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**ABSTRACT  
BOOK**

## Physiology of anaerobes

### BC-4/28

CONTINUOUS PRODUCTION OF ACETONE AND BUTANOL BY *Clostridium acetobutylicum* DSM 792 IN A MOLASSES MEDIUM. S. Dönmez, F. Özcelik, M. Balk and E. İc. Department of Food Science and Technology, Faculty of Agriculture, University of Ankara, 06110 ANKARA-TURKEY.

In recent years, much attention has been focused on continuous acetone-butanol-ethanol (ABE) fermentation. The use of continuous culture for investigating the physiology of ABE fermentation is well established mostly on glucose based mineral or complex media. Continuous ABE fermentation were carried out with *Clostridium acetobutylicum* DSM 792 in beet molasses media. Molasses medium without supplement gave approximately 3.5 g/L of solvents. At these conditions pH was between 4.8-4.9; optical density 1.2-2.0 with the dilution ratio of 0.015 h. Yeast extract addition increased total solvent production up to 7.9 g/L. pH was stable at 4.9 and cell density reached to 3.4 with the dilution ratio of 0.025 h. Phosphate was effective growth limiting factor for ABE fermentation. It was detected in a batch-wise fermentation. It was also detected in continuous system with the addition of 50 mg/L of  $\text{KH}_2\text{PO}_4$  to phosphate deficient molasses medium. It was showed that end product was butanol, no ethanol and acetone produced. Consequently at the phosphate deficient fermentation pH was stable between 4.4-4.6, OD 1.4 and butanol production ratio was 1.3 g/L at the dilution ratio of 0.016 h.

### BC-4/30

#### METHANOGENIC BACTERIA IN PADDY FIELD SOIL.

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Methane is one of the greenhouse gases, and paddy fields are reported to be an important source of atmospheric  $\text{CH}_4$ . But information is insufficient about ecology of methanogenic bacteria in paddy field soil. In this work, we isolated two methanogenic strains from a Japanese paddy field soil. The sampling site was the paddy field (Gray Lowland Soil) at Kyushu National Agricultural Experiment Station. Strain SA (=DSM 7056) was isolated from an enrichment culture on  $\text{H}_2\text{-CO}_2$  as a substrate inoculated with flooded soil taken from the plow layer. Cells of the strain were non-motile short rods and stained Gram-positive. The strain could use  $\text{H}_2\text{-CO}_2$  or formate as a methanogenic substrate. It required vitamins, but not acetate, for growth. Growth of and methane production by this organism were the fastest at 35-40 °C and pH 6.0-7.5 under the tested conditions. The mol % G+C content was 26.4. Strain SA showed more than 70% DNA relatedness to *Methanobrevibacter arbolophilus* DH1<sup>T</sup> (=DSM 1125<sup>T</sup>). On the basis of phenotypic and genotypic characteristics, we identified strain SA as *M. arbolophilus*. Strain TMA was isolated from an enrichment culture on trimethylamine as a substrate inoculated with the soil from the same field. Cells of the strain were pseudosarcina-like-shaped and stained Gram-positive. The strain could use methylamines, methanol,  $\text{H}_2\text{-CO}_2$ , and acetate. The optimum temperature and optimum pH for growth were 30-37 °C and 6.5-7.5, respectively. The mol % G+C content was 42.1. Strain TMA showed more than 90% DNA relatedness to *Methanosarcina mazei* S-6<sup>T</sup> (=DSM 2053<sup>T</sup>). On the basis of phenotypic and genotypic characteristics, we identified strain TMA as *M. mazei*. In the enrichment cultures on methanol and acetate, *Methanosarcina*-like cells were observed.

### BC-4/29

#### EVIDENCE OF A PROTON MOTIVE FORCE-ASSOCIATED GALACTOSE UPTAKE IN *CLOSTRIDIUM ACETOBUTYLICUM* P262

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In this laboratory, we are interested in the production of acetone-butanol-ethanol (ABE) from whey permeate, using the anaerobic bacterium *Clostridium acetobutylicum* P262. Unfortunately, lactose is a poor substrate for this process. Thus, prior hydrolysis of lactose to glucose and galactose is an attractive alternative. When present on their own, glucose and galactose are used by the organism at approximately equal rates. However, when mixtures of glucose and galactose are used, the organism suffers from some kind of catabolite repression. Fundamental studies on the mechanism of glucose and galactose uptake were carried out in order to understand the sugar uptake mechanisms and eventually overcome catabolite repression in this industrially important bacterium. Proton-conducting ionophores were used to demonstrate the involvement of a proton motive force in the transport of galactose. Both carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and *N,N*-dicyclohexylcarbodiimide (DCCD) were observed to inhibit galactose uptake but not that of glucose. Preliminary results showed that glucose is taken up by the phosphoenol pyruvate-phosphotransferase system.

### BC-4/31

#### EFFECT OF BIOGENIC AND ABIOTIC ORGANIC ACIDS ON THE GROWTH RATE OF *CLOSTRIDIUM ACETOBUTYLICUM* ATCC 824.

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Organic acids, such as acetic and butyric, play an important role in acetone-butanol fermentation, mainly because they contribute to the ionic equilibria of the medium and they have an inhibitory effect on growth. The exact mechanism by which acids inhibit bacterial growth is not completely understood. Thus, it seems interesting to compare the effects of biogenic (acetic and butyric) and abiotic (propionic, valeric and hexanoic) acids on the growth rate of *Clostridium acetobutylicum* ATCC 824.

The inhibitory effect of these acids was studied in batch cultures with uncontrolled pH. An ammonium salt of the corresponding acid was added to a synthetic medium with glucose as carbon source, growth being monitored by direct optical density measurements.

A threshold concentration ( $P_0$ ) had to be reached before growth inhibition occurred.  $P_0$  values ranged from 58 mM, for acetic acid, to less than 1 mM, for hexanoic acid. For higher values, the growth rate ( $\mu$ ) decreased with increasing acid concentration ( $P$ ), according to the linear relationship:  $\mu = \mu^* (1 - (P - P_0)/K_P)$ , where  $\mu^*$  is the growth rate without acid addition and  $K_P$  is the inhibition constant for the acid.

Values of  $K_P$  decreased from 0.46 M, for acetic acid, to less than 0.10 M, for hexanoic acid, which indicates that the inhibitory effect of the organic acids increases with the chain length and therefore with their hydrophobic nature. In addition, the natural logarithm of the inhibition constant and the natural logarithm of the threshold concentration were shown to be linear functions of the chain length of the acids.

It was also observed that  $K_P$  increases linearly with  $P_0$ , which seems to indicate that the same mechanism would be responsible for the growth inhibition of *Clostridium acetobutylicum* by biogenic and abiotic organic acids.