IJREAT International Journal of Research in Engineering & Advanced Technology, Volume 1, Issue 2, April-May, 2013
ISSN: 2320 - 8791
www.ijreat.org

A Comparative Overview of Ethanol Production from Cereal Grains and Potato by Enzymatic Treatment

Soumitra Banerjee[1], Debalina Kundu[2]

Dept of Food Technology, Techno India Saltlake, Kolkata 700091

Abstract

A comparative study was made for production of alcohol from cereals like Rice and Barley and starch rich tuber crop Potato. For this initially all the starches were subjected to gelatinization followed by Liquefaction by (Liquozyme® SC) and Saccharification by Spirizyme® Fuel.Two methods were studied for each sample like Individual Saccharification and Fermentation(ISF) and Simultaneous Saccharification and Fermentaion(SSF).Yield of alcohol and rate of alcohol fermentation were studied for different processes.The sugar depletion rate for each type of fermentaion was also studied to correlate with the yield of alcohol and production rate.

***Keywords**: Gelatinistion, dextrinisation, liquefaction, saccharification*

1. Introduction

Recent years have seen the introduction of large scale processing in the bioconversion of biomass resources especially starchy raw materials, to ethanol, which is expected to find a wide range of uses as a bio fuel and as starting materials for various chemicals.

However, the present process for ethanol production from starchy materials via fermentation consists of two or three steps and requires improvement to produce efficient product at low cost. There are two main reasons for the present high cost : one is that as the yeast Saccharomyces cerevisiae cannot utilize starchy materials, large amount of amylolytic enzyme namely, glucoamylase and α- amylase need to be added, the other is that the starchy raw materials need to be cooked at a high temperature (140 ^{o}-180^{o} C) to obtain high alcohol yield . To reduce the energy cost for cooking of starchy materials the non-cooking and low temperature cooking fermentation system have succeeded in reducing energy consumption by approximately 50 % , but it is still necessary to add large amounts of amylolytic enzyme to hydrolyze starchy raw materials to glucose.

Grain-based ethanol had to be produced using malt or koji as the enzyme source. The grain-based ethanol industry did not become a viable source of fuel until industrial microbial enzymes became readily available like today.The use of microbial enzymes for alcohol production from starch was first reviewed by Aschengreen (1) and various enzyme-based cooking processes were described in 1981 .A review of the production of ethanol from whole grain was made by Lyons in 1983 (3), and later by Lewis in 1996(4) .Fuel ethanol is recovered by distillation after anaerobic fermentation using yeast, primarily species of Saccharomyces cerevisia.

TABLE 1

Raw material	Typical starch content in % (as is)	Gelatinisation temperature, °C	Alcohol yield(litres per 100 kg)	Protein content in %
Barley	54 – 65	53° - 63°	34 - 41	9.0-14.0
Maize	60 – 63	68° - 74°	38 - 40	9 .0-10.0
Manioc/Tapioca (Meal)	65 – 80	51° - 65°	40 - 50	0.5-2.0
Rye	55 – 62	55° - 70°	35 - 37	8.0-16.0
Sorghum	55 – 65	70° - 78°	36 - 42	8.0-10.0
Triticale	63 – 69	55° - 70°	40 - 44	13.0-16.0
Wheat	58 – 62	58° - 65°	36 - 39	10.0-14.0

Table 1. Overview of starch content, gelatinization temperature and expected yield of alcohol for various raw materials used for alcohol production.

Source : www.biokemi.org

2. Materials & Methods

In the dry milling process hammer mills with screens grind the cereals so that 60-90 % has a particle size of 250-350 µm. The resulting meal is mixed with water to form a mash. In case of Potato the mash was prepared by peeling, slicing followed by wet-milling. The starch was liquefied and pre-saccharified using first alpha-amylase (Liquozyme® SC) and then glucoamylase (Spirizyme® Fuel). The resulting sugar is cooled and transferred to the conical flask where yeast is added. The fermentation process time was 48 hours. Alpha-amylase may be added during the pre-liquefaction at 70-90°C and again during the post liquefaction at 85°C.

