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**Optimization of Biofuel Production from Non-traditional
Biowastes.**

Project Report

Submitted in partial fulfillment of the requirements for the degree of

Master of Technology

in

Biotechnology

Submitted By

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DECLARATION

We hereby declare that the project entitled “**Optimization of biofuel production from Non-traditional biowastes**” is an authentic record of our own work carried out at School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, for the partial fulfillment of the award of Master of Technology in Biotechnology in Biotechnology under the guidance of Shashank Garg.

This work is our original work and has not been submitted for any degree/diploma in this or any other University. The information furnished in this report is genuine to the best of my knowledge and belief.

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CERTIFICATE

This is to certify that NehaThakur (11304545) is performing her work on the project entitled **“Optimization of biofuel production from Non-traditional biowates”** under my guidance and supervision. From the apart of my knowledge, the work which is done presently is the result of their original investigation and study.

No part of this report has ever been submitted for any other degree at any University. The report is fit for the submission and the partial form of fulfillment of the conditions for the award of M. Tech. Biotechnology.

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INTRODUCTION

IMPORTANCE:

There is need of Biofuel production because of increase of carbon dioxide concentration in the environment and the other green house gases. As the agricultural practices is considered as the good source of energy for the production of biomass which can be measured as Biofuel and as in renewable form of energy source[1].

The main importance of Biofuel is that as there is increasing use of energy or climate change due to emissions from fossil fuels, Biofuel is considered as renewable and non toxic in nature which are totally dependent on its production[1].

For the development, energy was considered as essential and major source. Moreover, global economy was fully dependent on energy. India copes up with nearly 70–80% of its total requirements of petroleum through imports. Over traditional fuels, benefits of biofuels related to the rural sector which includes greater form of energy security, reduction in the environmental impact, exchange of foreign exchange savings and socio economic issues. To both developing and industrialized countries the biofuels are very much relevant. For these reasons, over the next decade the share of automobile fuel are expected to grow rapidly. For all the countries the biofuels could be peaceful energy carrier[2]

Biofuels are renewable and they are available throughout the world. The fossil fuels usage are applied as unsustainable source of energy because of depleting sources and also due to depleting environment because of accumulation of green house gases. For environmental and economic sustainability the renewable and carbon neutral biodiesel are necessary. As a reservoir of fossil fuel the demand of biodiesel is constantly increasing [2].

As Biofuels are of two global liquid transportation which includes bioethanol and biodiesel that has the capacity to remove gasoline and even diesel fuel consumption from its unit. Transport is includes as the main consuming sector of energy. In the replacement of petroleum diesel the biodiesel is used and bioethanol is used in replacement of gasoline[3].

Biofuels production is amounted to about 2.9 billion liters onto which the bioethanol produced around at the rate of 630 millions of liters and the biodiesel was considered in 2.3 billion liters. For the production sector of ethanol, the beet of sugar is used in the form of feedstock as a cereal crop, on the other hand the biodiesel is manufactured mainly from rapeseeds. Biofuel is considered in the form of renewable energy source that can be produced from the natural materials. As the ethanol is produced from corn, wheat or sugar beet and biodiesel is produced from oil seeds. Bioethanol is considered as substitute of petrol that might be produced from biomass resources[2].

For the reduction of usage of both the crude oil and environmental pollution the production of bioethanol from biomass is it's one way to resolve the problem. For making biodiesel ,the vegetable oils are used which which are having low capacity of polluting than the conventional form of petroleum diesel fuel [3].

Biofuel is a term which is referred as in three form as solid form , gaseous form and liquid as in further (bioethanol, oil of vegetabl or biodiesel) fuels that can be emerged from biomass. The biggest difference Between biofuels and petroleum feedstocks the biggest difference is oxygen content [2].

AGRICULTURAL WASTES: As the agricultural wastes are cost effective and renewabe that are carried for the emergence of fuel of bioethanol. Bioethanol can be considered as the alternative fuel for gasoline. The production of bioethanol from agricultural residues that are used for the production of ethanol includes in many sectors of it [4].

Required form of pretreatment methods with proper form of parameters may increase the concentrations of required fermentable sugars. To make the whole process cost effective there is need of some new fermentation technologies for the Conversion of glucose to ethanol.

KITCHEN WASTES: In this different processes are followed for producing ethanol from kitchen wastes.The process consists of various steps for the fermentation of required

ethanol from biomass content. To test the effect on ethanol production, both forms of fermentation were carried out. The garbage which is produced from the kitchen is used in open form of fermentation to a certain extent. The kitchen wastes include potato peels, kinnow mandarin, banana peels, mango peels and orange peels[6].

2 . REVIEW OF LITERATURE

2.1 Types of Biofuels:

The biofuels are in three forms: solid, liquid (bioethanol and biodiesel) and gaseous(bio syngas).

