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Optimization of Fermentation Conditions for Ethanol Production from Renewable Biomass Using Response Surface Methodology

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Abstract

Potato Peel Waste (PPW) is a potential lignocellulosic biomass substrate for bioethanol production due to its high starch content and easy availability. In this study, we performed research optimization of fermentation process by Response Surface Methodology (RSM) using Plackett-Burman design. The process herein included acid-base pre-treatment of biomass, which was then followed by enzymatic hydrolysis as a potential step. The concentration of reducing sugar in the hydrolysate thus obtained was then analyzed by DNSA method. After fermenting the hydrolysate with *Saccharomyces cerevisiae* for several days, distillation was done. Analysis of hydrolysate was done by FTIR. Pre-treatment is used for lignocellulosic biomass for improving the hydrolysis of the potato as it contains a high amount of cellulose and removal of lignin and hemicellulose. Cellulose converts into the reducing sugars and then to ethanol. Saccharification and fermentation methods were performed to acquire maximum yield of ethanol. The potato peels were pre-treated with Sulphuric acid and sodium hydroxide solutions.

Keywords: Bioethanol; Process optimization; Response surface methodology; Potato peel waste; Hydrolysis

Introduction

The natural resources are limited in supply, but the demand for energy is increasing day by day. Also burning of fossils fuels releases large amount of CO_2 into the atmosphere which is the cause of increase in global temperature. In order to overcome all these problems, we need to look for all the available alternate energy resources. The ethanol production market worldwide grew from nearly a billion liters in 1975 to more than almost 39 billion liters in 2006 and is further about to reach upto 100 billion liters in 2025. In the present scenario, production of bioethanol is through extraneous sources like sugar cane juice, sugar beets, cereals, organic waste and cellulosic feedstock.

Production of ethanol from glycerol, glycerol-containing waste products, discharged from biodiesel manufacturing can be converted into hydrogen and ethanol by usage of *Enterobacter aerogenes* HU101 as the suitable organism, isolated primarily as increased-rate hydrogen producers from methanogenic sludge wastes. The ethanol concentration yield reported has been low, although many organisms in nature have been ideally identified as potential producers of ethanol that uses glycerol as substrate/source. Hao et al. [1] also isolated different microorganisms with potential of producing 1,3-propanediol by fermenting glycerol in aerobic conditions. But, the literary evidence regarding the aforesaid screening of potential microorganisms helpful in bio-conversion of glycerol into ethanol is highly limited.

The concept of "Second generation" bioethanol, with usage of lignocellulosic material as a primary feedstock, is a very potential alternative in contrary to first generation bioethanol. An overview study of the current status of bioethanol production reveals the bottlenecks that hamper its implementative processing and analysis. As part of the ongoing research and the literature provided by far specifies a method of conversion of biomass to bioethanol of 30 to ~50% yield only. Substantial novel processes have increased the conversion yield to up to 92% of the theoretical yield provided. These new combined processes thereby greatly reduce both the number of method-operational steps and the simultaneous production of inhibitors.

Recent research progress in genetically-modified microorganisms is highly promising in terms of increased alcohol tolerance and potential conversion efficiency. As a result, combining advanced research systems and by intensive research expertise to eliminate current congestion, naive second-generation biofuels (bioethanol) could efficiently surpass the traditional first-generation processes. In the present study, we investigate the co-production of bioethanol and probiotic yeast biomass from enzyme-pretreated grass juice. In addition to it, there has been a primary interest in the usage of lignocellulosic biomass from agriculture-related products as potential resource for the wide scale production of bioethanol(biofuel), more importantly because of the ever-increasing price rate for crop production and harvesting (e.g. corn, rice, etc.).

With reference to above stated, sugar cane bagasse and/or its products wheat straw husks, rice straw residues, corn stover, corncob products and also corn hull (i.e., corn fiber) are at present being researched investigated for their usage as important sources of high-quality bioethanol production [2-5]. Until now, as reported by their fermentable sugar composition, required optimal fermentation processes, and methods/strategies for their resulting hydrolysates have been examined. Before the bioethanol fermentation process, acid hydrolysis as a pre-treatment step of these byproducts has been carried out by using dilute range solutions of sulfuric acid (in particular), and phosphoric acid, or hydrochloric acid [2-4]. Addition to this acid hydrolysis, utilization of enzymatic hydrolysis as the next potential step and under combined hydrolysis methodologies using both the enzymes and dilute acids has been reported. Corn hull is a residue produced from the corn wet-milling process. Its sugar composition varies (glucose 10~ 50%, xylose 13~49%, arabinose 10~31%, and

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galactose 3~10%) depending on its origin, hydrolysis method, and corn processing method. Nowadays, most corn hull is used as animal feedstuff, and its utilization for bioethanol production has only recently been examined. Bioethanol provides a very good substitute as it can be produced from present fermentation technology.

