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POTENTIAL COST SAVINGS FOR FUEL ETHANOL PRODUCTION BY EMPLOYING A NOVEL HYBRID YEAST STRAIN

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SUMMARY

A hybrid yeast (Labatt culture collection strain 1393) was investigated for its ability to ferment a corn mash with reduced concentrations of added glucoamylase. It was found that glucoamylase additions could be decreased by as much as 50 percent. This reduction could represent significant cost savings in the production of fuel ethanol.

INTRODUCTION

The fermentation of starches to ethanol by yeasts requires pretreatment of the substrate in order to produce fermentable sugars. This pretreatment consists of three steps, gelatinization, liquefaction and saccharification. Gelatinization requires heat and free water and must precede liquefaction (Slack & Wainwright, 1980). Liquefaction, the dispersion of starch molecules into an aqueous solution (Norman, 1979), is accomplished by the use of heat and amylolytic enzymes. Heat stable bacterial alpha-amylases (1,4-α-D-glucan glucanohydrolase, E.C.3.2.1.1.) or malt enzymes may be employed (Saito, 1973; Norman, 1979; Slack and Wainwright, 1980; Yang *et al.*, 1982). During liquefaction starch molecules are only partially hydrolyzed producing a form of carbohydrate which cannot be assimilated by ethanol-producing yeasts such as *Saccharomyces cerevisiae*. Therefore, the partially hydrolyzed starch molecules must be converted to lower molecular weight sugars such as glucose or maltose by a process known as saccharification. This may be accomplished enzymatically, usually by the addition of fungal glucoamylases (1,4-α-D-glucan glucohydrolase, E.C.3.2.1.3.) to the fermentation vessel at the time of yeast inoculation.

The saccharifying glucoamylases represent a significant fraction of the total cost of producing ethanol. Reduction of the amount of added glucoamylase could significantly decrease the cost of the final product. It may be possible to decrease glucoamylase addition to starch mash fermentations by employing yeasts which actively produce and secrete glucoamylase. Such yeasts include strains of *Saccharomyces diastaticus* (Yarrow, 1984; Erratt and Stewart, 1981). A genetically manipulated yeast strain was investigated for such a purpose. This strain is known to contain three genes (*DEX1*, *DEX2* and *STA3*) which enable this yeast to produce extracellular glucoamylase (Stewart, *et al.*, 1982). The glucoamylase-producing yeast was compared to a commercial strain of *Saccharomyces cerevisiae* for its ability to ferment a corn mash containing approximately 20% (w/v) carbohydrate reduced concentrations of added glucoamylase.

MATERIALS AND METHODS

Yeast Strains. The yeast strains employed were Labatt culture collection strain 1393 (*DEX1/DEX1, DEX2/DEX2, SIA3/SIA3, MAL6/mal6*) a hybrid constructed using classical genetic manipulation techniques (patent pending) and Labatt culture collection strain 254, a commercial strain of *Saccharomyces cerevisiae* which was found to be unable to produce and secrete glucoamylase. Cultures were maintained on peptone-yeast extract (PY) agar slants composed of 3.5 g Difco peptone, 3.0 g Difco yeast extract, 2.0 g potassium phosphate monobasic, 1.0 g ammonium sulphate, 1.0 g magnesium sulphate septahydrate, 20.0 g Difco agar, and 20.0 g glucose in 1000-ml of distilled water.

Yeast Propagation. Cultures were transferred from slants onto PYN agar plates and incubated for 2 days at 28°C. Single colony isolates were used to inoculate 10-ml aliquots of PY broth. Broth cultures were incubated at 28°C overnight and used to inoculate 200-ml aliquots of brewer's wort in 500-ml flasks. These cultures were maintained at 21°C on a gyrotory shaker operated at an agitation rate of 150 rpm. After 2 days 400-ml of the wort cultures were used to inoculate 4-litre aliquots of brewer's wort which were stirred for 3 days at room temperature. Cells were recovered by centrifuging 1-litre aliquots at 10,200g and 5°C for 20 minutes.

