Biotechnology Letters Vol 9 No 1 59-62 (1987)
Received as revised November 4

FERMENTATION OF STARCH TO ETHANOL BY A COMPLEMENTARY MIXTURE OF AN AMYLOLYTIC YEAST AND *SACCHAROMYCES CEREVISIAE*

Mohamed M. Abouzied and C. Adinarayana Reddy*

Department of Microbiology and Public Health, Michigan State University, East Lansing, MI 48824-1101

SUMMARY: Synergistic coculture of an amylolytic yeast (*Saccharomycopsis fibuligera*) and *S. cerevisiae*, a non-amylolytic yeast, fermented unhydrolyzed starch to ethanol with conversion efficiencies over 90% of the theoretical maximum. Fermentation was optimal between pH 5.0 to 6.0. Using a starch concentration of 10% (w/v) and a 5% (v/v) inoculum of *S. fibuligera*, increasing *S. cerevisiae* inoculum from 4% to 12% (w/v) resulted in 35-40% (w/v) increase in ethanol yields. Anaerobic or "limited aerobic" incubation almost doubled ethanol yields.

INTRODUCTION

Large volumes of starchy feedstock such as corn and potato processing wastes offer an important renewable biomass resource for fuel alcohol production in the U.S. In commercial processes the starch is first hydrolyzed to glucose by commercial thermophilic enzymes (Laluce and Mattoon, 1984). This entails significant raw material, equipment, energy and labor costs which could be saved if this enzyme hydrolysis step could be eliminated. The purpose of this study was to evaluate a single-step process for enhanced fermentation of unhydrolyzed potato starch to ethanol using a synergistic coculture of *Saccharomycopsis fibuligera* or *Lipomyces kononenkoae*, which hydrolyzes starch to glucose, and *Saccharomyces cerevisiae* which ferments glucose to ethanol.

MATERIALS AND METHODS

Yeast strains and media. Amylolytic yeasts *S. fibuligera* (Y-1062) and *L. kononenkoae* (IGC-4052), and a non-amylolytic yeast, *S. cerevisiae* (ATCC 26603) were maintained on **YM agar medium** (Abouzied and Reddy, 1986). The **fermentation medium**, described previously (Abouzied and Reddy, 1986), contained potato starch (Forney and Reddy, 1977) the sugar content of which was shown to be 98.6 (w/w) based on the total carbohydrate estimation on an acid-hydrolyzed sample (Dubois *et al.*, 1956).

Preparation of inocula. Yeast strains were grown in 10 ml of YM broth contained in 50 ml foam plugged Erlenmeyer flasks for 24 hrs with shaking (200 rpm) at 30°C. A 5% (v/v) inoculum was used except where mentioned otherwise. Dried *S. cerevisiae* cells were obtained from Diamond-V Mills Inc. (Cedar Rapids, Iowa) and were used as the inoculum in some experiments.

Fermentation procedures. Yeast monocultures or cocultures were inoculated into sterile starch medium (200 ml in 500 ml Erlenmeyer flasks) and were incubated with shaking (200 rpm) under "limited aerobic" (see below) conditions, at 30°C up to 7 days. Samples were collected daily from a given flask, cells were removed by centrifugation and the supernatant fluid was used for analysis.

Analytical procedures. Amylolytic activity and residual starch were determined as previously described (Abouzied and Reddy, 1986. Ethanol

concentration was determined by gas chromatography (Wegienek and Reddy, 1982).

RESULTS AND DISCUSSION

Utilization of starch by mono- and cocultures. The results (Table 1) substantiated the hypothesis that cocultures of an amylolytic yeast and a non-amylolytic sugar-utilizing yeast can ferment starch to ethanol. Starch utilization and ethanol yields were low in monocultures of S. fibuligera

Table 1. Comparison of starch metabolism parameters in monocultures and cocultures of amylolytic yeasts and S. cerevisiae[a]

Yeast	S. cerevisiae	Ethanol g/L	Residual starch g/L	Amylolytic activity U/ml	Cell biomass g/L
S. fibuligera	-	4.5	11.0	10.2	13.8
S. fibuligera	+	17.7	3.7	12.8	6.8
L. kononenkoae	-	2.4	1.0	8.9	14.6
L. kononenkoae	+	12.7	5.4	8.8	8.9

[a]Experiments were conducted under "limited aerobic" conditions (Abouzied and Reddy, 1986) for 7 days using a 5% (v/v) inoculum of a given organism. Three separate experiments were conducted with two identical flasks per experiment. Variation was 5 to 10% between individual experiments and less than 5% in replicate samples. Mean values from a representative experiment are given here.

and L. kononenkoae whereas in cocultures of either of these organisms with S. cerevisiae, there was a dramatic increase in ethanol yield and decrease in residual starch. Substantially more carbon is used for cell production in monoculture, whereas in cocultures most of the substrate carbon is utilized for ethanol production. Coculture of S. fibuligera and S. cerevisiae was selected for further study because S. fibuligera consistently gave higher amylase activity and ethanol yield than L. kononenkoae.

The effect of initial pH on direct fermentation by the cocultures was determined by monitoring ethanol yields. Ethanol production was optimal in the pH range 5 to 6 (results not shown); pH 5.5 was used in most of the experiments.

Among the treatments tested in this experiment, the "limited aerobic" and "anaerobic-N_2" incubation appeared to be optimal for ethanol production. As expected, fermentation under aerobic conditions resulted in the least amount of ethanol production and the highest biomass yield (Table 2).

