SHORT COMMUNICATION

UTILIZATION OF SOME AGROWASTES FOR ALCOHOL PRODUCTION

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RINGKASAN : *Saccharomyces cerevisiae var. ellipsoideus dan Kluyveromyces fragilis masing-masing menghasilkan 59.8 dan 69.0* g l^{-1} *ethanol semasa proses fermentasi selenjar di dalam bahan buangan koko pada suhu 30°C. Penghasilan maksima etanol kedua-dua spesi diatas berlaku pada kepekatan biojisim sel 2.7 dan 2.9* g l^{-1} *masing-masing. S. cerevisiae (jenis liar) pula, di dalam jus nenas dan keadaan penumbuhan yang serupa menghasilkan etanol maksimanya, 70.0* g l^{-1} *pada kepekatan biojisim sel 3.9* g l^{-1}.

ABSTRACT : *Saccharomyces cerevisiae var. ellipsoideus* and *Kluyveromyces fragilis* produced 59.8 and 69.0 g l^{-1} ethanol respectively when grown in cocoa waste at 30°C by continuous fermentation. Maximum ethanol production by the species was achieved at cell biomass concentrations of 2.7 g l^{-1} and 2.9 g l^{-1} respectively. *S. cerevisiae* (wild strain) grown under similar conditions in pineapple juice produced 70.0 g l^{-1} of ethanol at a cell biomass concentration of 3.9 g l^{-1}.

KEYWORDS : Micro-organisms, utilization, agrowastes, biofuel production.

INTRODUCTION

Large amounts of waste materials are produced by agro-based industries in Malaysia mainly from the rubber, oil palm, cocoa and pineapple crops. These wastes cause environmental problems if they are not properly disposed. Therefore, it is desirable to treat the wastes for conversion into useful products such as fertiliser, feed and fuel. Fruit wastes, for instance, have been used in ethanol fermentation (Cooper; 1976). Organic wastes and agricultural residues are usually chemically and physically heterogenous (Playne and Smith, 1982), and therefore their conversion to industrially important chemicals and fuels by micro-organisms with good yields is complex. On the other hand, some industries may not necessarily require pure chemicals like ethanol, and a mixture of a family of chemical compounds may be quite acceptable.

Studies on alcohol fermentation using fruit juices such as sugarcane and grapes as raw materials have been carried out by many researchers (Maldonado *et al.,* 1975; Marchal, 1978). *Zymomonas mobilis* has been shown to ferment high concentrations of glucose rapidly to ethanol with high specific glucose uptake and ethanol production rates (Rogers *et al.*, 1979; Lee *et al.*, 1979). In this study, three different species of micro-organisms were grown under similar conditions of continuous fermentation for optimising alcohol production using cocoa and pineapple waste products.

MATERIALS AND METHODS

Substrate

Cocoa juice, a waste product of cocoa beans was obtained from Flemington Estate, Telok Intan, Perak, and kept in ice during transportation. Pineapple juice was extracted from 40 kg of pineapple skin peelings by homogenising in a Rx100 National blender. The homogenate was filtered using a cheese cloth and the 15 litre sample was autoclaved at 121°C for 30 min.

Micro-organism and Fermentation Procedure

Cultures of *S. cerevisiae var. ellipsoideus, K. fragilis* and *S. cerevisiae* (wild strain) were grown separately at a stirring rate of 300 rev min^{-1} in a 350 ml continuous fermentor (Chemostat Bio-Flo Model C). Each culture was first grown in batches to a predetermined optical density and then allowed to grow continuously at various dilution rates. All cultures were grown at 30°C and were maintained at steady state (constant cells optical density) for at least 48 h before changing to another dilution rate.

Sample Processing and Analytical Methods

In each case, samples from the fermentor were taken in replicates at the various dilution rates to determine the purity of the culture (by microscopic examination), biomass and alcohol concentration.