Potato peel utilization for bioethanol production is a considerable option as 58% of the dry weight is starch. Three types of fermentation process (batch, fed-batch, and continuous) are employed in ethanol production from these sugar juices. The most common microorganism used in fermentation from its history is the yeast, especially, *Saccharomyces cerevisiae*, though the bacterial species *Zymomonas mobilis* is also potentially used nowadays for this purpose. A number of factors related to the fermentation greatly influence the process and their optimization is the key point for efficient ethanol production from this feedstock. The several steps involved in production of ethanol are listed (Figure 1).

The important thing required for production of ethanol from peels is the efficient yield fermentable hydrolysate rich in glucose. Enzymatic hydrolysis although costly, but provides better yield in future. Pre-treatment methods are employed to increase efficiency of enzymatic hydrolysis. There are basically two pre-treatment methods physical and chemical methods. Of which acid-base hydrolysis is one of the most common methods followed by enzymatic hydrolysis. In which mineral acid is used in very low concentration.

Analysis

FTIR (Fourier Transfer Infrared Spectroscopy)

In the electromagnetic spectrum infrared region lies between visible and microwave region. The energy associated with molecular vibration fall in the IR region as they are lower than electronic transition. A molecular vibration absorbs IR radiation if the vibration causes change in dipole moment.

FTIR is used to identify organic molecules. Absorption by the molecules is measured against wavelength. An FTIR uses an interferometer which generates radiation; absorption of wavelength brings change in interferogram which gets detected. An interferogram is a time domain signal and is converted to frequency domain signal though Fourier Transformation. FTIR plots are usually % transmittance or absorbance versus wave number. Absorption bands in 4000-1500 cm^{-1} are generally due to functional groups, peaks below this region are due to complex vibrations of several atoms (Table 1). The sample can be used in different forms (solid, liquid or gaseous) depending upon the instrumentation used.

Bond	Molecules	Wavenumber (cm^{-1})
C-O	Alcohols, Ethers, Esters, Carboxylic Acids, etc.	1300-1000
C=O	Aldehydes, Ketones, Esters, Carboxylic Acids.	1750-1680
C=O	Amides	1680-1630
N-H(stretching)	Amines and Amides	3500-3100
-N-H(bending)	Amines and Amides	1640-1550
O-H	Alcohols	3650-3200
C-N	Amines	1350-1000
S-H	Mercaptans	2550

Table 1: Typical vibrational frequencies of functional groups.

Reducing sugar estimation

3,5-Dinitrosalicyclic acid used for reducing sugars estimation, involves oxidation of free carbonyl groups. DNSA is reduced to 3,5-amino-nitrosalicyclic acid which gives reddish brown color complex under alkaline conditions, with an absorbance maximum at 540 nm (Figure 2).

Response surface methodology

The study employed Response Surface Methodology for optimization of different conditions that affect fermentation temperature, acid concentration for hydrolysis, etc. Response surface method provides us scope for improvement and optimization of the desired response which is influenced by various variables [6]. Response surface methodology is defined as collection of techniques,

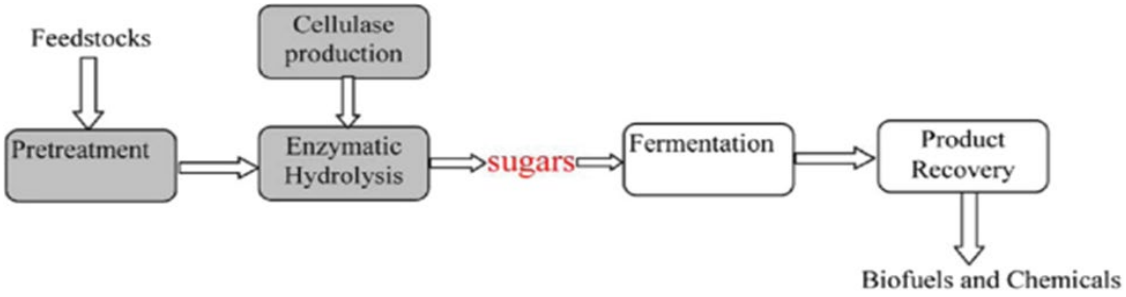

Figure 1: Process flow diagram.

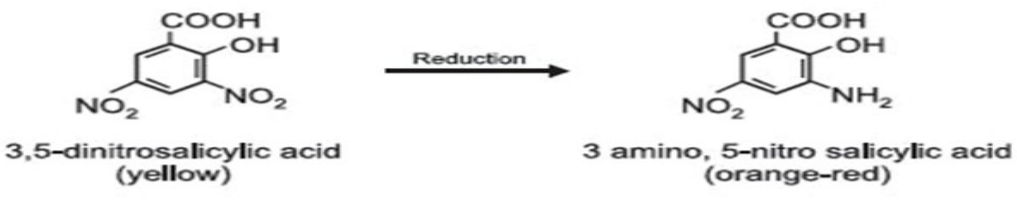

Figure 2: DNSA treatment.

mathematical and statistical both based on polynomial equation fit of the experimental data. The objective of this technique is to simultaneously optimize levels of variables for best possible results. Stages involved in RSM are as follows:

- Screening of variables - It is very difficult to study all the variables affecting the response. Therefore, the variables with major effect are selected.
- Choice of experimental design - A good experimental designs ensures that all variables are studied at three factor levels.
- Mathematical statistical treatment of data.
- Evaluation of fitted model.