Table 2. Effect of aeration condition on fermentation of starch by cocultures of S. fibuligera and S. cerevisiae[a]

Treatment[b]	Starch utilized g/L	Ethanol g/L	Cell biomass g/L
Aerobic	48.0	7.3	14.7
Limited aerobic	48.0	14.7	7.8
Anaerobic	46.0	12.7	6.9
Anaerobic-N_2	48.0	15.5	8.2

[a]Experimental conditions were as described in the footnote for Table 1.
[b]See Abouzeid and Reddy (1986) for a description of various treatments.

Effect of starch and S. cerevisiae concentration on ethanol yield. Using an inoculum of 5% (v/v) S. fibuligera and 6% (w/v) dry S. cerevisiae, and varying the starch concentration from 5 to 10% (w/v), the highest ethanol yield (close to 88% of the theoretical) was obtained in the fermentation with 5% starch (Fig. 1.A.). Lower ethanol yields of 79% and 70%, respectively, observed in fermentations with 7.5 and 10% starch suggested that at higher substrate concentrations, the yeast inoculum used (6% w/v) might be insufficient for the complete conversion of sugar to alcohol.

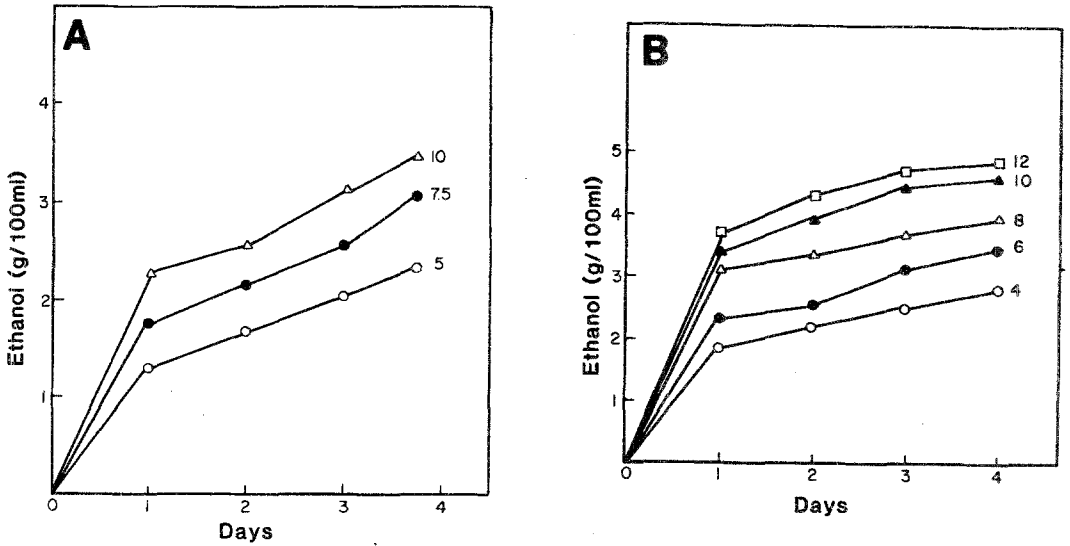

Fig. 1. A. Effect of starch concentration on ethanol yield in cocultures. Experimental conditions as in Table 1 except that 6% (w/v) dry S. cerevisiae cells were used as inoculum. B. Effect of varying S. cerevisiae concentration on ethanol production by cocultures. Experimental conditions as in Table 1 except that starch concentration was 10% (w/v) and 4 to 12% (w/v) dry S. cerevisiae cells per 100 ml of medium were used as inoculum.

Since it would be desirable for industrial applications to be able to utilize higher substrate concentrations, we used 10% (w/v) starch and increasing concentrations (4 to 12%) of dry S. cerevisiae cells (Fig. 1.B.). The results showed that as the concentration of S. cerevisiae inoculum increased, the yield of ethanol also increased. For example, at 12% yeast inoculum, the efficiency of starch conversion to ethanol was 92% of the theoretical maximum expected.

The following conclusions can be drawn based on results of this study:

1. Synergistic cocultures of an amylolytic yeast such as Saccharomycopsis fibuligera and a non-amylolytic, sugar utilizing yeast such as Saccharomyces cerevisiae could be employed for direct one step fermentation of unhydrolyzed starch to ethanol, eliminating the enzymatic hydrolysis step as currently employed and thereby significantly improving the process economy.
2. The efficiency of starch conversion to ethanol by the coculture could exceed 90% of the theoretical maximum.
3. The level of S. cerevisiae inoculum had a profound effect on ethanol yield suggesting that fermentation of sugar to ethanol is the rate-limiting step in the coculture fermentation.

ACKNOWLEDGEMENT

This research was supported in part by a grant from the Michigan Potato Industry Commission and a fellowship to MMA from the Egyptian Government. Journal article no. 12074 of the Michigan Agricultural Experiment Station.

REFERENCES

1. Laluce, C. and Mattoon, J. R. (1984) Appl. Environ. Microbiol. 48, 17-25.
2. Abouzied, M. M. and Reddy, C. A. (1986) Appl. Environ. Microbiol. 52, 1055-1059.
3. Forney, L. J. and Reddy, C. A. (1977) Dev. Industr. Microbiol. 18, 135-143.
4. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. F. (1956) Anal. Chem. 28, 250-256.
5. Wegienek, J. and Reddy, C. A. (1982) J. Clin. Microbiol. 15, 895-901.