2.1 Viscosity reduction of the pre-slurry

Viscosity reduction is essential for alcohol processes when raw materials like rice, barley and potato are used because of the importance of easy mash stirring, pumping and avoiding local overheating. Problems can be encountered during both mashing and liquefaction due to high viscosity, which reduces the efficiency of heat exchangers, enzyme kinetics, and fermentation. Reducing the viscosity of mashes and liquids in all stages of the process will facilitate use of higher content of dry solids, energy savings, and higher production capacity of alcohol in a given plant. Furthermore better pumping using smaller equipment, the avoidance of local overheating, more successful cleaning (CIP) and higher overall throughput of the process are obtained. The overall result is a greater yield of ethanol. The extraction/ solubilisation of all viscous polysaccharides such as starch, celluloses, pentosans or beta-glucans during the process very much depends on the composition of the raw materials

2.2 Gelatinization of Starch Followed by Liquefaction

Gelatinization was carried out by heating the starch at 80-85^{0} C with/without addition of water.

2.3 The Liquefying Amylases

Liquefaction is easily accomplished at 35-38% solids when using Liquozyme® SC from Novozymes. However, above 38 % solids the slurry becomes increasingly viscous. Liquozyme SC is a liquid enzyme preparation containing a heat-stable alpha-amylase expressed in and produced by a genetically modified strain of a Bacillus microorganism. Liquozyme SC can operate at lower pH (pH=4.5) and at lower calcium levels than conventional thermostable alpha-amylases. This brings advantages to its application which all result in reduced operating cost. Liquozyme SC was introduced on the market in 1999 especially designed to decrease viscosity rapidly

2.4 Simultaneous saccharification and fermentation (SSF)

Here the liquefied and saccharified starch mass was subjected to fermentation by S.cerevisiae without stopping the enzymatic action.The enzymatic action and fermentation were carried out simultaneously yielding more sugar and subsequently more ethanol.Both continuous fermentation and batch fermentation are successfully utilised in the dry-milling processes. The advantages of continuous fermentation include the full utilisation of fermentation vessel capacities (no filling / draining /sanitisation), the ease of controlling continuous flows and the consistency of the products. The disadvantages are the susceptibility to infection from the whole grain and stillage recycle, and the disruption caused to production by the occasional sanitisation of the fermenters.

2.5 Saccharifying amylases (glucoamylases) for ethanol production

Spirizyme® Fuel is used to saccharify whole-grain mashes for ethanol production. This glucoamylase is used in simultaneous saccharification and fermentation (SSF) as well as pre-fermentation saccharification processes. It is produced by submerged fermentation of a genetically modified microorganism. It has higher activity and greater thermostability than traditional glucoamylases from Aspergillus niger. It allows saccharification systems to be operated up to 70°C. A greater flexibility in operating conditions is an advantage for an SSF process to follow.

2.3 Method for Alcohol determination

Determination by Potassium Di-chromate Oxidation method(www.outreach.canterbury.ac.nz)

ISSN: 2320 - 8791
www.ijreat.org

ETHANOL PRODUCTION FROM RICE BY ENZYMATIC PROCESS

50 gm of Rice powder is mixed with 150 cc of Distilled water

↓

Gelatinized by Autoclaving at 15 psi pressure for 15 mins. At 121^{o}C

↓

Enzyme Alpha-amylase (liquezyme) is dosed at 1% v/v of slurry & it is heated at water bath at 60^{o}C for 2 hrs. liquefaction is also knows as dextrinisation of starch.

↓

Sugar is estimated to be about 5.7%

↓

Saccharifying enzyme Amyloglucosidase is applied at about 0.8% v/v volume of slurry. It is heated in a water bath at about 90 – 92^{o}C for 4 hrs

↓

After 2 hrs contenrt of the slurry 11.4%

↓

After 4 hrs. sugar content of the slurry 13% volume of slurry is 220 ml.

ETHANOL PRODUCTION FROM POTATO BY ENZYMATIC LIQUEFATION AND SACCHARIFICATION:

1 Kg potato taken and peeled off and 500 gm taken on wet basis

↓

Gelatinized by autoclaving at 121^{o}C at 15 psi for 15 minutes

↓

Liquezyme (α-amylase) enzyme is applied at 1% v/v & the slurry is heated in a water bath at 60^{o}C for about 3 hrs

↓

Sugar content (D.E.) of the slurry is estimated about 5.6%.By liquefaction, gelatinized starch macromolecules undergo debranching α-1, 6 linkages, and converting it to dextrin, making it more amenable and conducive to saccharification

↓

Spirizyme (Glucoamylase) enzyme is applied at about 0.8% v/v. Slurry is saccharified at about 90^{o}C for 3 hrs. in a water bath

↓

Sugar content is estimated about 9.2%

ETHANOL PRODUCTION FROM BARLEY

1 kg of whole Barley is taken

↓

Barley are ground to powder form in the Mixer.