Literature Review

The study discusses the issue of pre-treatment of lignocellulosic biomass so that desired sugar yield is obtained after enzymatic treatment. They performed consecutive dilute sulfuric acid-dilute NaOH treatment on Sugarcane bagasse. This enhanced the cellulase accessibility in embedded cellulose microfibrils [7]. Production of ethanol from potato pulp is mainly influenced by starch hydrolysis into monosaccharide. α-amylase and amyloglucosidase concentration affects hydrolysis greatly. The size of pulp and its concentration also influence hydrolysis [8]. Study on the very least studied *Pichia veronae*, to find optimum condition of ethanol production from this organism by response surface methodology based on central composite design [9]. This study basically focuses on utilization of high amount sweet potato residues generated in China for ethanol production. They studied various enzyme systems performance on SPRs hydrolysis and also role of cellulase and pectinase in it [10]. This study is about up-scaling of bioethanol and bio hydrogen production from *Enterobacter aerogenes* under semi-anaerobic conditions. Different conditions like fresh feed rate, liquid recycling etc. optimization was done to find the best possible conditions [11]. The study done utilized RSM and central composite face centered design to find best possible condition to maximize ethanol production in batch fermentation from sugarcane molasses using *Saccharomyces cerevisiae* Y-39 [12]. The paper focuses on feasibility of outdoor cultivation of carbohydrate rich microalgae *Scenedesmus obliquus* CNW N and its use as feedstock for ethanol production in long term batch operation. They studied time profile of content of carbohydrate under deficient conditions of nitrogen to check seasonal changes [13]. Water hyacinth is one of the most invasive weed of the world but die its high availability and large carbohydrate content it is also shows the ability to be a potential substrate for bioethanol production. In this study they tried to find out the solution of the problems associated with its hydrolysis and fermentation process. Pre-treatment of raw material and simultaneous saccharification and fermentation resolved both of the problems [14]. *Pagnum harmala* is a pharmaceutically important plant whose extract contains products of therapeutical effect but the leaves cannot be used as feed stock. So, they conducted studies to find optimal conditions to utilize this plant for ethanol production. Optimization of hydrolysis conditions were done with the help of response surface methodology and three levels Box-Behnken design [15]. Bioethanol production from algal biomass is a promising technology of future. Commercialization depends on the efficient production of monomeric sugars which will act as substrate for ethanol production. This paper used microalga *Microcystis aeruginosa* for optimization of pre-treatment and ethanol production. Pre-treatment with CaO before enzyme hydrolysis increases reducing sugar content for fermentation. A combination of microorganisms was used for fermentation [16]. The paper discusses the possibility of simultaneous utilization of glucose and xylose by *Saccharomyces cerevisiae* for production of ethanol on large scale,

S. No.	Biomass	Organism used	Optimal cond.	Productivity (g/l/h)	Reference
1	Sugarcane molasses	*Pichia veronae* Strain HSC-22	pH-5-5.5 Temp - 30°C	32	Hamoud et al. [10]
2	Potato waste	*T. reesei*	45°C	73.37	Fangzhong Wang et al. [11]
3	Biodiesel-Based Glycerol	*Enterobacter aerogenes*	pH-6.4 Temp - 37°C	--	Rujira Jitrwung and Viviane Yargeau [12]
4	Sugarcane Molasses	*Saccharomyces cerevisiae*	pH - 5.6 Temp - 38°C	--	El-Gendy et al. [13]
5	Microalgae	*2CVZ.mobilis*	pH - 6.0 Temp - 30°C	8.18	Shih-Hsin Ho et al. [22]
6	Gracilaria	*Saccharomyces cerevisiae*	--	4.93 g/l/d	Fang chan wu et al. [20]
7	Lignocellulosic	*Saccharomyces cerevisiae*	--	--	Chen et al. [14]
8	*Miscanthus x giganteus*	*S. stipitis*	pH- 4.8-5.0 temp - 45 - 50°C	0.13	Mohd Azhar et al. [23]
9	Water Hyacinth	*Saccharomyces cerevisiae*	38.87°C	1.289	Zhang et al. [14]
10	Glycerol	*E. coli*	37°C	6.5	Suhaimi et al. [24]
11	Lignocellulosic	*Saccharomyces Cerevisiae*	pH -5.0-6.0 Temp -25-35°C	1	Saini et al. [25]
12	Wheat straw	Pichia stipitis NRRL Y-7124	pH - 2.0-7.0 Temp- 25-35°C	Yield- 0.35 g/g	Saini et al. [26]
13	Green seaweed	*Cladosporium sphaerospermum*	pH-4.0 Temp - 25°C	Yield - 0.47 g/g	Trivedi et al. [28]
14	Corn Stover	*Phlebia brevispora*	28°C	Yield - 38.0 ± 0.2 g/g	Saha et al. [29]
15	*P. harmala* biomass	*Saccharomyces cerevisiae*	--	Yield -4.7%	Neifar et al. [15]
16	Biomass of Microcystis *aeruginosa*	*S. cerevisiae*, *K. oxytoca*, *B. custersainus* and *P. stipites*.	--	60 m M/ml	Imran Khan et al. [29]
17	Food waste	*Saccharomyces cerevisiae*	30°C	Yield - 0.39 g/g	Oya Nihan Uncu, & Deniz Cekmecelioglu [30]
18	Glucose and Xylose	*Saccharomyces cerevisiae*	--	37.1 ± 0.5	Ishola et al. [17]
19	Sweet sorghum bagasse (SSB)	*Pichia kudriavzevii*	--	26.02	Lavudi et al. [18]
20	Yellow poplar	*Pichia stipitis* CBS 6054	--	34.54	Jeong & Lee [19]

