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Ethanol Production from Corn, Potato Peel Waste and its Process Development

A.Meenakshi[1]*, R. Kumaresan[1]

[1]School of Chemical and Biotechnology, SASTRA University, Thanjavur,613402, Tamilnadu, India

Corres.author: meenakshiarun19@gmail.com(A.M), kumariisc@biotech.sastra.edu(R.K)

Ph no: 9003607214

Abstract: Corn is one of the richest sources for the production of ethanol. This project was carried out to study the optimum conditions for the production of ethanol. The parameters like, pH, Substrate concentration and particle size were optimized using response surface method in the MINITAB 16 software. Both solid and submerged fermentation were studied. Submerged fermentation turned out to be favourable. Yeast fermentation was employed simultaneously with the saccharification process (SSF) for 72 hours. An attempt was made to produce ethanol from Potato peel waste, however it proved corn as an efficient substrate. There was a considerable yield of ethanol of 15.88g/l using pH 5.5, an intermediate particle size of 0.157mm and at a substrate concentration of 10% (W/V). A process development for the entire production was made involving the reactor design and the equipments to be used at an industrial scale.
Keywords: ethanol, corn, simultaneous saccharification and fermentation, potato peel waste, process development.

1. Introduction:

The pursuits of sources for renewable alternate fuels have always been the mankind's interest. There is a growing concern about the price hikes and environmental problems due to the usage of petrol and diesel. So it is a necessity that we look up to each and every probable source of energy. Ethanol could be produced from rich sources like corn, sugar beet, sweet sorghum, sweet potato or from the abundant cheap cellulosic feedstocks like wheat straw, switch grass, wood etc known as cellulosic ethanol.In order to prevent the use of staple crops, agricultural wastes like corn cobs, stover, sorghum stalks and byproducts from sugar industries like sugarcane molasses etc are considered as cheaper sources of ethanol,.In this project corn was a choice of substrate as it requires very little processing when compared to the cellulosic substrates, moreover the yield is also higher.[1] Potato peel waste was also tried for ethanol production based on the fact that it consisted of about 58% of dry weight as starch.[2]

In India Ethanol is produced mainly produced from Sugarcane molasses which is a byproduct obtained from the sugar industry .Since it is rich in glucose it is easily converted into ethanol by yeast. They are known as First generation fuels as they are made from seeds, grains, sugars.[3] While corn is the most widely used feedstock in the western countries like Brazil and the USA. Petrol and diesel are the most widely used fuels for automobiles in India with exception of a very few places using Natural gas. Bioethanol can be blended with gasoline/petrol known as gasohol for use in vehicles. The amount of environmental pollution caused by the fossil fuels by means of its products of combustion like Carbon dioxide, carbon monoxide, nitrogen oxide and sulphur is more when compared to natural gas, bioethanol and biodiesel.

Apart from the known usage of ethanol as a fuel, about 45% of the produced ethanol is being used as potable alcohol, 40% in the industrial sectors and only the remaining is available for blending with petrol. In the industrial sectors ethanol is used by chemical, pharmaceutical industries etc.It is also used to produce Ethyl Tertiary-Butyl Ether (ETBE) [3]

There are three methods of pretreatment of the biomass namely; physical, chemical and Biological. At an industrial level biological process is the preferred since it does not produce any unnecessary byproducts. The targeted reactions are only carried out which is required for the product formation thus the remaining biomass after product extraction could be used for any other purposes like animal feed. Since all the biological reactions happens at optimum conditions, the production cost is also less when compared to other methods.[4] Starch is made up of amylose and amylopectin.It is made up of alpha 1,6 and alpha 1,4 linkages.When Starch is cooked at a high pressure and temperature, it is gelatinized, enabling the enzymes to access and digest the polymers[5].Dry grind method was used in this project for the production of ethanol, mainly because it is economical, gives high yield and also because the whole corn kernel could be used unlike the wet milling process that involves the separation of starch, germ,fibre etc.[6]

Milling
↓
Liquefaction
↓
Simultaneous saccharification and Fermentation
↓
Centrifugation
↓
Distillation