Fermentations. Fermentations were carried out in 2-litre flasks with a working volume of 700-ml. Flasks were agitated at 80 rpm on a gyrotory shaker at 28°C. The substrate was a corn mash prepared from refined brewer's corn grist having a carbohydrate extract value of approximately 82% by weight. The mash was supplemented with 2% (v/v) corn steep liquor and 1% (v/v) distillery stillage. The yeast inoculum was 4% wet weight per volume. Saccharifying fungal glucoamylase (Novo AMG 200L) was added to the fermentation vessel at concentrations varying from 0 to 0.1% by volume based upon substrate weight.

Mash Preparation. 320 grams of corn and 0.05% (w/w) Canalpha (Biocon 180L), a liquefying alpha-amylase, were suspended in 1-litre of brewery-treated water. The mash was heated to 65°C at a rate of about 1.8 Celsius degrees per minute. The mash was held at 65°C for 30 minutes, brought to boil and held at 100°C for 10 minutes. The mash was cooled to 65°C and held for 30 minutes after a further addition of 0.05% (w/w) Canalpha. The mash was sterilized by autoclaving at 100°C for 30 minutes after addition of 2% (v/v) corn steep liquor and 1% (v/v) distillery stillage.

Analytical Methods. Samples were prepared by centrifugation of 30-ml aliquots of mash at 18,000g and 5°C for 15 minutes. A portion of each supernatant was placed in a boiling water bath for 10 minutes. These samples were analyzed for carbohydrates using a Spectra-Physics SP8100 high performance liquid chromatograph (HPLC) incorporating a Bio-Rad oligosaccharide column (Aminex HPX-42A) measuring 300 mm by 7.8 mm, a Micromeritics Model 771 retractive index detector and a Spectra-Physics SP4270 computing integrator. The unboiled supernatant was employed for ethanol analysis using a Carle AGC Series 100 gas chromatograph operating at 180°C having an eight foot long column packed with Poropak Q (81,000 mesh) and a Hewlett Packard Integrator 3380A. Specific gravity values of these same samples were determined using an Anton Paar Calculating Density Meter DMA-55.

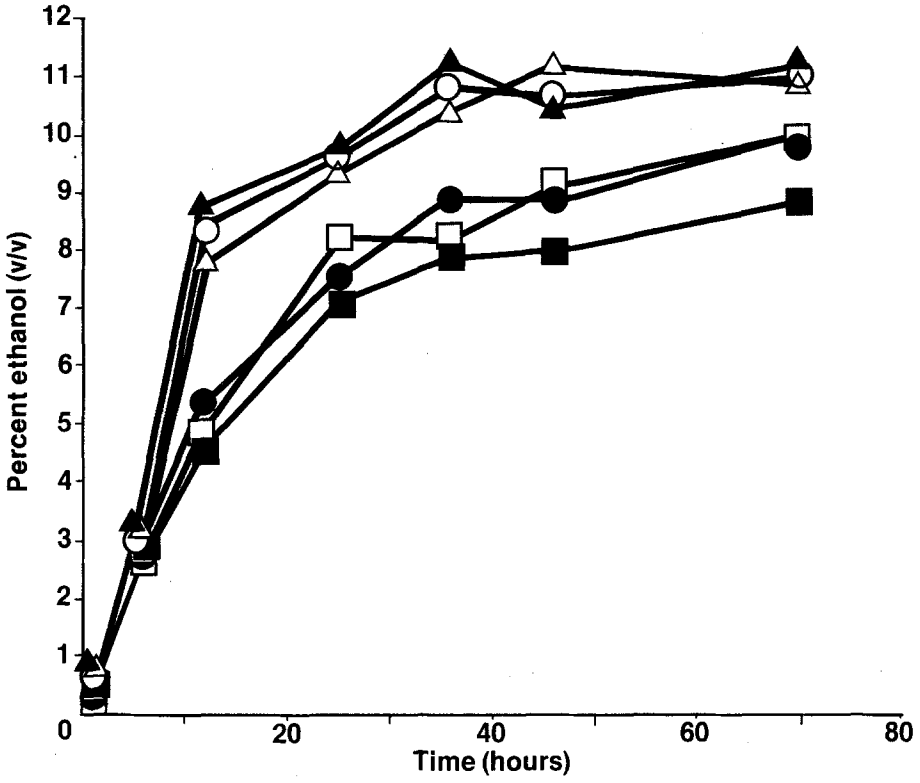

Figure 1. Ethanol Production by Hybrid Strain 1393. ■0.0% v/w AMG, □0.025% v/w AMG, ●0.04% v/w AMG, △0.05% v/w AMG, ○0.075% v/w AMG, ▲0.10 % v/w AMG

