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RESEARCH ARTICLE

PRODUCTION OF BIOETHANOL FROM BARLEY (*HORDEUM VULGARE*) USING YEAST *SACCHAROMYCES CEREVISIAE* IN BATCH FERMENTATION

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ABSTRACT

India is the world's second largest producer of ethanol next to China and has the potential of being the biggest, backed by its beverage and agricultural sector. Batch fermentation of cereals waste using yeast, *Saccharomyces cerevisiae* converts carbohydrates into carbon dioxide and alcohols. The enzymes of the malts break down starch in to sugar, which is ultimately converted into alcohol by yeast, the hybrid yeast improved ethanol tolerance, its glucose fermentation rate and yield more ethanol than those of its parent strain. The increased pH 5.0 to 6.5 & temperature 50 to 72 ^{0}C break down β-glucanase, α-amylase and natural protease in to malt sugar, and ultimately these enzymes convert malt sugar into glucose.

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INTRODUCTION

Fermentation processes are being extensively used in the biotechnology, pharmaceutical, food and beverage industries. Typically, fermentations utilize microorganisms (bacteria and yeast) to produce a desired product from a substrate. Ethanol produced from glucose in the presence of oxygen by aerobic fermentation. With a huge agricultural resources, abundant livestock, and cost competitiveness, India is fast emerging as a sourcing hub for processed beverage. The Indian beverage processing industry accounts for 32 per cent of the country's total beverage market. The alcoholic beverage is produced by the saccharification of starch and fermentation of the resulting sugar. The starch and saccharifying enzymes are often derived from malted cereal grains like barley and rice. *S. cerevisiae* is extensively used in batch fermentations to convert sugars in to ethanol for the production of beverages and bio-fuels. Despite the obvious importance of this process, the physiological constraints which limit the rate of glycolysis and ethanol production are not fully understood (Casey and Ingledew, 1986; Ingram and Buttke, 1984; Moulin *et al*., 1984.).

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Identification of these constraints represents an important step toward the development of improved organisms and process conditions for more rapid ethanol production. Such improvements could result in an increase in the ethanol production capacity of existing fermentation plants and a reduction in the cost of future facilities. *S. cerevisiae* is capable to increase rates of glycolysis and ethanol production under optimal conditions. Barley (*Hordeum vulgare*), has been used as animal fodder, as a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods. It is used in soups and stews, and in barley bread (Wolfgang and Rudolf, 2002). Barley was fourth both in terms of quantity produced (136 million tons) and in area of cultivation (566,000 square kilometers) of cereal crops in the world. Barley is easily identified in the field by its characteristics whisker. The barleycorn consists of embryo, together with a starchy endosperm, packed with in a protective layer. The fermentable material will be subsequently converted in to ethanol.

MATERIALS AND METHODS

The standard method of ethanol production was followed in present work as mentioned in flowchart.1 Ethanol percentage, original gravity, pH, cell count, colour, co_2, and DO were observed in bioethanol.

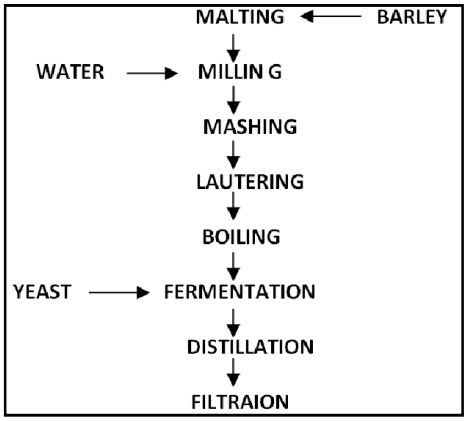

Fig.1. Flowchart of ethanol production

RESULT AND DISCUSSION

At initial concentration of 100 g/l cereal, 38 g/l of ethanol was obtained on 3 day by strain I of *S. cerevisiae* (Wild strain) and cereal was completely utilized. Where as in the case of 150 g/l cereals were completely utilized on 5 day by strain I of *S. cerevisiae* and 48 g/l ethanol was obtained. The maximum ethanol production of 49 g/l was obtained on day 5 by utilization of 200 g/l cereals. When the initial cereals concentration was increased upto 250 g/l, barley consumption and ethanol production were significantly inhibited and about 43 g/l ethanol was obtained on day 8. In addition, the recombinant strain II of *S. cerevisiae* demonstrated slightly higher rate of cereals consumption and ethanol production. In both of the above, the initial concentration of yeast was 20g/l. Approximately 42 g/l ethanol was obtained by initial cereals concentration 100 g/l on day 3. At initial cereals concentration 150 g/l they were completely utilized by strain 2 and 51 g/l ethanol was obtained on day 5. At initial cereals concentration 200 g/l, cereals were completely utilized by strain 2 and 55 g/l ethanol was obtained on day 5. When the initial cereals concentration was increased to 250 g/l, barley consumption and ethanol production by yeast were significantly increased and 61 g/l ethanol was obtained on day 8.