Fig.1. Outline of the dry grind process

When it comes to the fermentation process yeast is the most widely used organism. Simultaneous saccharification and Fermentation(SSF) process was used.In a single reactor, yeast is added along with the saccharifying enzymes which produces the glucose units and they were immediately converted into ethanol.In this way there are no chances of glucose accumulation and also the produced ethanol prevents the microbial contamination.[4]

The yield of ethanol could be calculated using the following equation. [7]

$$\text{Glucose} \xrightarrow{\text{Yeast}} \text{Ethanol} + \text{Carbondioxide} + \text{Heat}$$

1mole	2 moles	2 moles
180g	92g	88g

The selection of micro organism is one of the important factors for the production as it should be able to withstand the osmotic pressure and tolerance to ethanol. Yeast has been a commonly used organism since several decades and hence the same was used.[8]

Both solid and submerged fermentation were carried out to check the efficiency. Solid state fermentation was tried for the production of ethanol due to the suitability of the organism for such fermentation. Yeast very well adapts to a low moisture containing environment. It does not require agitation or aeration thus there are no energy requirements.[9]

2. Materials and Methods:

2.1. Materials:

Corn was obtained from a local market in Thanjavur.PPW was obtained from a local restaurant.

2.2. Enzymes:

Alpha amylase and Glucoamylase were obtained from S.V Biotech Pvt Ltd, Thanjavur.

2.3. Microorganism:

Baker's yeast *Saccharomyces cerevisae* was obtained from a local market in Thanjavur.

2.4. Methods:

2.4.1. Milling:

Thoroughly dried Corn kernels were milled in a flour mill to obtain a coarsely milled powder. Potato peel waste (PPW) was washed thoroughly, sun dried and powdered using a food processor. The corn flour and PPW powder were size separated using sieve shaker. The flour and powder retained in 3 different mesh size were collected and stored under air-tight condition.

2.4.2. Liquefaction:

In a 250ml conical flask, 5-15g of corn flour of a chosen particle size was added along with 100ml of distilled water. It was cooked at 121°C and 15 psi for 30 minutes in an autoclave. The gelatinized corn was then allowed to cool down followed by the addition of alpha amylase (579U/g) enzyme (0.08g/g of corn).The flask was maintained in the stirrer for 2 hours at 90°C and at 150rpm[10].At this step usually the pH is 6.0, if not it is adjusted using 3N NaOH or 3N Orthophosphoric acid.

2.4.3. Simultaneous Saccharification and Fermentation:

Glucoamylase enzyme (1346U/ml) was added to the liquified corn flour. Baker's yeast was also added (0.1g/g of corn).The mixture was allowed to ferment for 48-72 hours at room temprature in an orbital shaker at 150 rpm. Periodically 10ml of sample was withdrawn and centrifuged for reducing sugar and ethanol analysis by DNS (Dinitro salicylic acid) and potassium dichromate methods respectively.

2.4.4. Centrifugation and Distillation:

After fermentation, the broth was centrifuged at 6000rpm for 10 minutes. The supernatant was collected and fed into a simple distillation column. The boiling temperature of ethanol is 78°C hence distillation was carried out around that temperature to facilitate the evaporation of ethanol. The vapour was collected and got condensed by means of the circulation of cold water around the column. The distillate having ethanol was recovered in a conical flask at the other end of the column.

2.5. Potato Peel Waste (PPW) as feedstock:

Sieved PPW of particle size 0.211mm was used for the production. Same kind of procedure was followed using the optimized parameters of corn.

2.6. Solid state fermentation:

It is the type of fermentation which uses minimal amount of water.It is possible only if microorganisms could survive in low moisture content.Yeast is very much suitable for such conditions, thus solid state fermentation was carried out using corn and potato peel waste.

The amount of water to be added can be calculated using this formula:

$$\textit{Required moisture content} = \frac{\mathbf{X}}{\mathbf{X + amount\ of\ substrate\ used(g)}}$$

X- is the amount of water to be added (ml)

2.7. Analysis:

The compositional analysis of corn was done for estimation starch using anthrone reagent [11], protein using Lowry's assay [12], crude fibre [13], moisture [14] and ash content [15]

The reducing sugar concentration was found using DNS method.[16] The difference in sugars produced before and after the fermentation was used to find the amount of reducing sugars produced. The ethanol product confirmation was done using the potassium dichromate method.[17] It gave the percentage alcohol content of the sample.