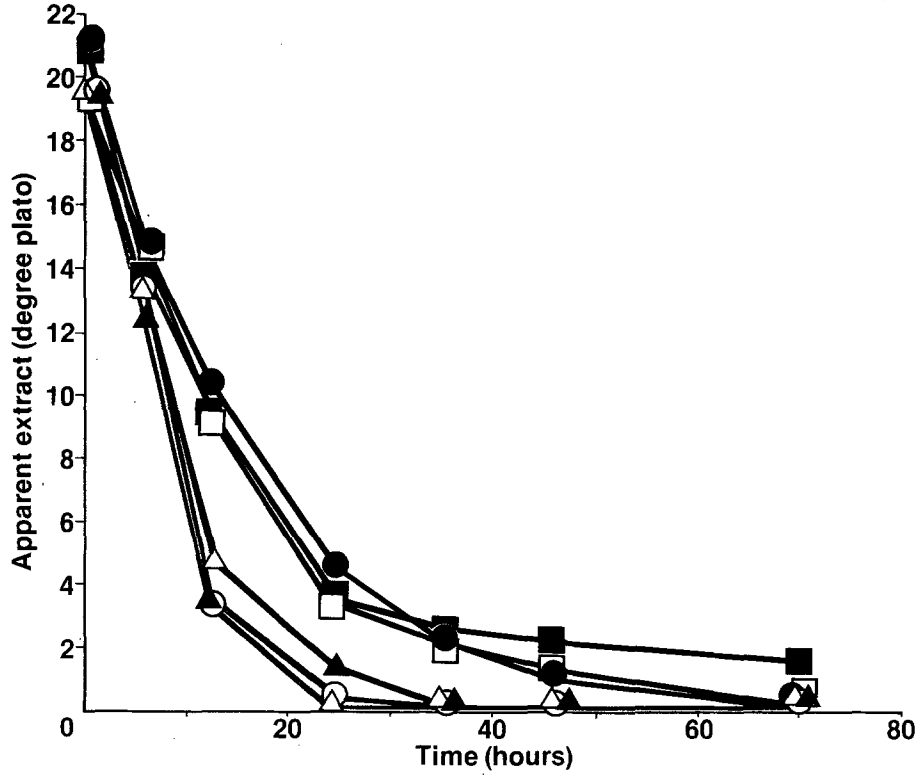

Figure 2. Apparent Extract Values for Hybrid Strain 1393. ■0.0% v/w AMG, □0.025% v/w AMG, ●0.04% v/w AMG, △0.05% v/w AMG, ○0.075% v/w AMG, ▲0.10% v/w AMG

