

Production of bioethanol by batch fermentation of cereals waste

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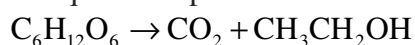
Abstract

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 species *Saccharomyces cerevisiae* converts carbohydrates to carbon dioxide and alcohols. The process of ethanol production includes malting, milling, mashing, lautering, boiling, aeration, yeast pitching, maturation, filtration and packaging. The enzymes of the malts break down starch in to sugar which is ultimately converted into alcohol by yeast, *Saccharomyces cerevisiae*. If pH increased 6.0 to 6.5 & temperature 50 to 72 °C then break down β -glucanase, β -amylase and α -amylase in malt sugar, these enzymes are converted malt sugar into glucose. Simple glucose is changed ethanol alcohols with slightly yellow-black colour, spicy sweet & lite bitter. Finally, some concluding considerations on current and future prospect in increased ethanol production by yeast species.

Keywords: *S. cerevisiae*, yeast, barley, glucose, ethanol

Introduction

Fermentation processes are used extensively in the biotechnology, pharmaceutical, food and beverage industries. Typically, fermentations utilize microorganisms (bacteria, yeast) to produce a desired product from a substrate. Butyl alcohol, acetone, citric acid, hydrogen, glycol, fuel alcohol, and beer are examples of the hundreds of biochemicals produced by fermentation. In many cases, fermentation is the more cost effective means to manufacture products. The amount of dissolved oxygen in the fermentation broth has major implications for the reactions that occur in yeast. When oxygen is present, respiration occurs converting simple sugars to cell mass and carbon dioxide. Ethanol may be produced from glucose in the presence of oxygen by aerobic fermentation, under some conditions. Under other conditions, ethanol may be consumed by yeast cells. These and other reactions are quite complex.



With a huge agriculture 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 saccharification enzymes are often derived from malted cereal grains like barley and wheat.

Barley (*Hordeum vulgare*), a member of the grass family, is a major cereal grain. It was one of the first cultivated grains and is now grown widely. Barley grain is a staple in Tibetan cuisine and was eaten widely by peasants in Medieval Europe. Barley has also 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 of various cultures (Dai, et al., 2012). Barley grains are commonly made into malt in a traditional and

ancient method of preparation. In a 2007 ranking of cereal crops in the world, barley was fourth both in terms of quantity produced (136 million tons) and in area of cultivation (566,000 square kilometers). Barley can be identified in the field by its characteristic whisker. The barley corn consists of embryo, together with an starchy endosperm, packed with in a protective layers. It is the food reserve that is the origin of the fermentable material that will be subsequently converted in to ethanol. In India, barley mainly grows in the states of Punjab, Haryana, and Rajasthan (2).

The main objective of this study is to produce ethanol in a batch fermentor. This experiment provides familiarity with the dynamic behavior biological systems, and allows for the determination of the appropriate kinetic parameters to describe the fermentation process at hand. In this experiment, *Saccharomyces cerevisiae* yeast are used to convert glucose into ethyl alcohol. The yeast cell contains enzyme catalysts that provide an energetically favorable pathway for the reaction.

Material and Methods

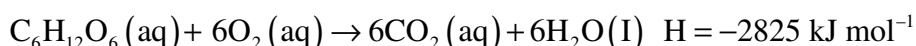
Malting is where the grains are made ready for brewing; there are three steps in malting first, the grains are allowed to soak in a vat of water for 40 hours; this process is called steeping. Next, the germination process beings spreading out the grain on a flat surface for around 5 day to allow

the starches in the grains to break down into shorter lengths. After this process, the grains are now called green malt. The last process of malting is Kilning. The green malt goes into a kiln gradually turning into high temperature to allow the malt to dry. Milling is the next process after malting. This is where the finished malt that is going to produce the ethanol is cracked. Milling is done for the grains to absorb water and to be able to extract sugars. Depending on the type of process used, milling can highly influence the outcome of the ethanol. Mashing After milling, mashing is the next step in order to convert the starches released during malting into sugars. The milled grain is transferred into a mash tune which is a large vessel filled with hot water to create a cereal mash.

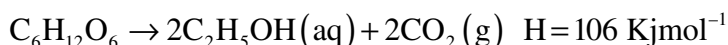
Lautering the left over sugar is then strained through the bottom of the mash in a process called "Lautering". Lautering separates the liquid containing the sugar extracted during mashing from the grains. Boiling At this point the liquid is called wort, and it is now time for the boiling process. The wort is moved into a large kettle where the it is mixed in with hops and other ingredients and set to a boil to stop enzymatic processes and to sterilize the wort. And then, the boiled hopped wort is moved into a whirlpool where in the solid particles are separated from the liquid.

Fermentation

After the boiling process, the wort is moved into a heat exchanger where it cools down to a temperature appropriate for fermentation. The wort is moved into a fermentation tank and then is added with yeast to start the fermentation process.