2.8. Gas chromatography-mass spectrometry:

A PerkinElmer Clarus 500 GCMS was used to analyse the double distilled ethanol.Helium was used as the carrier gas in a Capillary Column Elite-5MS (5%Phenyl 95% dimethylpolysiloxane) of length 30m.After the separation of compounds in the sample they were identified using electron ionization in mass spectrometry.

2.9. Response Surface Methodology

Response surface methodology was used for creating an experimental protocol using which the process variables were optimized. It determines the optimum conditions using the statistical techniques. It optimizes all the independent variables with a minimal number of experiments.[18] It combines special experimental designs with Taylor first order and second order equations. The experiments were designed using MINITAB 16 software in such a manner that several combinations of the process variables are taken into account. The important process variables which were included for optimization are:

1) Particle size 2) pH 3) Substrate concentration

Twenty experiments were carried out according to the RSM design.

3. Results and Discussion:

Table 1 Results for compositional analysis of corn:

Component	Percentage(%)
Starch	75
Protein	9.4
Crude fibre	1.6
Moisture	6.5
Ash	2.12

3.1 .Effect of Substrate concentration:

Substrate concentrations of 5,10 and 15% were used for the production of ethanol, out of which 10% gave the highest yield. Usually the yield is more as the substrate concentration increases, but in this study after a concentration of 10% (w/v) the yield reduced. This could be a result of substrate inhibition.[19]

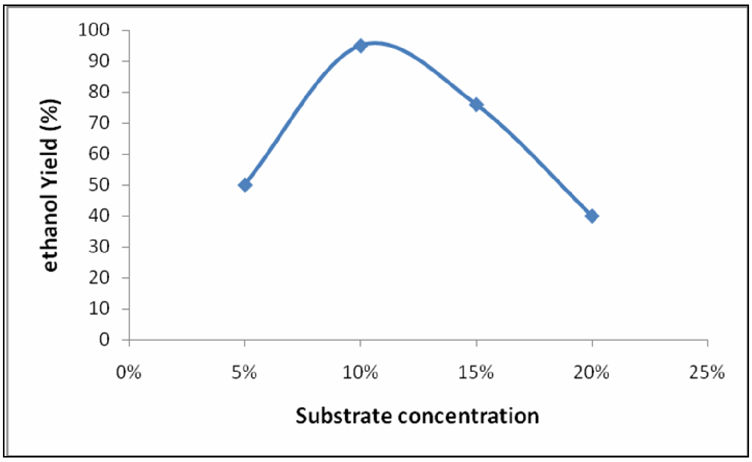

Fig 2. Effect of substrate concentration on ethanol yield

3.2. Effect of pH:

Yeast was capable of fermenting glucose best at an acidic environment.There was a maximum yield observed at a pH of 5.5.The yield was lower at an acidic pH like 2 or 3 and even lower when the fermentation was carried out at a basic pH like 7 or 8.At an acidic pH there is always a chance of death of yeast cells. This indicates that the glucose metabolism is affected with the changesin pH which eventually affects the ethanol production

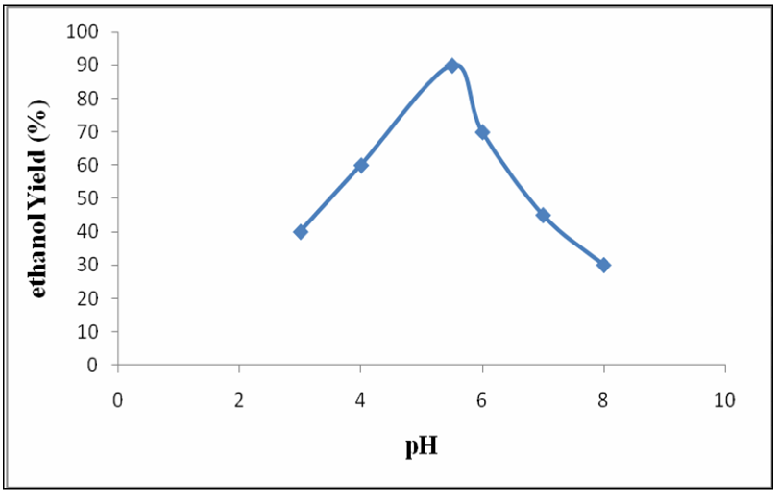

Fig 3. Effect of pH on ethanol yield

3.3.Effect of Particle size:

