Monitoring of Ethanol in Production of Baker's Yeast Using an Improved Membrane Gas Sensor *

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Previous work has shown the usefulness of membrane tubing methods for monitoring ethanol production in yeast fermentations.

The methods are based on the fact that the volatile ethanol can be separated from the fermentation broth by a gas permeable membrane and thereafter detected by a flame ionization detector[1] or a semiconductor gas detector.[2] The hydrophobic properties of porous Teflon and porous silicon have made these materials especially suitable, not only because of their permeability, but also due to their low degree of compatability with yeast cells.

Puhar *et al.*[2] have, among others, investigated the influence of factors such as aeration, impeller speed, carbon dioxide and oxygen concentration on the performance of the sensor. They found that only oxygen severely disturbs the detector response when nitrogen is used as carrier gas to the semiconductor.

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Data presented here show how this drawback can be considerably reduced by using a dilution system incorporated into the sensor system. Now the ethanol samples can be continuously diluted between 2 to 1000 times before the detector analysis, thereby diluting oxygen far below harmful concentration levels. The complete detector system was from the prototype series manufactured by a local workshop. A comprehensive description of two dilution system designs is given elsewhere.[3]

A recent report[4] has described the use of a dilution system in combination with a semiconductor detector for measuring ethanol levels in an anaerobic fermentation using immobilized yeast. The fermentation broth was diluted before reaching the permeable membrane. We have chosen to use the gas dilution method to monitor ethanol production in an aerobic fermentation of baker's yeast, and to demonstrate the sensor's capability for continuously following the variation in ethanol concentrations.

The yeast used was *Saccharomyces cerevisiæ*, a gift from Svenska Jästbolaget, Rotebro, Sweden. The yeast was cultivated in YM Broth (Difco Laboratories, USA) supplemented with 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.4 g/l KH_2PO_4 and 0.6 g/l $Na_2HPO_4 \cdot 2H_2O$.

The glucose concentration was 10 g/l. The inoculum was cultivated in the above medium for 20 h on a rotary shaker. Batch cultivation was performed in a fermentor (Chemoferm AB, Hägersten, Sweden) with a working volume of 2 l. Temperature and pH were controlled at 30 °C and 5.5 respectively; 0.5 M NaOH was used to keep pH constant. Foaming was controlled by adding, when needed, the antifoam Adekanol

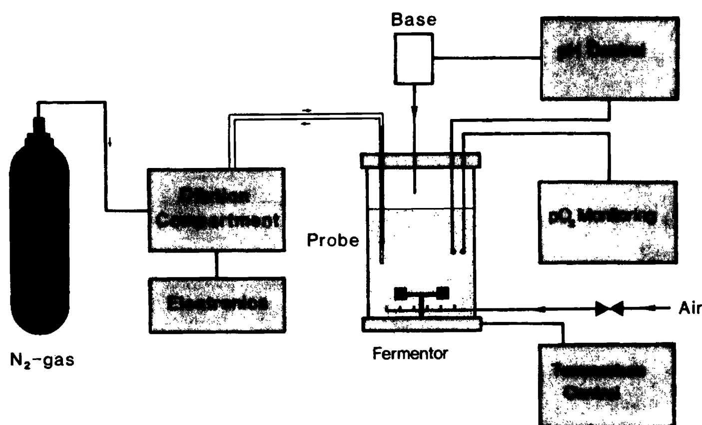

Fig. 1. Experimental set-up of the fermentor with the silicone tubing sensor and the equipment for temperature control, pH control and pO_2 monitoring.

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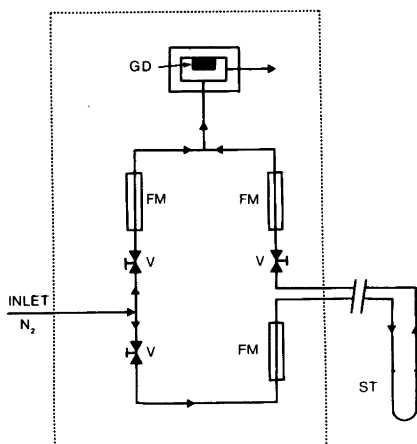

Fig. 2. Experimental set-up of the dilution compartment showing the gas detector in its insulated chamber (G.D.), precision valves (V), flow meters (F.M.) and the silicone tubing (S.T.).

