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Control of Acetic Acid Accumulation during Cultivation of Recombinant E. coli AT2471.

°Konstantin Konstantinov, Michimasa Kishimoto, Tatsuji Seki and Toshiomi Yoshida (International Center of Cooperative Research in Biotechnology, Japan, Osaka University, Yamada-oka, Suita-shi, Osaka 565)

During the fed-batch cultivation of recombinant E. coli AT2471 for phenylalanine production, much acetic acid was accumulated in the fermentation broth. This inhibited microbial growth and reduced efficiency. To prevent these negative consequences by control of the acetic acid concentration, the peculiarities of its accumulation were investigated. A teflon tubing sensor was used for on-line monitoring of the acetic acid concentration. We concluded that:

1. Acetic acid was accumulated only under conditions of oxygen limitation.
2. The rate of acetic acid accumulation was proportional to the level of oxygen limitation.
3. E. coli could metabolize glucose and acetic acid simultaneously.
4. When glucose and oxygen were in excess, accumulation of acetic acid caused by the Crabtree effect was not observed.
5. When the concentration of acetic acid was not high, a change from anaerobic to aerobic conditions resulted in its immediate consumption.
6. When acetic acid was left to accumulate up to high concentration, the reversal of anaerobic to aerobic conditions did not lead to its use, but the accumulation continued up to a very high final concentration.
7. The gas-chromatograph analysis proved the absence of any other volatile substances except acetic acid.

The correlation between the DO level and the acetic acid accumulation gives a possibility for the control of the acetic acid concentration by DO, which seems to be the simplest of known techniques.

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Histidine Fermentation by Brevibacterium flavum FERM 1564-II.

°Patoomporn Chim-anage, Yoichiro Shiba, Makoto Tsurumoto, Suteaki Shioya, Toshio Enatsu & Ken-ichi Suga.

Histidine production by Brevibacterium flavum FERM 1564 has been studied. It was previously reported that a high dissolved oxygen concentration and uracil were necessary for its growth and histidine production. Furthermore, in the absence of either acetate or glucose, the growth was frustrated.

It was presently understood that the microorganism needed acetate for the biosynthesis of the intermediates of citric acid cycle through glyoxylate shunt. Therefore, it is interesting to study on the optimum mole ratio of acetate to glucose (A/G) for histidine fermentation. The experiment was carried out by means of repeated exponential fed batch system using on-line control. At now stage, the A/G ratio of 2.3 was the best for cell and histidine production. An effect of specific growth rate on carbon dioxide production rate was investigated. The relationship tended to devide into 3 groups according to A/G ratio, ie the values of 2.3, higher and lower than 2.3 respectively. At the ratio of high acetate or glucose, the practical value of specific growth rate was lower than the expected dilution rate. This evidence might be due to the limitation of glucose or acetate in each case. By plotting various "overall" cell yield coefficients versus integral values of cell mass, the slope of the line indicated the maintaining constant. These values also appeared to be 3 groups, however the cell yield coefficient of 0.88 g cell/g carbon was obtained for every A/G ratio.

(Department of fermentation technology, Osaka University, yamada-oka, Suita-shi, Osaka 565).