The culture samples were centrifuged at 10,000 xg for 10 min at 4°C in a (Damon IEC Centrifuge, Model B-20, USA). The supernatant obtained was kept at -20°C for later analysis of alcohol concentration, whilst the pellets were washed twice with distilled water and recentrifuged in the above manner. The cells were then dried at 105°C until a constant weight for the determination of biomass. For alcohol determination, the supernatant collected earlier was filtered through a millipore filter (0.45μm, pore size membrane filter) and 5μl samples were injected in replicates into a gas chromatogram (GC); glass column (25 cm x 4.5 mm) containing 0.2% carbowax in 80/100 carbopack. Temperature of the oven GC Model 5840-A (Hewlett Packard Co., USA), was programmed at 135°C isothermally while the gas carrier, nitrogen, was maintained at a flow rate of 25 ml min^{-1}, hydrogen and air flow rates were at 40 ml min^{-1} and 10 ml min^{-1} respectively. The GC was operated with a flame ionization detector. The quantities of alcohol were determined against known amounts of the respective standards prepared from a standard kit (PolyScience Corporation, Illinois, USA).

RESULTS AND DISCUSSION

Figures 1 and 2 show ethanol production increasing with dilution rates. Maximum ethanol production by *S. cerevisiae var. ellipsoideus, K. fragilis* and *S. cerevisiae* (wild strain) were 59.8, 69.7 and 70.0 g l^{-1} at cell concentrations of 2.7, 2.9 and 3.9 g l^{-1} respectively. Aiba *et al.* (1968) showed similar results for the growth of *S. cerevisiae* in 10% glucose medium where ethanol production also increased with dilution rate. The activity of the enzyme, alcohol dehydogenase, has been reported to increase in the presence of alcohol (Paca *et al.*, 1983). The possibility of the enzyme stimulating

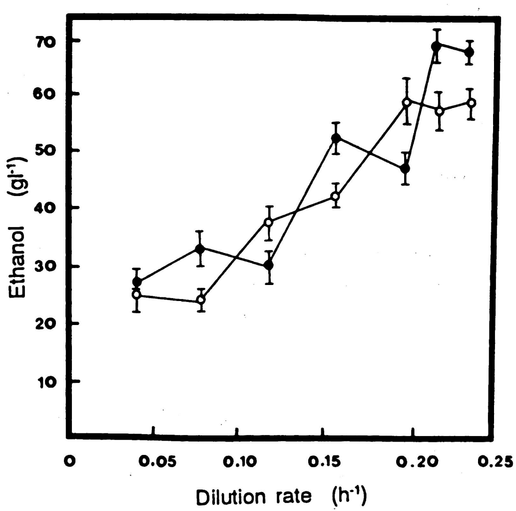

Figure 1. *Effect of dilution rates on ethanol production in continuous fermentation using cocoa waste*
(●) *K. fragilis*
(o) *S. cerevisiae var ellipsoideus*

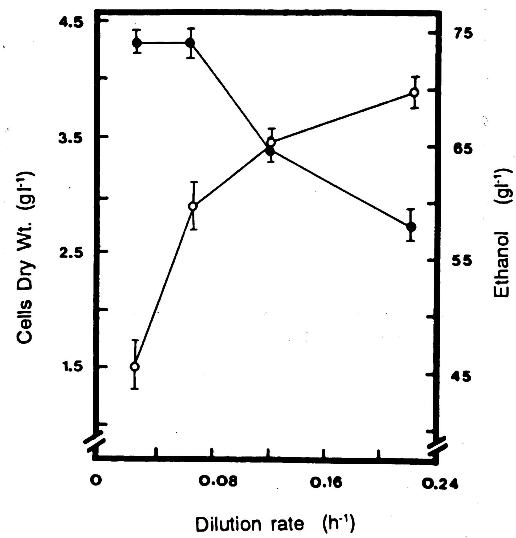

Figure 2. *Effect of dilution rates on biomass concentration and ethanol production in continuous fermentation using pineapple waste by S. cerevisiae (wild strain)*
(●) *biomass*
(o) *ethanol*

alcohol production and influencing the quantity produced has been suggested. Lee and Rogers (1983) reported that substrate inhibition effects on ethanol production were small and therefore could be neglected.

On the other hand, the production patterns of methanol and 1-propanol using cocoa waste were different. Both *K. fragilis* and *S. cerevisiae var. ellipsoideus* demonstrated a gradual but not very significant increase in methanol until a dilution rate of about 0.20 h^{-1}, after which its production decreased (Figure 3). Both species however, exhibited a marked difference in the production of 1-propanol (Figure 4). A two-fold increase in 1-propanol production by *K. fragilis* was observed as dilution rates were increased from 0.08 to 0.20 h^{-1}. *S. cerevisiae var. ellipsoideus* produced 1-propanol at much lower quantities compared to the former.