LG-109 (Asahi Electro Chemical Co., Japan). The medium in the fermentor was inoculated with 5 % (v/v) inoculum. The level of dissolved oxygen was measured continuously with a galvanic oxygen electrode.[5] Aeration rate was 1.0 volume/volume min. Stirrer speed was set at 300 rpm.

The fermentor was also equipped with an ethanol probe (Fig. 1). This consisted of a stainless steel bar designed to fit the fermentor connections and suitable for autoclaving. The membrane tubing was mounted on this probe and was a 45 mm long and 0.2 mm thick silicone tubing connected to the continuous dilution system, which was connected to a SnO_2-semiconductor gas detector (Figaro 812, Figaro Eng. Inc. Japan) (Fig. 2).

Due to chemisorption of the gas onto its surface, the semiconductor underwent changes in its conductivity. Since the semiconductor is only able to detect small concentrations of ethanol (500–5000 ppm), above which it becomes saturated, dilution of the gas is quite favourable. By setting the dilution degree to 10 times, the detector gave a linear characteristic up to 20 g/l, whereby the signal response was 0–10 V. To investigate the performance and ability of the sensor for continuously and accurately reflecting the ethanol concentration, it was tested in a 26 h batch fermentation.

Fig. 3 shows the time course of the cultivation and the different parameters which were followed. The ethanol and glucose concentrations, as well as the optical cell density at 620 nm, were measured each hour. After sterile filtration (0.45 μm, Millipore), the glucose samples were analysed enzymatically, (glucose kit, Boehringer-Mannheim, FRG) and the ethanol samples were analysed in a GLC (Varian).

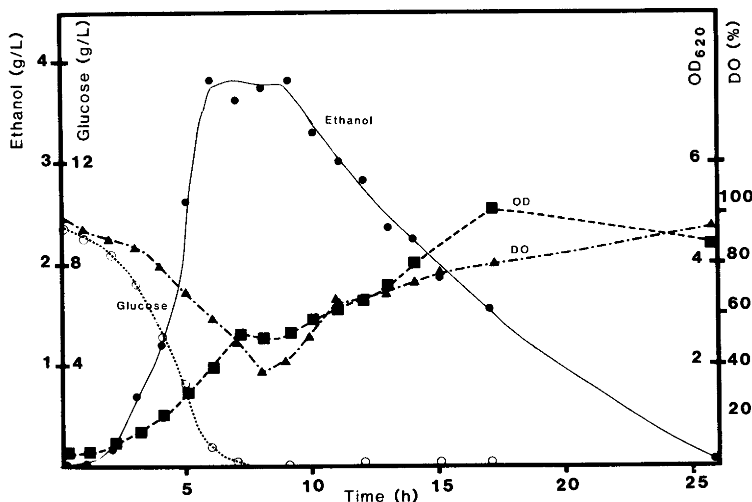

Fig. 3. Fermentation kinetics of ethanol production using baker's yeast, showing: glucose concentration (○); ethanol concentration continuously recorded with the sensor (–) or measured with GLC (●); optical density at 620 nm (■) and dissolved oxygen (▲).

The time course in Fig. 3 clearly shows the production of cell mass at the expense of glucose, but at the same time yielding ethanol concentrations up to 3.5 g/l. This ethanol can later serve as a second substrate when the glucose has been completely consumed after 9 h.

However, the ethanol consumption rate is lower, resulting in a lower growth. The diauxic effect also indicates the change of substrate. After 26 h the ethanol is completely consumed and the final cell mass reaches an optical density of 4.3. The dry weight was estimated to be 4.8 g/l, giving a total cell yield of 0.48 g dry weight/g glucose. This is in agreement with other reported results.[6]

The oxygen consumption during the first 8 h parallels that of glucose, and thereafter shows a slight increase as the ethanol is utilized more slowly.

The continuous ethanol signal agrees with the off-line performed GLC-analyses. The deviations are small and negligible. The changing oxygen concentration in the medium does not influence the sensor signal, nor does the cell density seem to cause fouling of the silicone membrane, which otherwise would have hampered the transfer of ethanol.

The results above show the advantages of using this type of ethanol sensor. Besides, being unaffected by several disturbances in the environment, this method of ethanol detection is also easy, inexpensive to operate, and has few maintenance requirements.

The ethanol sensor thus seems to be a suitable tool for continuous monitoring and later also control processes involving continuous production of ethanol, as is the case in ethanol fermentation[4] and production of baker's yeast.[7]

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