Three different particle sizes were used for optimization 0.104, 0.157 and 0.211mm.As the particle size decreases the surface area available for the hydrolysis reaction is more, producing maximum glucose units that is possible eventually it is all fermented to ethanol. In this study ethanol yield was observed higher using an intermediate particle size (0.157mm).Using a bigger particle size reduces the degree of gelatinization as a result the substrate is not fully available for hydrolysis by the enzymes.[20]

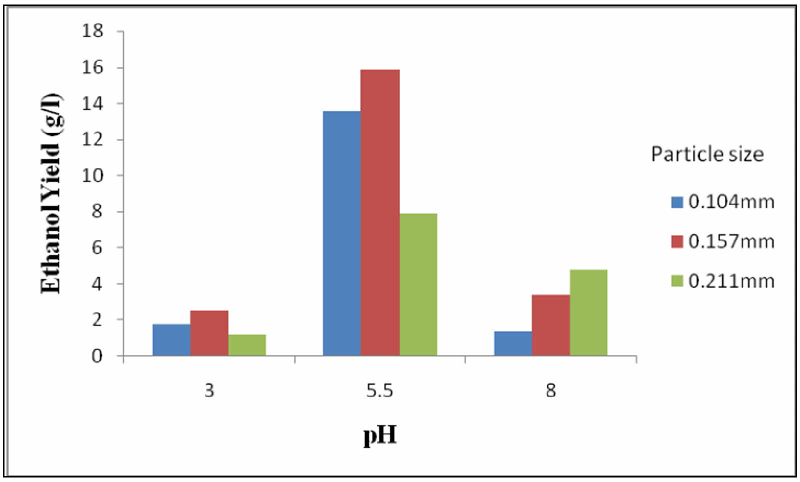

Fig 4. Effect of particle size on ethanol yield

3.4. Solid state fermentation:

A substrate concentration of 10%(W/V) with a moisture content of 75% gave a better yield in both corn and potato peel waste(PPW) .

Table 2 Ethanol yield at various moisture conditions

Feedstock	Substrate concentration(%)	Moisture (%)	Ethanol yield (g/l)
Corn	15	70	3.22
Corn	10	75	4.15
Corn	15	80	2.86
PPW	10	75	0.40
PPW	15	80	0.25

3.5. MINITAB Results:

The table 3 shows the experiments designed using MINITAB 16.The observed ethanol yield for the experiments were recorded for analysis using the regression coefficients and P values.

Table 3: Results for the production of ethanol

StdOrder	RunOrder	PtType	Blocks	particle size (mm)	pH	Substrate concentration(%)	Ethanol yield(g/l)
1	1	1	1	0.104	3	5	1.639
2	2	1	1	0.211	3	5	3.223
3	3	1	1	0.104	8	5	2.124
4	4	1	1	0.211	8	5	7.998
5	5	1	1	0.104	3	15	4.567
6	6	1	1	0.211	3	15	7.478
7	7	1	1	0.104	8	15	3.4567
8	8	1	1	0.211	8	15	7.8934
9	9	-1	1	0.067524	5.5	10	1.897
10	10	-1	1	0.247476	5.5	10	7.884
11	11	-1	1	0.1575	1.295518	10	2.879
12	12	-1	1	0.1575	9.704482	10	5.669
13	13	-1	1	0.1575	5.5	1.591036	3.234
14	14	-1	1	0.1575	5.5	18.40896	7.456
15	15	0	1	0.1575	5.5	10	15.34
16	16	0	1	0.1575	5.5	10	15.88
17	17	0	1	0.1575	5.5	10	14.667
18	18	0	1	0.1575	5.5	10	15.234
19	19	0	1	0.1575	5.5	10	13.98
20	20	0	1	0.1575	5.5	10	15.23

R-Sq = 99.13%   R-Sq(pred) = 95.64% R-Sq (adj) = 98.35%
The responses were expressed in the form of second order polynomial equation and the variability in R^2 value is explained by the equation given below.

$$\text{Ethanol yield} = -53.9181 + 386.683P_S + 6.50351pH + 3.21121C_S$$
$$-1212.75P_S^2 - 0.590266pH^2 - 0.132420C_S^2 + 5.43523P_S \times pH - 0.0595490pH \times C_S$$