↓

Wt of the powder obtained = 816 gms.

↓

Powdered Barley are sifted for dehulling and degerming purpose in Sieve shaker

↓

Wt. of pure barley powder → 567 gms

↓

250 gms of powder taken + 700 cc of DW is added

↓

Gelatinized by autoclaving at 121^{0}C at 15 psi for 15 minutes.

↓

Gelatinized Starch liquefied by applying α-amylase enzyme at 1% v/v and liquefaction carried out at 60^{0} C for 3 hrs.

↓

Sugar content of slurry is estimated about 6.6%

↓

Liquefied slurry is further saccharified by applying Amyloglucosidase enzyme at about 90^{o}C for 3 hrs.

↓

Sugar content of the saccharified slurry is found to be 9.8%

↓

Growth culture is inoculated at the F.M

3. Tables, Figures and Equations

- Fermentation efficiency= Actual ethanol recovery/ Theoritical recovery x100
- Theoritical recovery= Total sugars x 0.64
- Actual ethanol recovery= Actual ethanol obtained

TABLE 2

SSF			
Name of Raw Materials	***Sugar Content***	***Alcohol Yield***	***Fermentation Efficiency(%)***
RICE	13 ±0.2	8.0±0.2	93.9
POTATO	10.0±0.2	6.0±0.2	96
BARLEY	9.8±0.2	6.0 ±0.2	91.2
ISF			
Name of Raw Materials	***Sugar Content***	***Alcohol Yield***	***Fermentation Efficiency(%)***
RICE	11±.2	7.5±0.2	91.7
POTATO	9.2±0.2	7.0±0.2	94.5
BARLEY	8.7±0.2	6.0±0.2	89.8

Table 2: Shows the summarized result of the processes carried out

Kinetic study of Fermentation Process of RICE

RICE		
RESIDUAL SUGAR		
TIME(hrs)	**ISF**	**SSF**
0	11	13
6	9	9.1
12	7.5	7.2
18	6.1	6
24	5.5	5.2
30	3.9	3.7
36	2.6	2.5
42	1.4	1.2
48	0.5	

ALCOHOL PRODUCTION V/V		
TIME(hrs)	**ISF**	**SSF**
0	0	0
6	1.2	1.8
12	1.5	2
18	3.1	3.5
24	3.8	4
30	4.3	4.7
36	5.2	5.5
42	6.5	6.8
48	7.5	8

ISSN: 2320 - 8791
www.ijreat.org

Kinetic study of Fermentation Process of BARLEY

BARLEY

RESIDUAL SUGAR		
TIME(hrs)	**ISF**	**SSF**
0	9.8	9.8
6	8.1	7.8
12	6.1	5.9
18	3.9	3.7
24	2.4	2.2
30	1.8	1.7
36	1.1	1
42	0.5	0.4
48	0.3	0.2