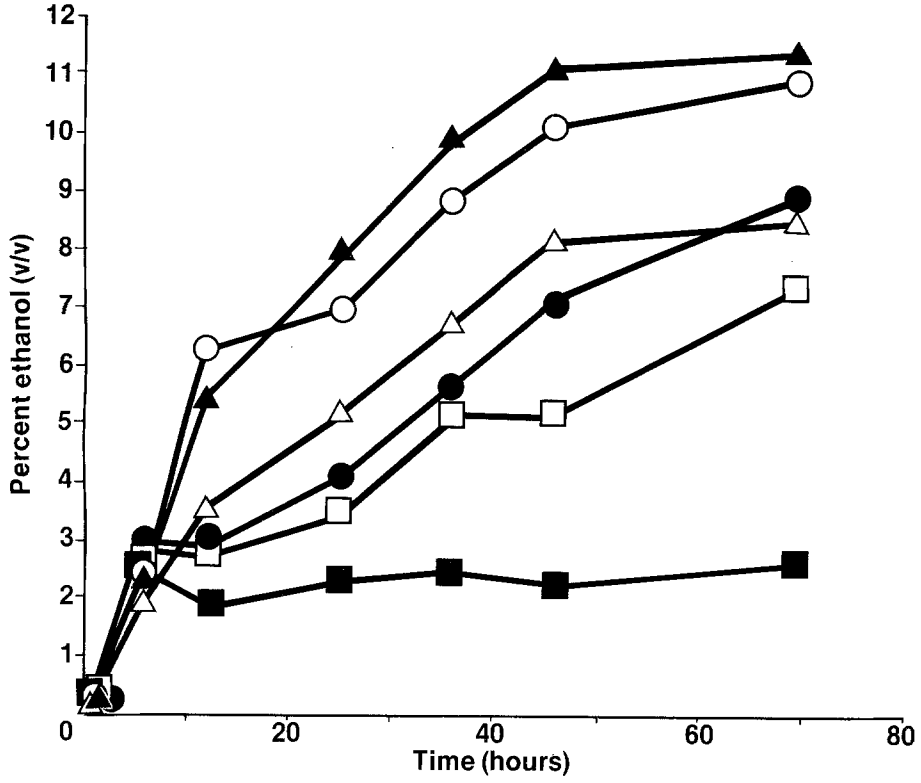

Figure 3. Ethanol Production by Commercial Strain 254. ■0.0% v/w AMG, □0.025% v/w AMG, ●0.04% v/w AMG, △0.05% v/w AMG, ○0.075% v/w AMG, ▲0.10% v/w AMG

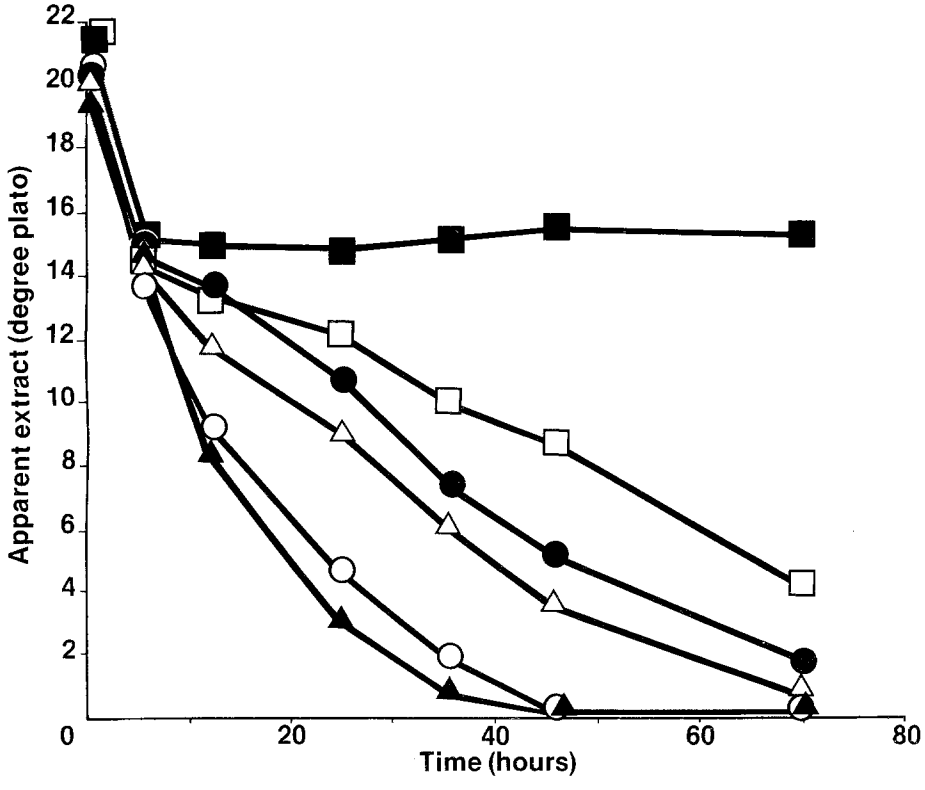

Figure 4. Apparent Extract Values for Commercial Strain 254. ■0.0% v/w AMG, □0.025% v/w AMG, ●0.04% v/w AMG, △0.05% v/w AMG, ○0.075% v/w AMG, ▲0.10% v/w AMG