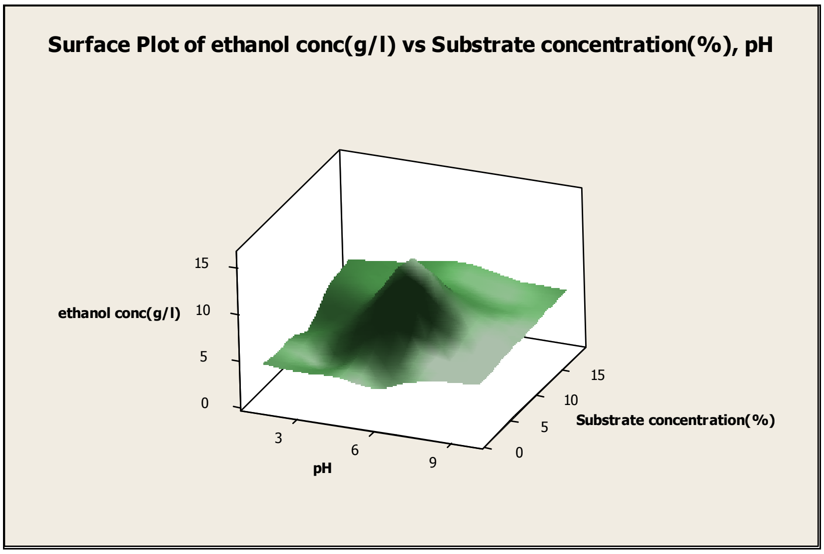

Fig.5 Surface plot of Ethanol concentration Vs substrate concentration and pH

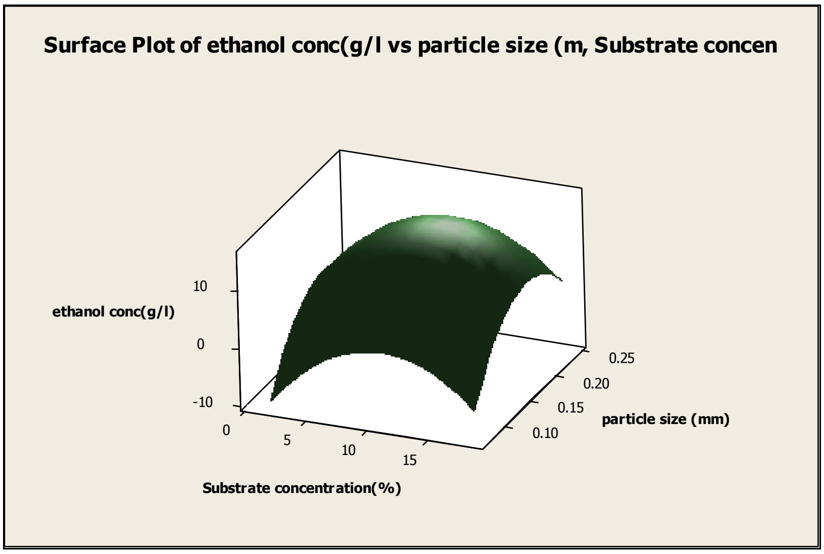

Fig .6 Surface plot of Ethanol concentration Vs particle size and substrate concentration