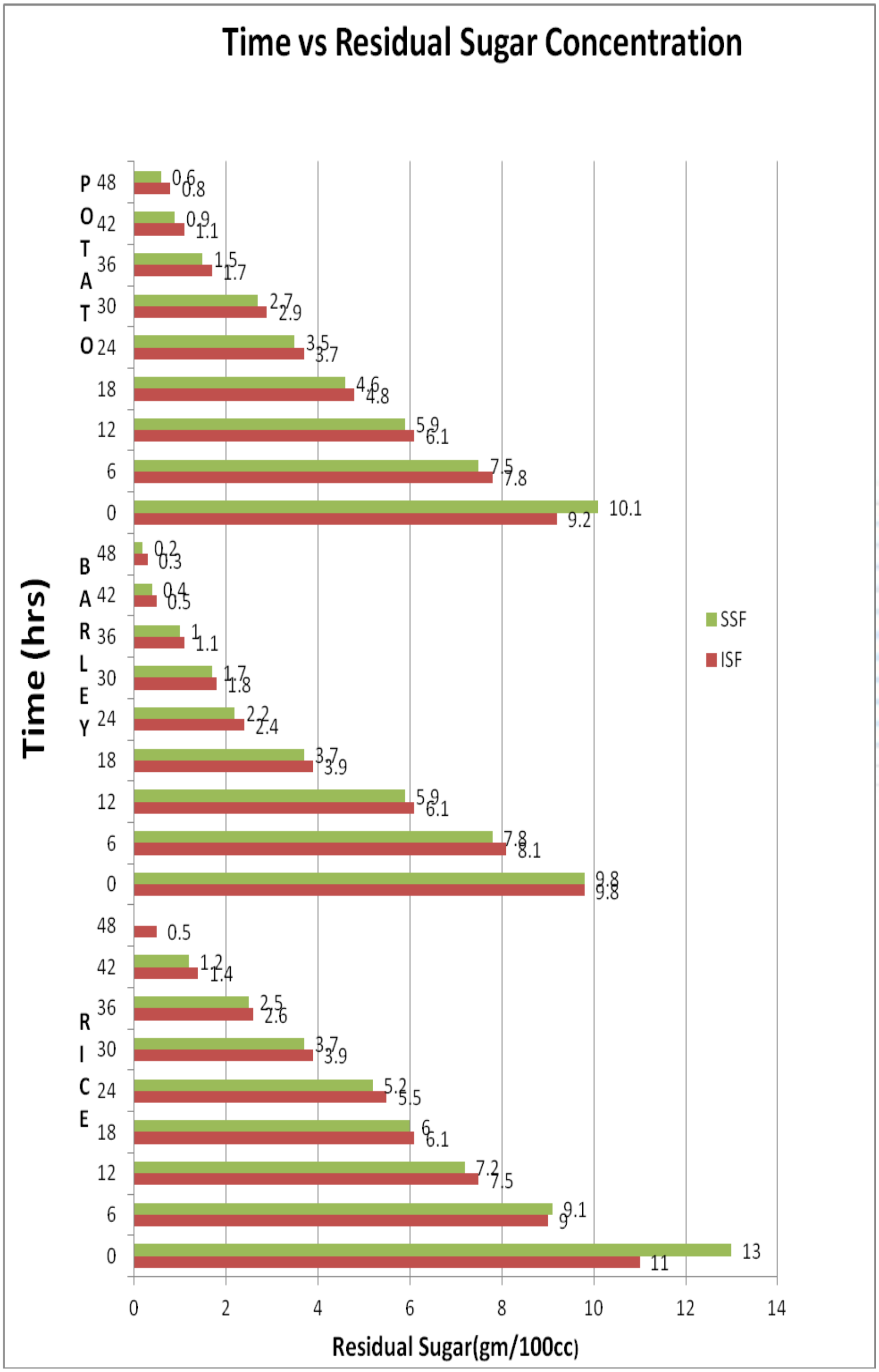
ALCOHOL PRODUCTION V/V		
TIME(hrs)	ISF	SSF
0	0	0
6	1.1	1.3
12	2.5	2.8
18	3.8	3.9
24	3.7	4.1
30	4.2	4.4
36	4.8	5
42	5.4	5.5
48	6	6