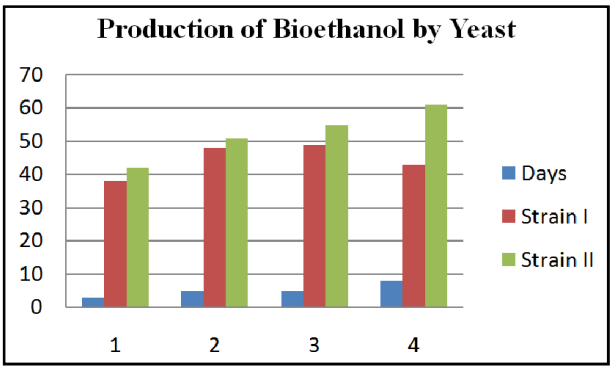

Fig.2. Production of Bioethanol by yeast (Strains I & II)

S. cerevisiae is the best working horse for production of ethanol. The hydrolyses of biomass containing both hexoses and pentose, the wild-type strains of *S. cerevisiae* cannot utilize hexose, such as glucose. Utilization of glucose is very important to improve the ethanol yield from biomass hydrolyses, making the process economically viable. Numerous recombinant *S. cerevisiae* strains were constructed by heterologous expression of glucose utilization pathways from yeast and over expression of endogenous XKS gene through rational metabolic engineering in combination with evolutionary engineering (Eliasson *et al*., 2000; Wumpelmann and Kjaergaard, 1979). Although the hybrid yeast improved ethanol tolerance, its glucose fermentation rate and more ethanol yield than those of its parent strain.

Fermentation

α amylase, β glucanase and natural protease were mixed in the malts of cereals to convert complex sugar into simple sugar. The wort was collected for fermentation. The revelations of fermentation analysis for alcohol %, pH, gravity, Real extract and cell count were analysed (table1). The maximum pH 5.52, alcohol content 44.4 and original gravity 1.069 were found in unit tank 4, whereas primary gravity and cell count were the highest in unit tank 6.

Table 1. Analysis of bio ethanol at fermentation stage

Parameter	UT no.1	UT no.2	UT no.3	UT no.4	UT no.5	UT no.6
pH	5.50	5.52	5.50	5.52	5.50	5.51
Alcohol	40.5	41.6	41.2	44.4	44.3	44.1
Primary Gravity	1.021	1.002	1.001	1.002	1.001	1.021
Original Gravity	1.067	1.069	1.023	1.069	1.023	1.067
R.E.(Real extract)	1.014	1.010	1.170	1.010	1.171	1.014
Cell count	2000	2500	2400	2600	2800	3000

Filtration

Filtering the ethanol stabilizes the flavor and brilliance. The result of filtered ethanol quality for CO_2, alcohol, gravity, R.E., and DO in ethanol production is given in table 2. The highest parentage of carbon dioxide and pH were found in unit tank 2 where as the highest alcohol % was found in unit tank 5. The primary gravity and original gravity of ethanol were maintained in all unit tanks. The maximum DO was found in unit tank 4.

Table 2. Revelation of filtered ethanol

Parameter	UT no.1	UT no.2	UT no.3	UT no.4	UT no.5
CO_2 (%)	2.90	2.92	2.90	2.90	2.90
Alcohol (%)	42.20	43.52	45.40	47.02	51.20
pH	5.3	5.5	5.3	4.3	4.3
Primary gravity	1.005	1.005	1.005	1.005	1.005
Original gravity	1.057	1.057	1.057	1.057	1.057
R.E.	1.016	1.016	1.016	1.014	1.014
D.O.	26ppb	26ppb	28ppb	30ppb	29ppb

A Japanese study found that low alcoholic drinks possess strong anti-cancer properties. At initial concentration of 100 g/L cereals & 20 g/L yeast were completely utilized on day 3 by both strains and 38 g/L of ethanol was obtained by Strain type I and 42 g/L by strain type II. The maximum ethanol production of 51 g/L was found on day 5 in 150 g/L cereals. Whereas 48 g/L ethanol was obtained by strain I under the same conditions. The recombinant strain type II demonstrated higher rates of cereals consumption and ethanol production in same yeast concentration. When the initial cereals concentration was increased further to 200 g/L, the difference between the rates of yeast consumption and ethanol production by Strain II became more noticeable. Approximately 49 g/L ethanol was obtained by strain I on day 5 and 55 g/L by strain

2. Whereas 43 g/L ethanol was obtained on day 8 in 250 g/L cereals by strain I and 61 g/L by strain II. The similar findings was reported that the high xylose content only slightly inhibited xylose consumption and ethanol production by ScF2 with the maximal ethanol concentration of 47 g/L on day 6. The highest ethanol titre of 51 g/L was obtained by the recombinant strain ScF2 in 150 g/L initial xylose concentration. Further increase of the initial xylose concentration triggered a slight decrease in the maximal ethanol titre and an increase of the fermentation time Zhang and Geng (2012).

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