Fermentation is the processes by fermentable carbohydrates are converted by yeast into alcohol, carbon dioxide, and numerous. The byproducts have a considerable effect on the taste, aroma, and other characteristic properties of the ethanol.



This is where the sugars are turned into alcohol, carbon dioxide and other components of ethanol. Conditionings are the next stages into the process. It is where the brewer takes wort now turned into ethanol and racks it into a conditioning tank where the ethanol is left to age, making its taste smoother and removing unwanted flavors. After weeks to several months of conditioning, the ethanol starts its finishing stage, the last stage of the brewing process. In this process, the ethanol is filtered and it given time to get its natural color. Then it undergoes carbonation and is moved to a holding tank until it's time for the ethanol to be bottled.

Analysis of bio ethanol

The following parameters are involved in the final quality analysis:

Ethanol percentage

Take 250 ml of ethanol sample in a 1000 ml round bottom flask added 200 ml of water. Collect the extract in 250 ml volumetric flask through distillation up to the mark. Collective material contains alcohol which is further taken into 250 measuring cylinder.

Original gravity of ethanol

$$\text{Original Gravity} = 2A + E - C$$

Where A = alcohol %, w/v

E = Real extract

C = Correction factor

Get answer in degree Plato

Colour

Colour of the ethanol sample was taken using UV spectrophotometer. Distilled water was taken as blank. Absorbance at 430nm of the ethanol sample was taken. Let the value be 'A'.

$$\text{Calculation: } B = A \times 1.27 \times 10$$

$$\text{Colour units (EBC)} = (2.65 \times B - 1.2)$$

Bitterness

Take 10ml of degassed ethanol + 1ml 3NHCL + 20 ml of is-octane. Shake it for 10

minutes at a moderate speed. Take the absorbance at 275 nm with is-octane as blank. Say the value is 'A'.

Calculation:

$$\text{Bitterness} = A \times 50 \text{ BU}$$

Where BU is bitterness units

CO₂

Bottle was shaken first then inserted into the slot provided in the Zahm and Nagel instrument. Now the instrument as a whole was shaken and the cork subsequently punctured. The CO₂ escapes, registering a pressure in the pressure measuring device. Temperature of the bottle was also taken. Using these values of temp and pressure CO₂ content was found out.

Calcium

Took 20ml. of beer sample add 100ml. of distilled water + 3ml. of NaOH + 0.5 ml. of calcium indicator. Titrate std EDTA (0.2N) solution till green florescence (should be done against black background) say the value is "A"

$$\text{Calcium content in ppm} = \frac{A \times 0.2004}{\text{Vol. of sample}} \times 100$$

Free SO₂

Take 25 ml. of just opened ethanol add 10 ml. cons. HCL. Do the distillation and collect the distillate. Take 15 ml. distilled water + few drops starch + 0.1 ml. of 0.025N iodine. Titrate the distillate in to the solution until the blue colour persists. Get the value say

$$\text{Free SO}_2 \text{ in ppm} = A \times 12.8 \text{ ppm}$$

Result and Discussion

The present investigation has been planned to study Production of ethanol by fermentation of barley the result and discussion has been discussed in following heads.

Wort analysis

The mashing allows the enzymes in the malts to break down the starch in the grain in to sugars, typically maltose to create malty liquid called wort. The result of quality assessment of wort for sp. G.V.,pH, iodine test and colour observation from step I II III by wort analysis are given in the following tables.

Table 1 Effect of pH on wort analysis and Colour observation

	Date	pH
Process water		7.02
Mash kettle [MK]	01/03/2014	6.02
	02/03/2014	6.32
	03/03/2014	6.2
	04/03/2014	6.1
	05/03/2014	6.0
	06/03/2014	6.5

Table 2 Fermentation performance of yeast in ethanol production containing 500 HL/ tank

Product	Yeast	F1	F2	F3	F4	F5	F6	F7	F8
Ethanol yield (g/g)	0.27±	0.28±	0.29±	0.28±	0.29±	0.29±	0.29±	0.29±	0.31±
Ethanol productivity (g/L/h)	0.32±	0.33±	0.35±	0.34±	0.35±	0.36±	0.35±	0.36±	0.38±

Table 3 Specific gravity and pH at different stages

Wort kettle (WK)	DATE	Before boiling		After boiling		Last run	Iodine test
		pH	Sp.Gv.	pH	Sp. Gv.	Sp. Gv.	
	01/03/2014	5.41	1.046	5.24	1.060	1.009	Ok
02/03/2014	5.39	1.047	5.31	1.044	1.009	Ok	
03/03/2014	5.41	1.046	5.29	1.061	1.009	Ok	
04/03/2014	5.41	1.046	5.29	1.054	1.009	Ok	
05/03/2014	5.40	1.044	5.24	1.059	1.008	Ok	

Fermentation

Fermentation is the process in which fermentable carbohydrate are converted into alcohol by yeast. The revelation of fermentation analysis for alcohol %, pH, gravity, taste, colour, Real extract, cell count, viability, given in table 4.

