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"Preparation of a novel sanitizer from the ethanol obtained from potato starch"

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Abstract

In order to add value to potato Starch and also curb their contagious disease, this study investigated the ethanol enrichment of potato starch[1] with the fungal strains *A.niger, A. awamori and A. foetidus* were taken and compared from the results when bulk dried yeast*S.cerevisiae* was used from other sources. Fermentation process involves solid matrix and Incubation has been done in limited aerobic condition at 30°C with shaking at 200 rpm for 5 days with *S. cerevisiae* at 30˚C, pH of 5-6.For each set of experiment 10 ml of sample solution was centrifuged at 4°C for 20 min with a speed of 5,000 x g. The study for the determination of ethanol, reducing sugar and amylolytic activity was performed.Gas chromatography technique was used for the determination of ethanol concentration in experimental sample.The results showed that amylase activity in the culture of *Aspergillus species* caused inhibition by glucose produced from starch hydrolysis. The test for co-culture of an *Aspergillus species* in starch-containing growth medium could prevent the accumulation of inhibitory concentration of reducing sugar and increase in amylolytic activity as well as the percentage of ethanol yield, in the main objective for the study.This isconcluded that utilization of starch, amylolytic activity and ethanol yields are low in the studies with *A. niger*, *A. foetidus* and *A. awamori* whereas in study with *S. cerevisiae* the excellent increase in the amylolytic activity and ethanol production have been observed.Starch was used upto 96.1% this value is much higher than those obtained in monocultures.

Keywords

Potato Starch, fungal strains: *A.niger, A. awamori, A. foetidus* and *S. cerevisiae*, yeast extract, agar medium, Crude Protein, Ethanol, culture broth, flame ionization detector.

Introduction

Large volumes of priggish feedstock such as corn represent an important biomass resource for fuel alcohol production, because the chemical composition and high density of starch, compared to other forms of biomass, facilitates prolonged storage and decreased transportation and pretreatment costs. Large quantities of starch-rich agro-industrial residues such as potato-processing wastes represent another important resource which could be fermented to yield ethanol. For example, an estimated 3.6 x 109 kg of wet potato-processing wastes is generated

annually in the United States[1] which is potentially convertible by fermentation into about 126 x 109 gallons (476.9 x 109 liters) of ethanol. The production of industrial and fuel ethanol from starchy biomass commonly involves a three-step process[2]: (i) liquefaction of starch by an endoamylase such as a-amylase; (ii) enzymatic saccharification of the low-molecular-weight liquefaction products to produce glucose; and (iii) fermentation of glucose to ethanol. Commercial amylases (frequently those produced by *Aspergillus species*) are used for liquefaction and saccharification of starch and represent a significant expense in the production of fuel alcohol from starchy materials.

Initial studies aimed at the elimination of the enzymatic liquefaction and saccharification step by using symbiotic coculture of amylolytic and sugar-fermenting organisms have been promising. For example, in the "Symba" process for single-cell protein production from potato-processing wastes, Jarl[3] and Skogman[4] eliminated the enzymatic liquefaction and saccharification step by using a coculture of Endomycopsisfibuligera (an amylolytic yeast) and *Candida utilis* (a nonamylolytic sugar utilizer). Laluce and Mattoon[2] suggested the use of *Saccharomyces diastaticus* for direct conversion of manioc starch to ethanol; however, prior treatment of the starch with a-amylase was required for obtaining efficient fermentation of starch. The purpose of this study was to develop and enhanced fermentation of potato starch to ethanol by using symbiotic culture and co-cultures of fungal strains such as *A.niger, A. awamori, A. foetidus*which hydrolyze starch to glucose, and *S. cerevisiae*, which is amylolytic but efficiently ferments glucose to ethanol.

Materials and methods:(Potato starch)

Potato starch was obtained from the water of the potato chips manufactured industry the collected starch was dried in the open air and processed by using a waring blender. After Pulverization the starch was stored at room temperature.

Microorganism:

The fungal strains *A.niger, A. awamori and A. foetidus* were taken from I.V.R.I. Bareilly India. The strains have maintained a slant on a specific agar medium. The medium has yeast extract (3gm.) malt extract (3g): glucose (10g) peptone (5g) and agar (20g). The dried yeast (*S. cerevisiae*) was obtained from Sigma chemical co and used as inoculum in our experiments and the conclusion obtained were compared from the results when bulk dried yeast was used from other sources.

Medium:

The experimental procedure involved the growth medium having potato starch (1g/100 ml), peptone (0.1 g/100ml), extract of malt(0.1g/100ml), yeast extract (0.2 y/100ml) calcium carbonate (0.2g/100ml) magnesium chloride Hexa hydrate (0.1g/100ml), ammonium phosphorate (0.2g/100ml) and ferrous sulphatehapta hydrate (0.001g/100ml). All the chemicals were purchase from Sigma chemical and are of AR grade. The composition of the growth medium for the fermentation process to get the ethanol was kept the same as above, only the concentration of starch was valid starting from 1 gm up to 10gm. for various set of experiments. Variation of pH was done by using a fixed volume of 1 normalHCl or 1 normal NaOH to the medium.

Inocula preparation procedure:

For a different set of the experiment the slant cultures were inoculated in 20 ml of sterile medium contained in an Erlenmeyer flask of 50 ml capacity. The containers were kept at 30°C with shaking at 200 rpm for 5 days. In the case of *S. cerevisiae* YM broth (of pH 5.5) was used in place of the growth medium and it was incubated only for 24 hours.