RESULTS AND DISCUSSION

Figures 1 and 2 illustrate that when the hybrid yeast strain 1393 was employed added glucoamylase concentrations could be significantly decreased without reducing ethanol production or sugar uptake. Reduction of the added glucoamylase concentration from 0.1% by volume, based upon the substrate weight, to 0.05% resulted in no significant decrease in ethanol yield or sugar uptake. For example, after 70 hours approximately 11% (v/v) ethanol was produced by the hybrid yeast whether the mash was supplemented with 0.1, 0.075 or 0.05% added glucoamylase. As seen in Figures 3 and 4, ethanol yield and sugar uptake by the non-diastatic yeast strain were significantly decreased when the added glucoamylase concentration was decreased. For instance, after 70 hours, reduction of the added glucoamylase concentration from 0.1 to 0.05% resulted in a decrease in ethanol yield of approximately 25%. Examination of HPLC carbohydrate profiles of fermentations by both yeasts reveal similar results. Reduction of the added glucoamylase from 0.1 to 0.05% resulted in no signficant increase in residual carbohydrate in the fermentations completed by the hybrid yeast (Table I). However, when the commercial yeast was employed a 50% reduction in added glucoamylase resulted in an increase in residual sugars. After 70 hours approximately four times more residual carbohydrate remained in fermentations in which the added glucoamylase was reduced by 50%. This residual carbohydrate was composed of dextrins having four or more glucose moieties (Table I). The reduction of added glucoamylase is possible because the hybrid yeast is able to produce and secrete its own glucoamylase. Secretion of glucoamylase by similar yeasts has previously been demonstrated in the laboratory (Erratt and Stewart, 1981).

Table 1. HPLC ANALYSIS OF INITIAL AND FINAL DEXTRIN CONCENTRATIONS

Added Glucoamylase Concentration (% v/w)	Dextrin Concentrations (g/100-ml)			
	Hybrid Yeast		Commercial Yeast	
	0 h	70 h	0 h	70 h
0.00	20.3	4.9	21.5	21.5
0.025	19.6	2.0	19.5	8.9
0.04	19.5	1.5	20.6	4.9
0.05	19.3	0.9	21.1	3.8
0.075	20.9	0.8	19.5	0.7
0.10	20.6	0.7	19.5	0.6

Production of glucoamylase by the hybrid yeast and subsequent reduction of added glucoamylase represent a potential financial savings for the ethanol producer. Since the cost of glucoamylase may exceed $3.75 (U.S.) per litre a 50% reduction of this enzyme can result in significant decreases in costs. For example, an ethanol manufacturer that is producing 100 million litres of 95% ethanol per year uses 190 million kg of starch [assuming that the fermentation of 20% (w/v) starch yields 10% (v/v) ethanol]. For 190 million kg of starch, 0.19 million litres of glucoamylase are required (based upon an addition of 0.1-litre of glucoamylase per 100 kg of starch). This results in an annual enzyme cost of approximately 0.7 million dollars [assuming an enzyme cost of $3.75

(U.S.) per litre]. Reduction of the added glucoamylase by 50 per cent represents an annual savings of about $350,000 (U.S.).

Further research regarding glucoamylase reduction employing the hybrid yeast and other novel yeast strains is currently underway. Scale-up of the experiments described was an initial concern. Trials employing 10,000-litre mashes are now being completed. Initial results show similar trends as described above. Substrates other than corn are also being examined. These include wheat, potato, rice and cassava. Glucoamylase savings may vary depending upon the source of the starch. Using the various techniques of selection and genetic manipulation the hybrid yeast is presently undergoing further improvements to increase both its ethanol production and its ethanol tolerance.

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