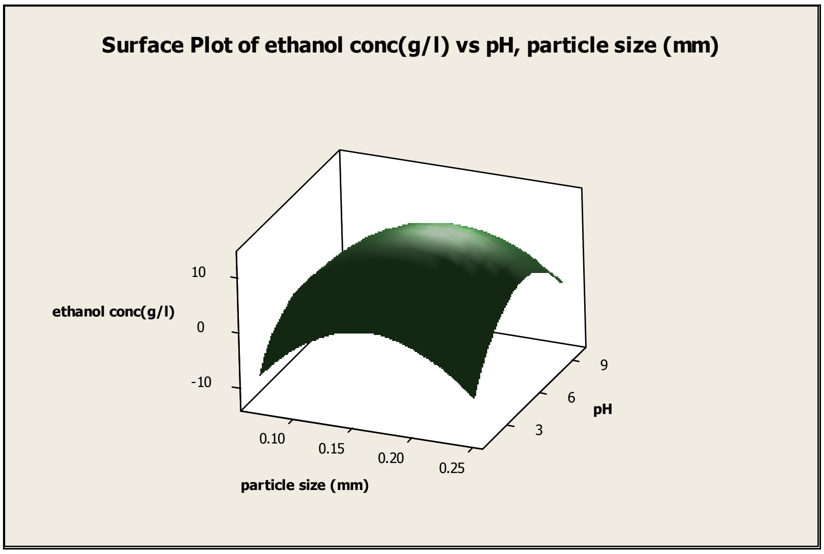

Fig.7 Surface plot of Ethanol concentration Vs pH and particle size

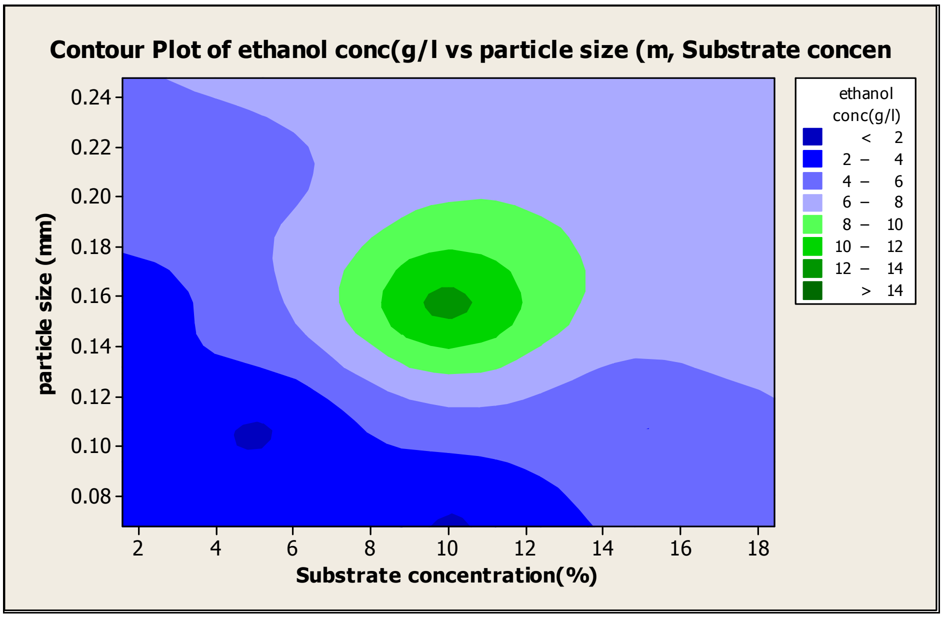

Fig.8. Contour plot of Ethanol concentration Vs particle size and substrate concentration

3.5.1. Interpretation:

The above plots were obtained using MINITAB 16 depicting the effect of particle size, substrate concentration and pH on ethanol production.Fig.5,6,7 is a surface plot depicted in a 3-D form. It visually demonstrates the effect of various parameters (independent variables) on the dependent variable. It was observed that a maximum yield of ethanol of 15.8g/l was obtained. In fig.8 the contour plot indicates the yield of ethanol using different colours.The dark green spot at the centre shows the corresponding maximum ethanol production among the other corresponding colours indicating lower yields. At 10% of substrate concentration and 0.157mm particle size and pH 5.5, the ethanol production is above 14g/l.

According to alternate and null hypothesis,the p value obtained from p-test were less than 0.05, which proves that all the parameters considered for optimization had a significant effect on the ethanol produced.

3.6. Gas chromatography-mass spectrometry(GCMS):

The results showed that the sample contained a significant amount of ethanol of 97.84% along with three other impurities that constitutes only 2.15%. This proves that corn is an efficient substrate for the production of ethanol.