Table 2: Ethanol production from various lignocellulosic biomass.

as xylose is an important component of most agricultural residues. They used the genetically modified strain of *S. cerevisiae* T0936 and employed Simultaneous Saccharification, Filtration and Fermentation technique for simultaneous utilization of glucose and xylose. They also tested the elimination of bacterial contamination by high solid loading and inhibitory medium effect on the modified strain of bacteria used [17]. This paper basically focuses on optimization of condition through response surface methodology for production of ethanol from sweet sorghum bagasse as it is cheap and widely available resource. They performed a two stage optimization, in first they chose conditions for pre-treatment and at second stage they chose different conditions for enzymatic hydrolysis so that they could setup a parameter for further scale up studies [18]. The study in the paper was to find the optimal pre-treatment condition at which yellow poplar can be used for ethanol production by simultaneous saccharification and fermentation with the help of response surface methodology. Combined severity factor of pretreated biomass was one of the main factors to check the effect increasing acid concentration with time [19]. This study is on *Pterocladiella capillacea* as a third-generation biomass for bioethanol production. They performed optimization of saccharification by sulfuric acid and its detoxification. For fermentation yeast *Kluyveromyces marxianus* was used as it is thermo tolerant, higher growth rate and has broad substrate spectrum [20]. Seaweeds contain a variety of monomeric sugar making it a potential substrate for bioethanol production. This study was done to find out the microorganism which could ferment these sugars in seaweed and its hydrolysate into ethanol [21]. They used the plant *Phragmites australis* (a perennial grass) as a source of ethanol production due to its high cellulose content, fast growth, high biomass yields and low nutrient & water requirements. Technique of steam explosion was used for pre-treatment as it requires low capital investment and gives complete sugar recovery. Optimization of result was done through RSM and Pre saccharification and simultaneous saccharification and fermentation was done to get improved ethanol yield [6] (Table 2).

Water (ml)	Stock Solution (ml)	Total Volume(ml)
10	0	10
9	1	10
8	2	10
7	3	10
6	4	10
5	5	10
4	6	10
3	7	10

Table 3: DNSA experiment table.

S. No.	Glucose Concentration	Absorption at 540 nm
1	0	0.002
2	1	0.124
3	2	0.164
4	3	0.203
5	4	0.301
6	5	0.336
7	6	0.361
8	7	0.464
9	8	0.479

Table 4: Reducing sugar estimation.

Materials and Methods

Potato Peel Waste (PPW) was collected from the household wastes and local food vendors from Lovely Professional University, Punjab. The PPW was washed properly with running water to remove any unwanted dust particles. It was air dried for a period of 1-2 days and then dried in hot air oven at a temperature of 110°C for 2 hours so as to remove excess moisture. The dried PPW was then grinded with Pestle and Mortar and converted to a fine powder and was stored at room temperature till required.

Two duplicates of the solutions were prepared to check for best results. The simultaneous acid-base pre-treatment method for lignocellulosic biomass has been effective in removal of hemicellulose and lignin ultimately releasing high amount of fermentable carbohydrates, which could be further carried out by enzymatic hydrolysis [22-31] (Figure 3).