Figure 5 shows the biomass profile of *K. fragilis* and *S.cerevisiae var. ellipsoideus* in continuous fermentation. A gradual increase in biomass concentration with dilution rates was observed and was seen to be parallel to the increase in ethanol production and, to a smaller extent, to methanol production. Besides ethanol, methanol and 1-propanol, other forms of alcohols were also produced in smaller quantities. It is suggested that the alcohol composition produced from the waste materials by these species is suitable for consumption as beverage.

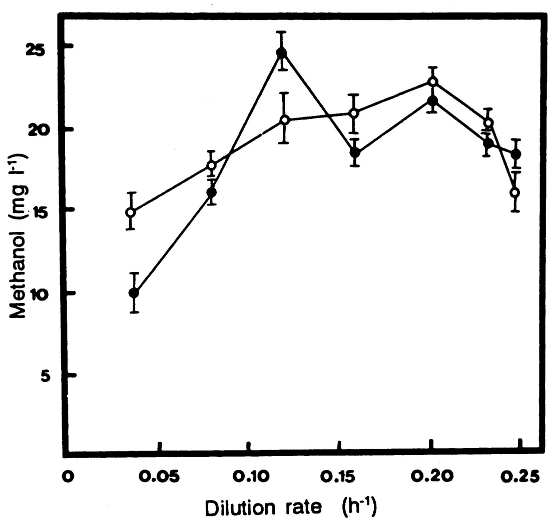

Figure 3. *Effect of dilution rates on methanol production in continuous fermentation using cocoa waste*
(●) *K. fragilis*
(o) *S. cerevisiae var. ellipsoideus*

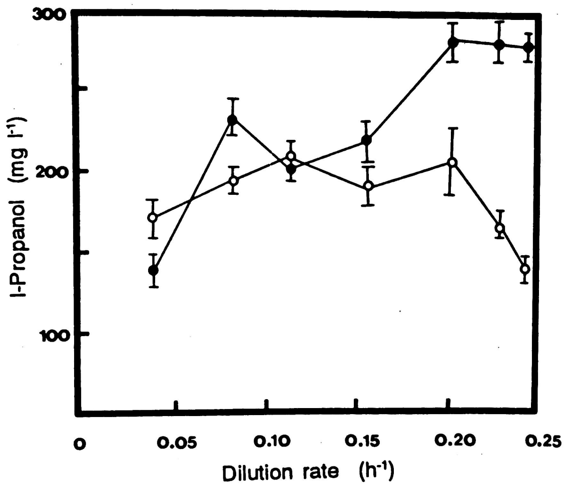

Figure 4. *Effect of dilution rates on 1-propanol production in continuous fermentation using cocoa waste*
(●) *K. fragilis*
(o) *S. cerevisiae var. ellipsoideus*

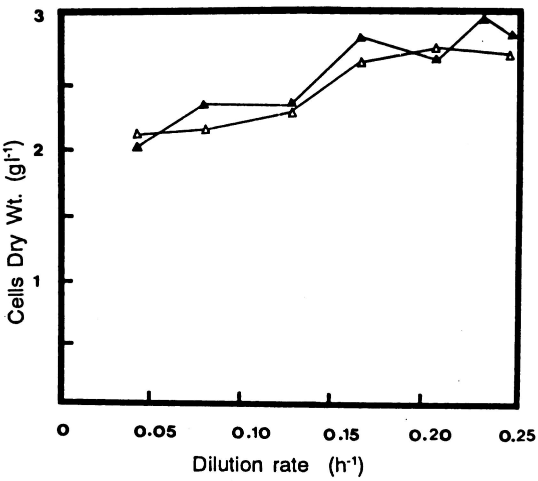

Figure 5. *Biomass profile in continuous fermentation using cocoa waste at various dilution rates*
(▲) *K. fragilis*
(△) *S. cerevisiae var. ellipsoideus. Each point represents the average of two readings.*

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