RAPID DETERMINATION OF GLUTAMIC ACID BASED ON IMMOBILIZED *ESCHERICHIA COLI* AND A QUADRUPOLE MASS SPECTROMETER

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Large quantities of glutamic acid are produced by the fermentation process so that rapid determination of glutamic acid is important for the control of the fermentation. Previously, we reported a microbial sensor consisting of immobilized *Escherichia coli* containing glutamte decarboxylase activity and a carbon dioxide gas-sensing electrode(1). The sensor has a major disadvantage of slow speed, 10 samples per hour, due to slow response of the gas-sensing electrode. We require more rapid sensors to deal with many samples from the fermentation laboratories. In stead of the gas-sensing electrode, a quadrupole mass spectrometer was employed to measure carbon dioxide gas produced from glutamic acid by the immobilized *E.coli*(2). The method has very high speed, more than 100 samples per hour, and has been applied to the assay of glutamic acid in the fermentation broths.

MATERIALS AND METHODS

Freeze-dried cells of *E.coli* (ATCC 8739) were prepared according to the procedure described previously. The probe containing immobilized E.coli is illustrated in Fig.1. A nylon mesh coated the cells of *E. coli* paste was placed on the surface of the carbon dioxide permeable membrane (silicone) and covered with a cellophane membrane to entrap the microorganisms between the two membranes. The probe was inserted to a flow cell and the gas conduit of the probe was connected to the inlet of the quadrupole mass spectrometer (MSQ-150A, Nippon Shinkuh). The temperature around the probe was maintained at 30°C and a buffer solution (pH 4.40) was transferred through the flow cell at the flow rate of 2 ml/min together with 500ml/min of nitrogen gas. A sample solution was pumped into the flow system at the flow rate of 0.8 ml/min for a certain period.

RESULTS AND DISCUSSION

When a sample solution containing glutamic acid was introduced into the flow cell, glutamic acid diffused into the the layer of E. coli and was metabolized to produce carbon dioxide by glutamate decalboxylase. The reaction was carried out at pH 4.4 which was sufficiently below the pK value of carbon dioxide, it permeated through the gas permeable membrane of the probe and was detected by the mass spectrometer. As a result, the output current of the detector increased with time and became constant which was corresponded to the concentration of glutamic acid in the sample. The time required to obtain 90% of the current response was 10 sec. When the pure buffer flow returned to the flow cell, the output current, decreased to the initial value. The measurements were carried out with a shorter introduction period for example 8 sec as shown in Fig. 2. In this case, the calibration plot of the output current vs. glutamte concentration were linear in the range 1-10,000 mg/l and 120 samples could be assayed per hour. When a sample solution was measured in replicate, standard deviations of the current responses were about 1% for several experiments. The selectivity of the response and the reusability of the probe(more than 2 weeks) were excellent similarly to the microbial sensor reported(1). The results obtained with the method and by the Auto-analyzer for the fermentation broths were in good agreement(r=0.99).

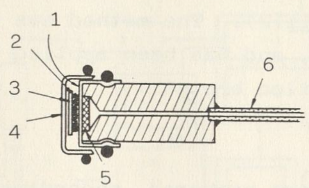

Fig.1. Structure of the probe: 1.silicone membrane;2.microbial cells;3.nylon mesh;4.cellophane membrane;5.porous metal;6.gas conduit.

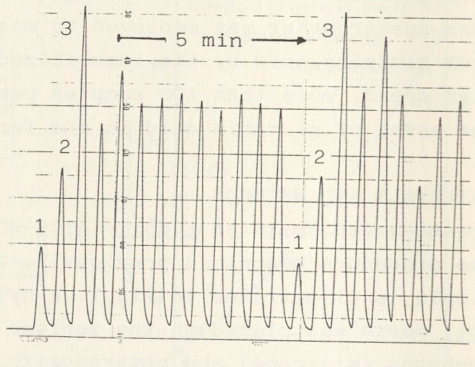

Fig.2. Response curve of the system: 1.62.5mg/l;2.125mg/l;3.250mg/l of glutamic acid standard solution.

REFERENCES

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