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Enzymes Coimmobilized with Microorganisms

The Bioconversion of Whey Permeate to Ethanol with β-Galactosidase and *Saccharomyces cerevisiae*

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Abstract

The enzyme β-galactosidase was coimmobilized with the yeast *Saccharomyces cerevisiae* in alginate. The coimmobilized system was used to produce ethanol from cheese whey permeate.

Index Entries: Enzymes, coimmobilized with microorganisms; coimmobilization, of enzymes and microorganisms; microorganisms, coimmobilization with enzymes; whey permeate, bioconversion to ethanol; ethanol, from whey permeate; β-galactosidase, in production of ethanol from whey permeate; *Saccharomyces cerevisiae*, in production of ethanol from whey permeate.

Introduction

The utilization of complex substrates for the production of specific chemicals can no longer depend on bioconversions involving only one enzyme or one microorganism. The traditional approach has been mixed culture fermentations (*1*). The development of immobilized biocatalysts during the last decade (*2*) has brought about further expansions of this area. Thus, procedures for coimmobilizing two microorganisms (*3*) as well as procedures for coimmobilizing enzymes (*4*) have been developed. The present communication describes the coimmobilization of

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one enzyme and one microorganism. This approach has recently been applied to β-glucosidase and *Saccharomyces cerevisiae* in an enzyme–microbe reactor that, when connected with a membrane reactor for enzymatic saccharification of cellulose, was found to improve the bioconversion of cellulose to ethanol (*5*).

Experimental

The present study involves the coimmobilization of β-galactosidase (E.C.3.2.1.23) and *Saccharomyces cerevisiae* for the bioconversion of whey permeate to ethanol. The enzyme was covalently bound to alginate (*6*). This alginate–enzyme complex was then co-entrapped with yeast in alginate precipitated in the shape of 2-mm beads (*7*). The beads were filled in columns and sweet as well as acidified whey permeate was pumped through. Table 1 shows that 1.3% ethanol was produced from sweet whey permeate and 1.5% from acidified whey permeate. The experiments were run for three weeks with no decline in ethanol production (*8*). At room temperature these reactors have been found to operate for more than 2 months with no decline in productivity. The table also shows that the residual lactose and glucose concentrations in the eluate are lower at pH 4.5, reflecting the fact that both enzyme and microorganism have pH optima between 4 and 5.

The lactose concentration in whey permeate is around 4.5%, corresponding to a theoretical yield of 2.4% ethanol. Thus, in both experiments more than 50% of the theoretical yield of ethanol has been obtained, indicating that not only glucose, but also galactose has been fermented. *S. cerevisiae* has been reported to ferment galactose only when adapted to this carbon source (*9*). The design of the enzyme–microbe reactor as a column might therefore have resulted in galactose-adapted *S. cerevisiae* cells in the upper part of the column because the available glucose has been consumed in the lower part of the column.

Kluyveromyces fragilis ferments lactose directly to ethanol. This yeast has however a lower sugar as well as ethanol tolerance than *S. cerevisiae* (*9*). Recently strains of *K. fragilis* with high lactose and ethanol tolerance have been isolated (*10*). Work is presently underway to compare the productivity and long-term stability of *S. cerevisiae* coimmobilized with lactase and similarly immobilized *K. fragilis* (generously supplied by Dr. Kosikowski) in order to evaluate the potential advantages of a coimmobilized enzyme–microbe reactor for the bioconversion of whey permeate to ethanol.

TABLE 1
Eluate Composition (%, w/v) of Whey Permeate[a]

pH	Ethanol	Lactose	Glucose
6.3	1.3	0.4	0.05
4.5	1.5	0.1	0.01

[a]7.5 mL bed volume; 5.25 g alginate beads; flow rate, 2.3 mL/h; 30°C.

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