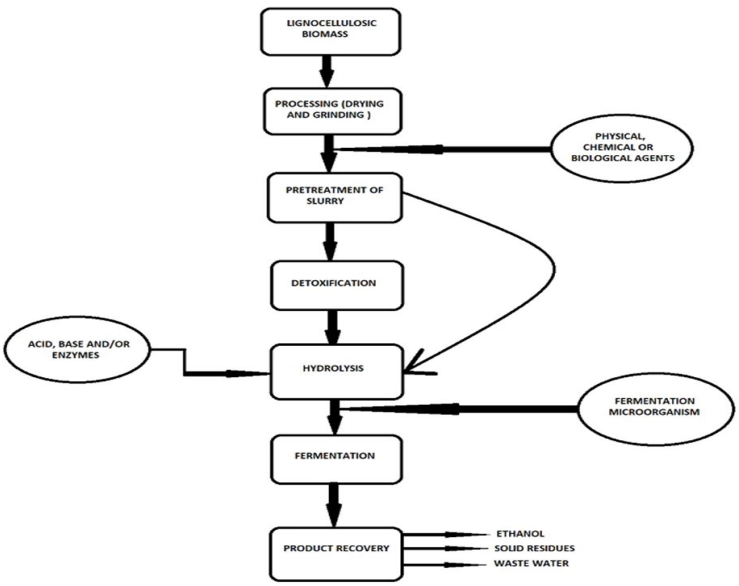

Figure 3: Flowchart representation of process/methodology adopted in ethanol production by response surface methodology.

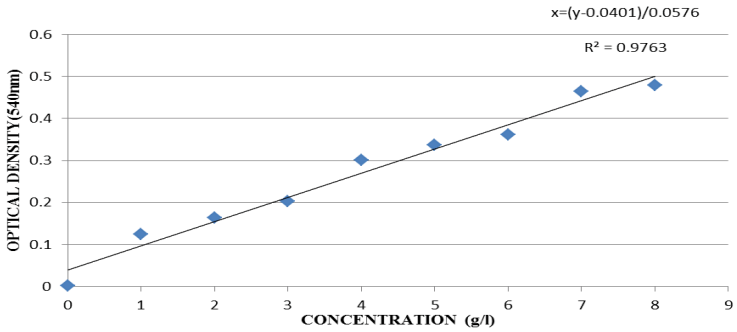

Figure 4: DNSA method graph.

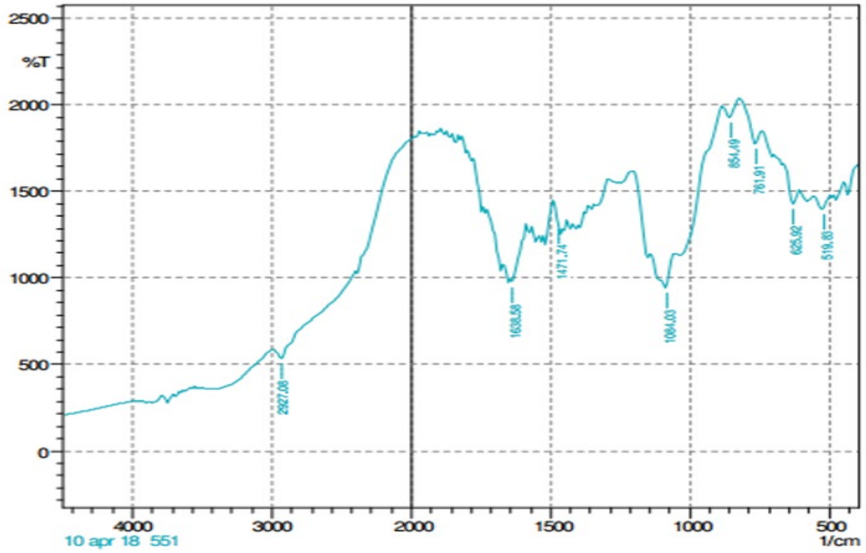

Figure 5: Graph for un-treated biomass.

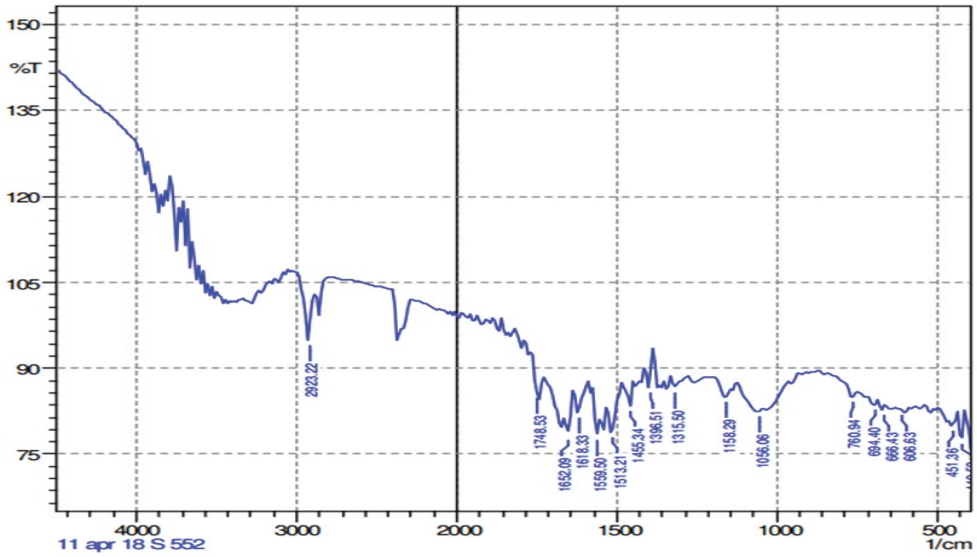

Figure 6: Graph for treated hydrolysate.

