Enzymes Co-immobilized with Microorganisms for the Microbial Conversion of Non-metabolizable Substrates *

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Complex substrates can be fermented in mixed cultures as has been reported for the conversion of starch to microbial protein.[1] *Endomycopsis fibuliger* hydrolyses starch to maltose and glucose, which products are fermented by *Candida utilis* to produce cell mass. *Candida utilis* cannot by itself metabolize starch.

The present communication describes a similar approach. An enzyme and a microorganism, *Saccharomyces cerevisiae*, were co-immobilized in calcium alginate,[2] with the concept that this design will allow the enzyme to hydrolyze a substrate which the microorganism cannot metabolize. The hydrolysis product, however, provides a metabolizable substrate for the organism. This design offers several advantages: in cases where the enzyme is product-inhibited co-immobilization with a microorganism prevents inhibitory concentrations of the intermediate to accumulate; immobilization of enzyme and microorganism allows the process to be run continuously preventing inhibitory concentrations of the end product to accumulate; immobilization as such facilitates recovery as well as efficient utilization of the biocatalysts.

The use of an enzyme co-immobilized with a microorganism will be discussed with respect to two substrates: Cellobiose and whey permeate. *Saccharomycses cerevisiae*, which ferments neither cellobiose nor lactose, was co-immobilized with the enzymes β-glucosidase (β-D-glucoside glucohydrolase, E.C. 3.2.1.21) and lactase (β-D-galactoside galactohydrolase, E.C. 3.2.1.23), respectively.

Besides glucose, the major product in an enzymatic hydrolysis of cellulose is cellobiose.[3,4] β-Glucosidase, the enzyme that hydrolyses cellobiose to glucose, is subject to product inhibition.[5] β-Glucosidase from sweet almonds was co-immobilized with Bakers yeast, *Saccharomyces cerevisiae*, in calcium alginate. The alginate gel

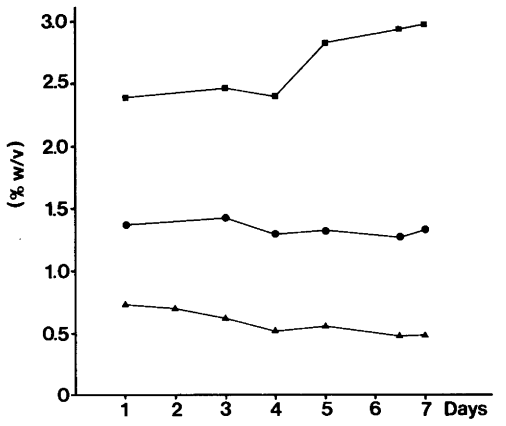

Fig. 1. Conversion of 5 % cellobiose (0.1 M acetate buffer, pH = 4.8, 0.01 M Ca (II)) in a co-immobilized β-glucosidase-yeast column at 22 °C. Concentration of ethanol (▲), glucose (●) and cellobiose (■) in eluate. Alginate beads (5.25 g wet weight) in a 7.5 ml column. Flow rate 2.3 ml/h. 8 U β-glucosidase and 65 mg yeast cells/g beads.

containing enzyme and yeast cells was obtained in the shape of beads, 2 mm in diameter. These beads were filled in a column and the substrate, 5 % cellobiose, was continuously pumped through the column. Fig. 1 shows the ethanol production at 22 °C in such a column. The remaining cellobiose and glucose concentrations, measured with the enzyme thermistor,[6] are also shown in the diagram. During the first three days, ethanol and glucose concentrations are somewhat higher than the steady state level which is then reached. This is presumably due to excess non-immobilized β-glucosidase being continuously washed out from the column. It appears that at 22 °C about 50 % of the cellobiose is enzymatically hydrolyzed to glucose and that about 40 % of the resulting glucose is fermented to ethanol at this temperature. This reflects the fact that at 22 °C neither β-glucosidase nor yeast are operating at optimal temperatures. When the flow rate was decreased from 2.3 to 0.6 ml/h, it was possible to achieve an ethanol concentration of 2.2 % (w/v) corresponding to 80 % of the theoretical yield (Fig. 2). Also in this experiment initially higher concentrations of ethanol were obtained.

