	2.8	6.0	3.8	1.4
Acetone (g/l)	0.5	0.7	0.5	1.6	0.9	1.7	0.4	1.3	1.0	1.8	1.0	0.4
Solvents Yield*	0.12	0.16	0.27	0.27	0.24	0.32	0.22	0.27	0.40	0.35	0.32	0.20

*$Y = \frac{\text{Solvents produced}}{\text{Lactose metabolized}}$