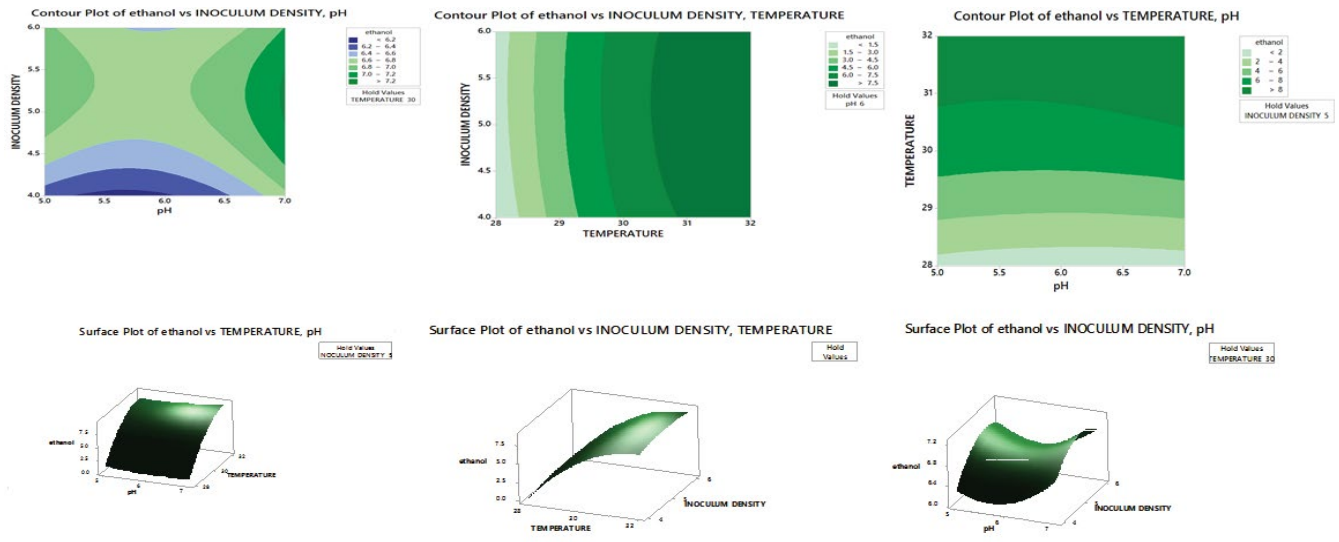

Figure 7: Response surface methodology plots (Contour and surface plots).

S. No	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Area
1	519.83	1393.9	21.1	525.62	503.44	-25.5	0.1
2	625.92	1428.3	141.4	658.71	603.74	-64.8	1.1
3	761.91	1773.5	133.3	818.81	739.73	-101	1
4	854.49	1926.6	85.7	881.5	818.81	-81.2	0.6
5	1084.03	944.2	95	1100.43	1051.24	-49.7	0.7
6	1471.74	1313.9	12	1480.42	1470.77	-10.9	0
7	1638.58	974.7	22.1	1640.51	1631.83	-8.6	0
8	2927.08	532	71.9	2990.73	2872.1	-89.8	2.7

Table 5: FTIR reference table for un-treated biomass.

No	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Area
1	419.53	77.56	5.184	432.07	406.99	2.451	0.389
2	451.36	79.974	0.497	456.18	447.5	0.83	0.012
3	606.63	82.61	0.117	613.38	604.7	0.714	0.003
4	666.43	82.812	1.162	681.86	655.82	2.067	0.102
5	694.4	83.524	1.17	711.76	681.86	2.251	0.1
6	760.94	84.945	1.244	838.1	749.37	5.172	-0.014
7	1056.06	82.362	1.194	1107.18	1040.63	5.209	0.273
8	1158.29	84.911	2.196	1191.08	1138.04	3.386	0.269
9	1315.5	86.708	1.605	1327.07	1300.07	1.591	0.126
10	1396.51	86.607	5.038	1407.12	1384.94	1.106	0.271
11	1455.34	83.292	2.603	1460.16	1443.77	1.16	0.122
12	1513.21	78.74	2.145	1519.96	1508.38	1.148	0.093
13	1559.5	78.275	2.999	1572.04	1555.64	1.487	0.165
14	1618.33	82.126	3.474	1629.9	1602.9	2.04	0.217
15	1652.09	78.397	1.479	1659.8	1649.19	1.054	0.045
16	1748.53	85.8	0.6	1762.03	1747.57	0.736	0.026
17	2923.22	94.816	9.627	2993.62	2879.82	-0.715	1.65

Table 6: FTIR reference table for treated biomass.