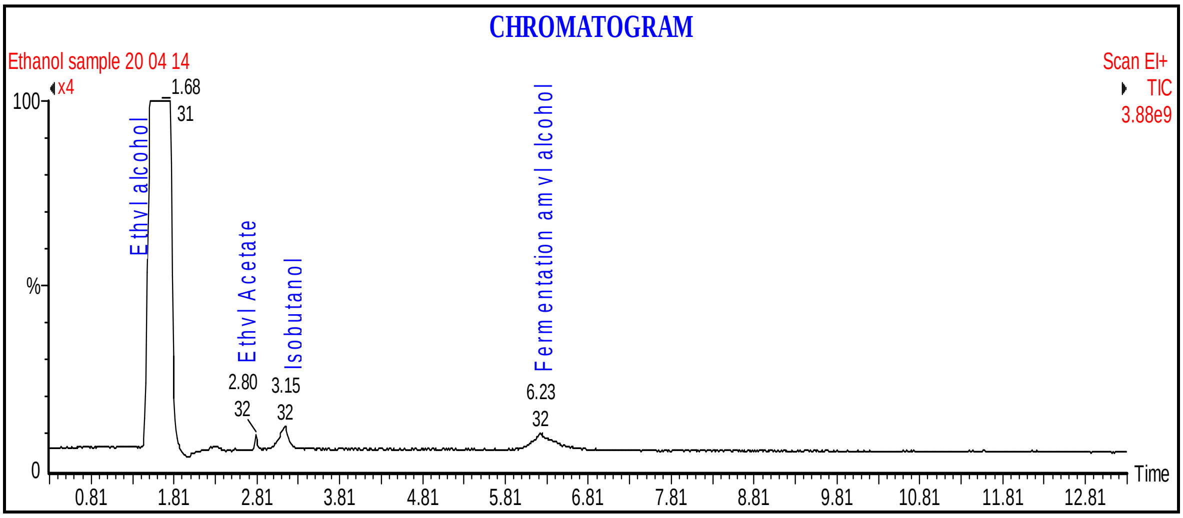

Fig.9. Chromatogram showing % Concentration vs. Retention time

Table 4 List of compounds:

S.no	Compound	% composition
1.	Ethyl alcohol	97.84
2.	Ethyl acetate	0.1
3.	Isobutanol	1
4.	Fermentation amyl alcohol	0.9

3.7.Process Development for Scale up of production:

A small study was made on scaling up of this project to an industrial level. Using simple calculations based on the results of the project, a process was developed to produce 1000litres of ethanol. This requires 5 tonnes of corn as feedstock, 397.5 kgs of enzyme α-amylase, 99.37litres of glucoamylase and 745 kgs of Baker's yeast. Water consumption would be 50kilolitres.The production process was designed for a batch production.Since the reactions are anaerobic, agitation alone was sufficient in the reactors.

As shown in the fig.10 a hammer mill was chosen for milling the corn to a flour. A jet cooker using a high pressure and temperature could be used for gelatinization of corn. For liquefaction a batch reactor was designed, Then the feed is passed on to another similar reactor for simultaneous saccharification and fermentation.72 hours later, the fermented mash is sent to distillation column for the separation of ethanol via evaporation and condensation. The left over heavy stillage containing the solids along with the liquor is centrifuged using a disc centrifuge. The settled solids are dried in a rotary dryer and is collected in the form of Distillers Grains (DG). The thin stillage is passed on to the evaporator to separate the Distillers soluble from the fine solids so that it could be combined with Distillers Grains to produce Distillers Dried Grains with Solubles (DDGS).To the ethanol separated, a denaturant is to be added to make the alcohol unfit for potable purposes.