Table 4 Analysis of bio ethanol.

Parameter	UT no.1	UT no.2	UT no.3	UT no.4	UT no.5	UT no.6
Ph	5.5	5.52	5.5	5.52	5.5	5.5
Alcohol	6.8	6.3	3.7	6.3	3.7	7.4
P.Gravity	1.021	1.002	1.001	1.002	1.001	1.021
O.Gravity	1.067	1.069	1.023	1.069	1.023	1.067
R.E.	1.014	1.010	1.17	1.010	1.17	1.014
Cell count	3000	2000	1800	2000	1800	3000
Colour	Spicy yellow	Light Yellow	Brown	Light dark	Dark	Yellow

Filtration & BBT Filtering the ethanol stabilizes the flavor and gives beer its polished shine, and brilliance. The result of test of beer quality by filtration and BBT for CO₂, alcohol, gravity, taste, colour, R.E., Bitterness, DO, in beer production is given in table 5.

Table 5: Revelation of bright beer tank in filtration

Parameters	1	2	3	4	5
CO ₂	2.90%	2.92%	2.90%	2.90%	2.90%
Alcohol	7.4%	6.4%	7.4%	7.02%	7.02%
pH	5.3	5.5	5.3	4.3	4.3
P. gravity	1.005	1.005	1.005	1.005	1.005
O. gravity	1.057	1.057	1.057	1.057	1.057
Celerity	0.24	0.24	0.24	0.24	0.24
Taste	Ok	Ok	Ok	Ok	Ok
Colour	6.35	6.35	6.35	6.41	6.41
RE	1.016	1.016	1.016	1.014	1.014
Bitterness	14.19	14.14	14.15	14.18	14.19
DO	26ppb	26ppb	28ppb	30ppb	29ppb

Discussion

In case s2005 Japanese study found that low alcohol beer may possess strong anti-cancer properties. Another study found nonalcoholic beer to mirror the cardiovascular benefits associated with moderate consumption of alcoholic beverages. However much research suggests that the primary health benefit from alcoholic beverages comes from the alcohol they contain.

The results are shown in Figure 1. At initial concentration of 100 g/L, cereals & 5 HL/L yeast was completely utilized on day 3 by both strains and 42 g/L of ethanol was obtained by ScF2 and 38 g/L by strain type 2. The maximum ethanol production of 51 g/L was obtained on day 5 in 150 g/L yeast by ScF2, whereas 48 g/L ethanol was obtained by yeast under the same conditions. In addition, recombinant strain ScF2 demonstrated slightly higher rates of cereals consumption and ethanol production in both of the above initial 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 ScF2 and yeast became more noticeable. Approximately 49 g/L ethanol was obtained by

ScF2 on day 5, whereas 43 g/L ethanol was obtained by yeast on day 8. At initial yeast concentration of 250 g/L, barley consumption and ethanol production by yeast were significantly inhibited by the high content of yeast and about 20 g/L of ethanol was obtained on day 7. On the other hand, the high yeast & barley content only slightly inhibited glucose consumption and ethanol production by yeast with the maximal ethanol concentration of 47 g/L on day 6.

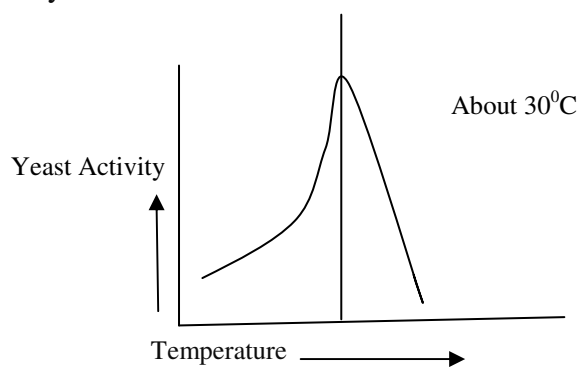


Fig. 1 Performance of yeast in ethanol production

S. cerevisiae is the best working horse for ethanol industrial production (12). However, hydrolysate from biomass contains both hexoses and pentoses, and 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 hydrolyzate making the process economically viable. Numerous recombinant *S. cerevisiae* strains were constructed by heterologous expression of glucose utilization pathways from yeast and overexpression of endogenous XKS gene through rational metabolic engineering in

combination with evolutionary engineering (4,13,14). Although the hybrid yeast was improved in ethanol tolerance, its glucose fermentation rate and ethanol yield were lower than those of its parent strain 16. In addition, it was discovered that the mononucleate fusants were able to quickly segregate into their parental type strains (17).

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