S. No.	Sample	Concentration (G/L)
1.	Sample 1	0.50
2.	Sample 2	5.97
3.	Sample 3	6.80
4.	Sample 4	6.90
5.	Sample 5	7.32
6.	Sample 6	8.00
7.	Sample 7	8.90

Table 7: Titration results.

Acid pre-treatment

Reagent used: 1% and 2% H_2SO_4 (v/v) solution has been prepared.

Acid pre-treatment is used because it's inexpensive as the availability of the acid used is widely available and at a cheap cost. Mainly Sulphuric acid is used for acid treatment. The treatment of lignocellulosic biomass by using dilute Sulphuric acid is a rapid and effective process for the removal of hemicellulose from it, thereby leaving only cellulose and lignin together in a complex form known as

Source	DF	Adj SS	Adj MS	F-Value	p-value
Model	9	140.681	15.631	79.67	0
Linear	3	123.536	41.179	209.88	0
pH	1	0.186	0.186	0.95	0.375
Temperature	1	123.01	123.01	626.97	0
Inoculum Density	1	0.34	0.34	1.73	0.245
Square	3		5.565	28.36	0.001
pH × pH	1	0.437	0.437	2.23	0.196
Temperature × Temperature	1	15.567	15.567	79.35	0
Inoculum Density × Inoculum Density	1	0.448	0.448	2.28	0.191
2-Way Interaction	3	0.45	0.15	0.76	0.561
pH × Temperature	1	0.403	0.403	2.06	0.211
pH × Inoculum Density	1	0.024	0.024	0.12	0.741
Temperature × Inoculum Density	1	0.023	0.023	0.11	0.749
Error	5	0.981	0.196	--	--
Lack-of-Fit	3	0.897	0.299	7.09	0.126
Pure Error	2	0.084	0.042	--	--
Total	14	141.662	--	--	--

(a) ANOVA results.

S	R-sq.		R-sq.(pred)
0.442943	99.31%	98.06%	89.74%

(b) Model summary

Term	Coef	SE Coef	T-Value	p-value	VIF
Constant	6.727		26.3	0	
pH	0.153	0.157		0.375	1
Temperature	3.921	0.157	25.04	0	1
Inoculum Density	0.206	0.157	1.32	0.245	1
pH × pH	0.344	0.231	1.49	0.196	1.01
Temperature × Temperature	-2.053	0.231	-8.91	0	1.01
Inoculum Density × Inoculum Density	-0.348	0.231	-1.51	0.191	1.01
pH × Temperature	0.318	0.221	1.43	0.211	1
pH × Inoculum Density	-0.077	0.221	-0.35	0.741	1
Temperature × Inoculum Density	-0.075	0.221	-0.34	0.749	1
Regression equation in uncoded units Ethanol = -491.7-8.35 pH + 32.00 Temperature + 5.28 Inoculum Density + 0.344 pH × pH - 0.5133 Temperature × Temperature - 0.348 Inoculum Density × Inoculum Density + 0.159 pH × Temperature - 0.077 pH × Inoculum Density - 0.038 Temperature × Inoculum Density.					

(c) Coded coefficients

Table 8: Response surface methodology results.

cellulignin [32]. This test can be carried out at different concentrations and at different temperatures.

For acid pre-treatment, the chemical used here is H_2SO_4: The constituents added in the 250 ml flask were 24 g PPW which was dissolved in 120 ml distilled water containing 1% and 2% H_2SO_4. Sterilization of both the mixtures are done in at 121°C, over a time period of 40 minutes (for 1% H_2SO_4) and 60 minutes (for 2% H_2SO_4) respectively. Solutions must be cooled to T< 40°C and neutralized under using tap water and filtered. The pH of both the samples is adjusted between; 6.9-7.1 for optimum growth of yeast.

Alkali pre-treatment

Reagent used: 1% and 2% NaOH (w/v) solution has been prepared.

Potato peel is a by-product, containing a high level of starch, cellulose and hemicelluloses. The alkali used here is NaOH. The NaOH helps dissolve carbohydrates. It is used thereby for biological conversion of cellulose into glucose. Pre-treatment done for PPW at lower temperatures enhances the process [33].

In this experiment 1% and 2% NaOH solutions were used for pre-treatment. The constituents added in the 500 ml beaker were NaOH solution was added to 24 g PPW which was dissolved in 120 ml distilled water having 1% and 2% NaOH. All the constituents were treated at 100°C for 1 hour. The treated solution was then cooled down to T<40°C and then neutralized with tap water and filtered. The filtrate was used for further enzymatic hydrolysis treatment.