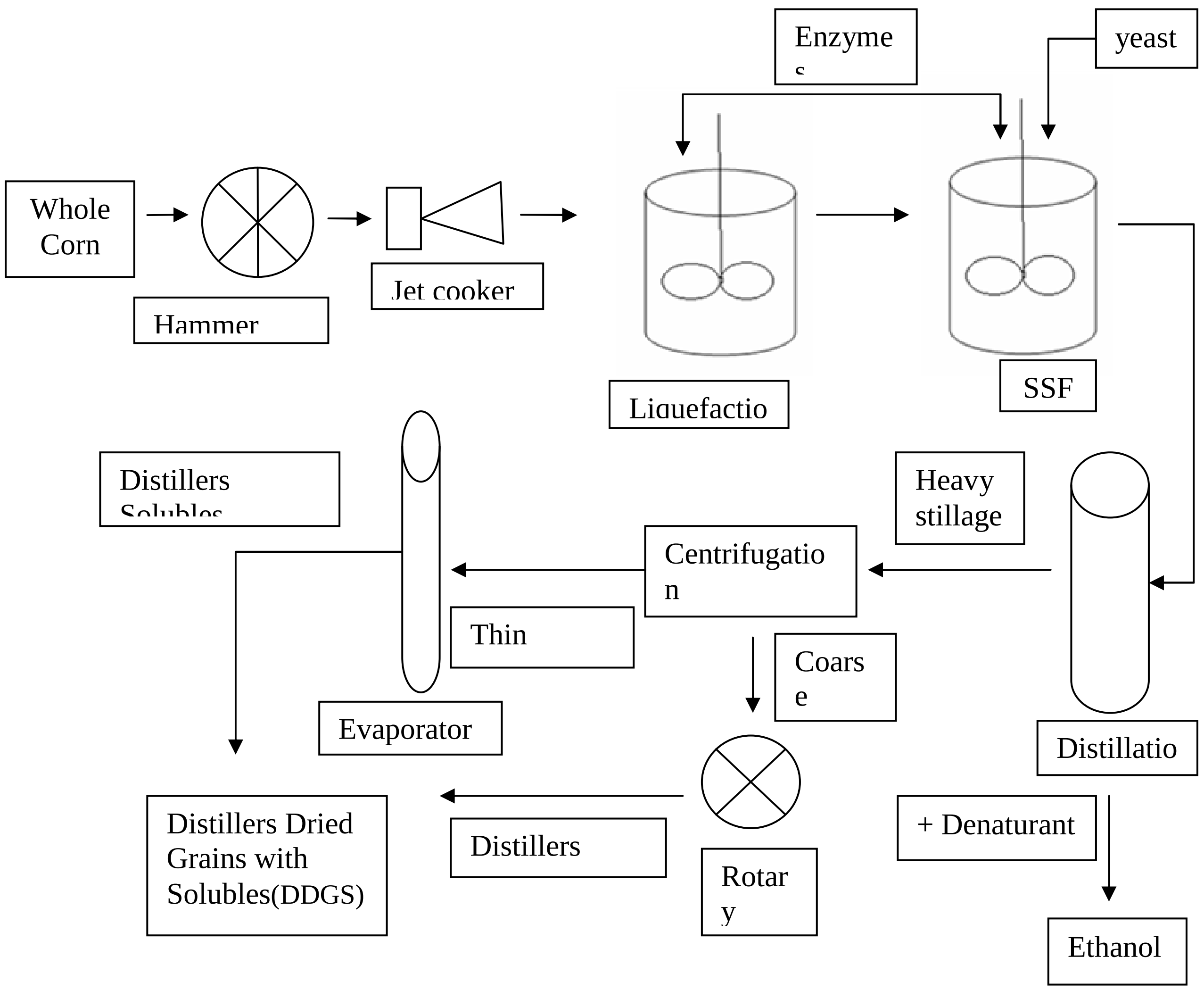

Fig.10. Design of Process development

3.8. Fermenter Design:

A batch fermenter for operation under anaerobic conditions was designed for a volume of $3m^3$. The height to diameter of the tank ratio was taken as 2.5.The tank diameter to impeller diameter ratio was taken as 3.[21] The dimensions of the fermenter components and the power requirements were calculated whose details are given below in Table 5.

Table 5. Calculated Design parameters.

S.No	Design parameters	Values
1.	Tank diameter	1.15m
2.	Impeller diameter	0.38m
3.	Height	3.16m
4.	Ungassed power	60.16KW
5.	Shaft diameter	24.1cm
6.	Shell thickness	4.1mm
7.	Head thickness	1.845mm
8.	Number of impellers	6

4. Conclusion:

The potential of corn as feedstock for the production of ethanol was studied. The choice of substrate, enzymes, microorganisms and the conditions under which they operate were found to be crucial for the yield of ethanol. Under the optimized conditions of pH, particle size and substrate concentration of 5.5, 0.157mm and 10% (W/V) respectively, the ethanol yield was 15.88g/l. Potato peel waste was also used for the production, however the yield was not comparable with that of corn. Among the methods of fermentation, submerged and simultaneous saccharification and fermentation were found to be more productive compared to solid state. A process development was made for a case of a scale up of the entire production process to an industrial level. This includes a calculation for a 1000litres of ethanol production, choice of equipments and the design of fermenter.

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