Instead of operating the co-immobilized enzyme-microbe column at a low flow rate to achieve high yields the operating temperature can be increased. This was done in the second application converting whey permeate, which holds 4.5 % lactose, in a co-immobilized lactase-

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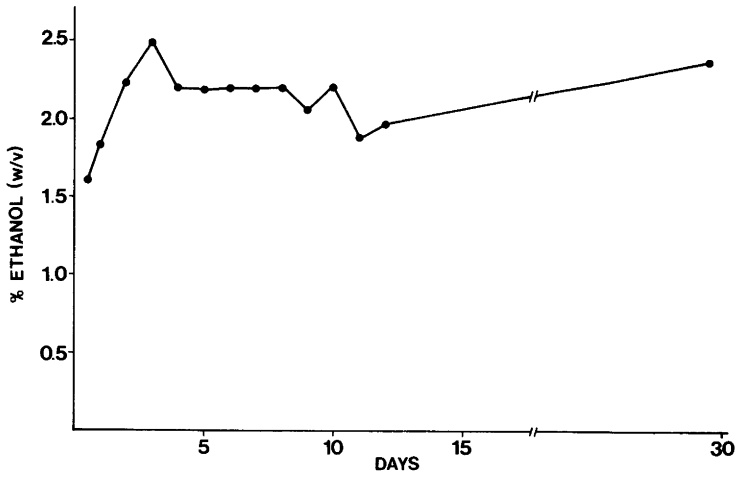

Fig. 2. Same conditions as in Fig. 1. Flow rate 0.6 ml/h.

yeast column. Lactase from *Aspergillus niger* was used. The enzyme has a broad pH optimum in the range of 3.2 to 5.2 as well as a broad temperature optimum in the range of 40 to 55 °C. Two substrates were used: Sweet whey permeate which naturally has a pH of 6.3, and acid whey permeate with a pH of 4.5 (Fig. 3). Acid whey permeate was converted to 1.5 % (w/v) of ethanol, corresponding to 66 % of the theoretical yield and 1.3 % (w/v) of ethanol corresponding to 57 % of the theoretical yield was obtained from sweet whey permeate. Less than 0.01 % glucose was found in the eluate at pH = 4.5 and less than 0.05 % was found at pH = 6.3. The corresponding figures for lactose were 0.1 % at pH = 4.5 and 0.4 % at pH = 6.3. This indicates that the lower pH is more favorable for the activity of both the enzyme and the yeast. To some extent the results also point out that the hydrolysis of lactose is the rate limiting step in the coupled process of lactose hydrolysis and yeast fermentation.

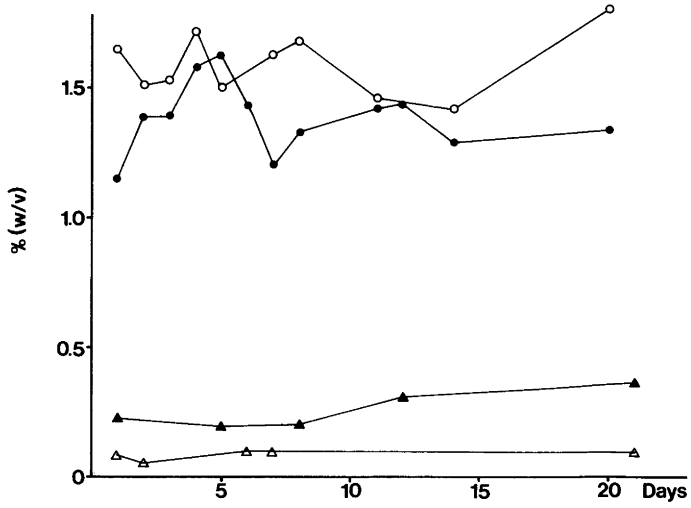

Fig. 3. Conversion of sweet whey permeate (filled symbols) and acid whey permeate (open symbols) in a co-immobilized lactase-yeast column at 30 °C. Concentration of ethanol (● ○) and lactose (▲ △). 0.01 M Ca (II) added. Flow rate 2.3 ml/h. Column design as in Fig. 1. 24 U lactase and 65 mg yeast cells/g beads.

The fact that yields higher than 50 % were obtained in both cases indicates that not only glucose, but also galactose is fermented by *Saccharomyces cerevisiae*. This organism has been reported only to ferment galactose when preadapted to this substrate.[7] In the present study no such adaptation was done. The results might rather reflect that glucose is fermented in the lower part of the column, while in the upper part of the column – due to the absence of a glucose substrate – the yeast becomes adapted to galactose.

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