Fermentation:

This is a very important step and very much dependent on the experimental condition. The Erlenmeyer flasks (of 500 ml capacity) with 200 ml of growth medium were used for the process. First, the flasks were sterilized at 121°C USING autoclave for half an hour and after that, 5% inoculums of a genius or yeast was used. The experiments with dried *S. cerevisiae*(4% to 12% w/v) used in different sets of experiments. Incubation has been done in limited aerobic condition at 30°C with shaking at 200 rpm for days. In those experiments in which the effect of aerobic or limited aerobic condition was studied, the flasks were fitted either with foam plugs or with a stopper having a hole to maintain the incoming of air in the flasks (aerobic). For maintaining the limited aerobic condition the flasks were maintained as in the case aerobic study except that cotton plugged end of the glass tube was placed under water. After the incubation of 24 hours in aerobic condition with nitrogen before incubation in anaerobic condition. Flushing with nitrogen requires only 2 min.

Analysis:

For each set of experiment 10 ml of sample solution was centrifuged at 4°C for 20 min with a speed of 5,000 x g. This process enabled the removal of cells and we got the supernatant liquid for the study. The study for the determination of ethanol, reducing sugar and amylolytic activity was performed. The calculation of theoretical yield was done assuming complete conversion of glucose produced by starch hydrolysis to control.

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

1mole 2 mole

The reaction involves the production of two moles of ethanol for a single mole of glucose. The concentration of residual starch was determined in either undiluted or in a diluted sample of culture broth by using the phenol-sulfuric acid method. The amount of starch in the sample was determined by using the keer method[5]. glucose (g/100ml) x 0.93 = starch (g/100ml)

The amount of reducing sugar released from starch was the indicator for the calculation of extracellular lipolytic activity during the process. It was determined by Lemmel's method[6]. The curve for the colorimetric study was drawn by assuming glucose as standard. The enzyme in 1 ml of that liberated 1 µ mol of reducing sugar from starch in a fixed time interval (in this case, 3 min.) was taken for one unit of amylolytic activity. Total of three sets of experiments were used to get the mean value for two replicate flasks per experimental conclusion[7].

Cell biomass:

The value of cell biomass was calculated by centrifugation, drying (at 70°C under vacuum) to constant weight. It was expressed as g/100ml of growth medium.

Ethanol concentration:

The gas chromatography technique was used for the determination of ethanol (Varian Aerograph series 2400; varian Co.). The column of stainless steel packed with chromosorb w (acid-washed and 80/100 mesh) and a flame ionization detector was used. Nitrogen (30 ml/min) was used as carrier gas.

Results and Discussion

Consumption of starch by monoculture and co-cultures:

It was observed in recent studies that amylase activity in the culture of *Aspergillus species* caused severe feedback inhibition by glucose produced from starch hydrolysis. The test for co-culture of an *Aspergillus species* in starch-containing growth medium could prevent the accumulation of inhibitory concentration of reducing sugar and increase in amylolytic activity as well as the percentage of ethanol yield, in the main objective for the study.

The conclusion was in favour of the fact that utilization of starch, amylolytic activity and ethanol yields are low in the studies[8, 9] with *A. niger, A. foetidus* and *A. awamori* whereas in study with *S. cerevisiae* the excellent increase in the amylolytic activity and ethanol production have been observed. The results are shown in table.1.

Starch was used upto 96.1% this value is much higher than those obtained in monocultures. These results indicate that *Aspergillus species* with *S. cerevisiae*can be useful method for our purpose.

Table.1: Comparison of starch metabolism parameters:

Fungus	*S. cerevisiae*	Ethanol (g/100ml)	Residual starch (g/100ml)	Amylolytic activity (U/ml)
A. niger	-	0.29	1.7	ND
A. niger	+	2.12	0.29	11.2
A. foetidus	-	0.56	1.95	ND
A. foetidus	+	1.59	0.63	11.2
A. awamori	-	0.31	2.8	ND
A. awamori	+	1.61	0.91	11.2

Table.2: Different concentration of starch utilization by mono and co-cultures of *A. niger* and *S. cervisiae*

Monoculture *A. niger* (g/100ml)			Co-culture *A. niger* + *S. cerevisiae*(g/100ml)		
Starch consumption	Cell biomass	Ethanol	Starch consumption	Cell biomass	Ethanol
0.9	1.0	0.18	0.98	0.9	0.20
2.1	1.7	0.18	0.98	1.1	0.52
2.7	2.0	0.31	3.2	1.3	0.74
3.9	2.3	0.31	4.1	1.6	1.08
4.4	2.9	0.33	5.2	1.9	1.45

Table.2 represents the results of mono and co-culture of *A. niger* and *S. cerevisiae*. The incomplete utilization of starch in monoculture of *Aspergillus species* perhaps due to availability of low oxygen level. The production of ethanol is higher in co-culture experiment.

Preparation of hand Sanitizer

Prepare Ethanol Antiseptic Topical Solution containing ethanol 80% (v/v) as follows*:*
Measure the quantities of Ethanol obtained from potato starch, Hydrogen Peroxide, and Glycerol in suitable containers. Transfer the Ethanol and Hydrogen Peroxide into a suitable calibrated container and mix gently. Transfer the Glycerol stepwise and quantitatively into the calibrated container and mix gently after each addition. Rinse the container containing glycerol several times with Water and add the contents to the calibrated container. Add sufficient Water to bring to final volume, Mix well. Transfer the solution into suitable containers.

Table.3: Composition of Alcohol based hand sanitizer (80%)

Contents	Quantity
Ethanol (96%)	833.3 mL
Hydrogen Peroxide (3%)	41.7 mL
Glycerol (98%)	14.5 mL
Water[a], a sufficient quantity to make	1000 mL

[a] Water may be distilled water, cold boiled potable water, reverse osmosis water, or filtered water.

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