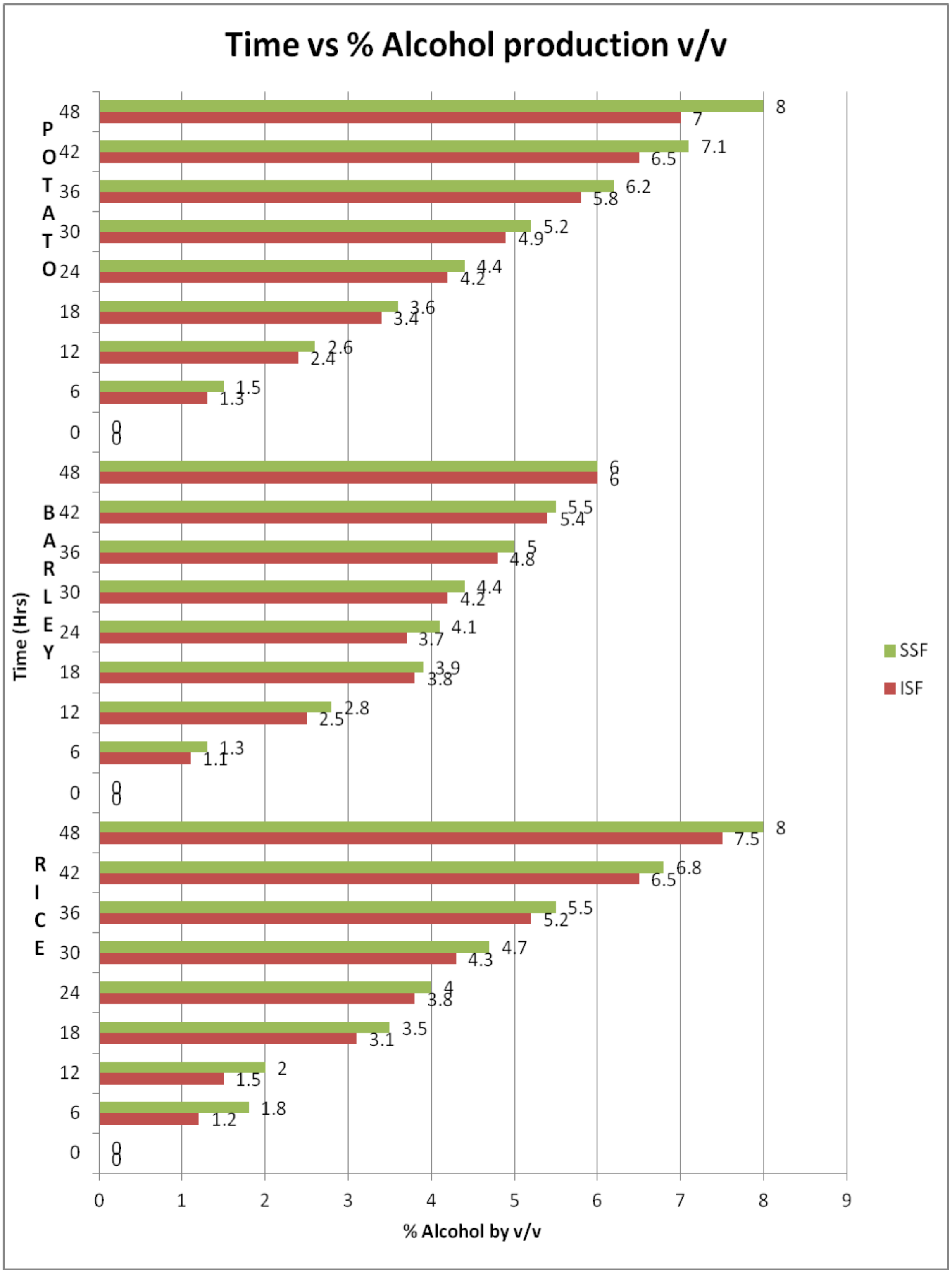
Kinetic study of Fermentation Process of POTATO

POTATO

RESIDUAL SUGAR		
TIME(hrs)	**ISF**	**SSF**
0	9.2	10.1
6	7.8	7.5
12	6.1	5.9
18	4.8	4.6
24	3.7	3.5
30	2.9	2.7
36	1.7	1.5
42	1.1	0.9
48	0.8	0.6

ALCOHOL PRODUCTION V/V		
TIME(hrs)	**ISF**	**SSF**
0	0	0
6	1.3	1.5
12	2.4	2.6
18	3.4	3.6
24	4.2	4.4
30	4.9	5.2
36	5.8	6.2
42	6.5	7.1
48	7	8


Time vs Residual Sugar Concentration
Time (hrs)
POTATO
BARLEY
RICE
48
42
36
30
24
18
12
6
0
SSF
ISF
0.6
0.8
0.9
1.1
1.5
1.7
2.7
2.9
3.5
3.7
4.6
4.8
5.9
6.1
7.5
7.8
10.1
9.2
0.2
0.3
0.4
0.5
1
1.1
1.7
1.8
2.2
2.4
3.7
3.9
5.9
6.1
7.8
8.1
9.8
9.8
0.5
1.2
1.4
2.5
2.6
3.7
3.9
5.2
5.5
6
6.1
7.2
7.5
9.1
9
13
11
0
2
4
6
8
10
12
14
Residual Sugar(gm/100cc)


Time vs % Alcohol production v/v
Time (Hrs)
POTATO
BARLEY
RICE
% Alcohol by v/v
SSF
ISF

References

(1) Aschengreen N.H. Microbial Enzymes for Alcohol production. Process Biochemistry, August 1969 3pp.(1969).

(2) Hagen, H. A. "Production of Ethanol from Starch-containing Crops - Various Cooking Procedures". Paper given at a Meeting on bio-fuels in Bologna, June 1981. Available as Available as Article A-5762a GB from Novozymes A/S, (1981).

(3) Lyons, T. P Alcohol – Power/Fuel. In Industrial Enzymology. (Ed. Tony Godfrey & Jon Reichelt), Macmillan Publishers Ltd., England, (1983).

(4) Lewis, S. M. Fermentation alcohol. In Industrial Enzymology. (Ed. Tony Godfrey & Stuart West), Macmillan Publishers Ltd., England, (1996).

(5) www.outreach.canterbury.ac.nz

(6) www.biokemi.org