Enzymatic hydrolysis

The enzymatic hydrolysis of PPW was done with two-component enzyme system. Two enzymes, α-amylase (E.C. 3.2.1.1) and amyloglucosidase (E.C. 3.2.1.3), are used as the best treatment method applicable for enzymatic hydrolysis of lignocellulosic mass and starch treatment. α-amylase specifically catalyzes the hydrolysis of α-1,4 glycosidic bonds in starch to dextrins, maltose, and a small amount of glucose. Then amyloglucosidase catalyzes the hydrolysis of maltose and higher DP (degree of polymerization) maltodextrins to glucose since it can hydrolyze α-1,6 linkages [31].

To 100 mg of dry potato peel hydrolysate (obtained from previous acid-base treatments), 100 µl α-amylase enzyme solution and 100 µl of amyloglucosidase solution, containing different ranges of enzyme concentration, was used. The ratio of α-amylase to amyloglucosidase enzyme was taken fixed at 15:1 and evenly used for starch treatment. The PPW was then treated by enzyme hydrolysis for 48 hours at 37°C.

Estimation of glucose concentration by DNSA method

Preparation of reagents:

3, 5-dinitrosalicylic acid [DNS]-DNS reagent composition (for 100 ml)

- Sodium hydroxide: 1 g
- Sodium potassium tartarate: 19.2 g
- Dinitro salicylic acid: 1 g
- Phenol: 0.2 g
- Sodium sulphite: 0.05 g

- Make up the volume to 100ml with distilled water.
- Stock Solution of Glucose – 1000 ppm=1000 mg/l
- Working solution – 100 ppm =100 mg/100 ml
  - Take 8 clean test tubes and add 0 ml, 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, and 7 ml of standard glucose solution and label the test tubes. Now, make up the volume 10 ml in each test tube. Add 1 ml of DNS to each test tube. Incubate the test tubes in boiling water for 10 minutes. Take spectrophotometer reading at 540 nm. After getting the results we plot the graph by plotting glucose amount on X-axis and optical density on y-axis (Table 3).

Determination of ethanol concentration by titration

- Acid dichromate solution (0.01 mol/L in 5.0 mol/L Sulphuric acid)
- Starch indicator solution (1.0% solution)
- Sodium thiosulphate (0.03 mol/L)
- Potassium iodide solution (1.2 mol/L)

Dilute the solution samples in 1:20 (10 ml in 200 ml) with distilled water. To a 250 ml conical flask, add 10 ml of acid dichromate solution and seal flask with rubber stopper. To a 5 ml beaker, pipette out 1 ml of sample solution and prepare three samples accordingly. Suspend the 5 ml beaker over the acid dichromate solution. Incubate the flask overnight at 25-30°C. After incubation, keep the flask at room temperature and loosen the stopper and discard the 5 ml beaker. Rinse the walls with distilled water; add to it about 100 ml of distilled water and 1 ml of potassium iodide solution and vortex slightly to mix. Prepare 3 blank titrations of same by adding 10 ml of acid dichromate solution and 100 ml of distilled water and 1 ml potassium iodide solution and mix it. Fill the sodium thiosulphate solution in the burette and titrate against each solution. Add 1 ml of starch solution when the color of solution changes to yellow and titrate until the blue color disappears (Table 4).

Results and Discussion

Concentration vs. Absorbance

This graph is the standard glucose curve. The given graph provides us our samples concentration obtained after biomass acid-base and enzymatic treatment (Figure 4).

Sample 1: -Absorbance - 2.908; Concentration - 48.7189 g/l.

FTIR analysis was performed to check the presence of functional group and do the comparative study of the functional groups in the raw material and hydrolysate (Tables 5 and 6). For the raw sample, the peak at 2927.08 cm^{-1} shows presence of medium Carbon-Hydrogen stretching vibration bond. 1638.58 cm^{-1} peak is for the presence of monosubstituted alkene (-C=C-) (Figure 5). The peak at 1084.03 cm^{-1} show strong stretching of -C-O- bond and confirms the presence of primary alcohol groups in the sample. While the notable difference in the peaks of hydrolysate is visible at 1748.53 cm^{-1}, which represents strong stretching of esters and also the peaks at 1652.09 cm^{-1} medium stretching of cyclic alkene, 1618.33 cm^{-1} strong stretching of α, β-unsaturated ketone (Figures 6 and 7). One of the important differences is noted in 1395.91 cm^{-1} peak which is for medium bending of aldehyde groups. The broad peak at 1056.06 cm^{-1} in hydrolysate graph shows strong stretching of anhydride group (Tables 7 and 8).

Conclusion

Lignocellulosic biomass proved to be an efficient source for second-generation biofuels, as an alternative to petroleum-oil based fossil fuels. The utilization of agricultural wastes, household wastes and industrial solid residues for bioethanol production is a highly cost-efficient and potentially environment-sustainable approach for coping up with the ever-increasing global energy demands. As far as the needs of alternative fuels are concerned, the recent research progress in enzyme production outputs, the pre-treatment methodologies involved and the metabolic engineering and analysis of yeasts.

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