1. Liquid Form:

Bioethanol:

As the renewed forms of energy from the biomass that are derived is ethanol which is considered or having the requirement to be taken a sustainable fuel or moreover as the alternative as fuel oxygenates that can cope in gasoline replacement. Furthermore it is considered as oxygenated which has the potential to reduce particulate emission in the compression ignition

engines. Bioethanol can be produced from any sugar or starch crop. Lignocellulosic biomass is considered as the another potential resource for ethanol production which includes the agricultural materials such as (straws of crops, bagasse of sugarcane) and other wastes[7].

Different percentage of ethanol production is formed from different sources as : about 60% of global bioethanol production is formed from sugar cane and 40% is formed from other crops. Bioethanol is majorily produced in different countries and the major producers of ethanol are dependent upon them only, which accounts for 63% of whole production. The different sources includes for ethanol production in Brazil is sugarcane on the other hand corn grain is the feedstock in the US. For the production of bioethanol, different feedstocks with its potential of production are given in Table 1[8].

Table 1

Different feed stocks for ethanol production and its production potential.

FEEDSTOCKS	BIOETHANOL PRODUCTION POTENTIAL (L/TON)
1. Sugarcane	60
2. Sugar beet	100
3. Sweet Potato	120
4. Potato	100
5. Cassava	170
6. Maize	360
7. Rice	420
8. Barley	240
9. Wheat	330
10. Sweet Sorghum	70
11. Bagasse and other cellulose biomass	290

Microorganism taken for the bioethanol processing is the yeast *Saccharomyces Cerevisiae*. As *Saccharomyces Cerevisiae* is unable to utilize starched materials, so the enzymes are generally added to convert cellulose materials into simple sugar and the enzymes generally includes glucoamylase and alpha amylase. For bioethanol production , the Process for conversion of lignoCellulosic material to bioethanol consists various steps and the four units includes: pretreatment, hydrolysis, fermentation and product separation or distillation [8].

Biodiesel:

For the transportation of India's fuel requirement it was an inique steps in the world. In India Biodiesel consumption is almost five times more than gasoline and in the whole world, in all other countries uses more gasoline than biodiesel . During the last two years the use of diesel is more due to which in place of petroleum biodiesel the use of biodiesel ,considered to be as more important for us than forests of the countries[2].

Biodiesel is monoalkyl form of esters which are fatty acids that are derived as the renewable form of feed stocks which includes oils of vegetables or animals. If we visualize the environment, biodiesel was determined as neutral carbon source because the carbon dioxide which get released in the processing time is sequestered for growth or formation of vegetable oil crops[10].

The most prominent and biggest advantage of biodiesel over the other alternative transportation fuel is that it can be use in the existing diesel engines without any modifications [2].

The National Biodiesel Mission (NBM) was the centerpiece for biodiesel development and commercialization which was given by the government of India . Furthermore, from the carbon neutral biomass the process of production of biodiesel will provide biodegradable and safe or non-polluting environment and will boost up the rural economy. In terms of reduction in pollutants emissions, the benefits of 100% biodiesel and 20% biodiesel are given in Table 2 [10].

As in worldwide biodiesel production the raw materials which are generally used are comes in the oil crops: rapeseed and also soybean even palm also. Various different oils are being used in different countries for its biodiesel production. For biodiesel production, soybean is used in united states and in many European countries, rapeseed oil is used. In Malaysia and Indonesia, the palm oils and coconut oils are used [2].

Table 2

BIODIESEL WITH RESPECT TO PETROLEUM BIODIESEL:

FORMS OF EMISSIONS	B100(PURE FORM OF BIODIESEL)	B20 WHICH IS MIXED FORM OF BIODIESEL(INCLUDES 20% OF BIODIESEL AND 80% OF PETROLEUM BIODIESEL)
Regulated Form of Emissions (%)		
Total Unburned Hydrocarbons	-94	-29
Carbon Monoxide	-60	-30
Particulate Matter	-40	-32
NOx	+12	+3
Non Regulated form of Emissions (%)		
Sulphates	-100	-19
Polycyclic Aromatic Hydrocarbons	-80	-15
Nitrated Aromatic Hydrocarbons	-80	-40
Life Cycle form of Emissions(%)		
Carbon Dioxide	-60	
Sulphur Dioxide	-99	

Biodiesel looks like a clear amber yellow colored liquid which is non-inflammable and having the viscosity which was same like an petrol diesel is having and it was considered as non explosive. The properties of biodiesel was given in Table 3[11].

Table 3

Technical form of properties of biodiesel:

Common name	Biodiesel
Common chemical name	Fatty acid (m) methyl ether
Common chemical formula	C14-C24 methyl esters
Viscosity range	3.4-5.3(mm ² /sec)
Density range	859-895(kg/m ³)
Boiling point range(K)	>475
Vapour pressure	<5mmHg
Water solubility	Insoluble in water
Physical appearance	Light to dark yellow or clear liquid
Biodegradability	More biodegradable than that of the petroleum diesel
Reactivity	Stable in nature
Flash point range(K)	410-460
Distillation range	480-600
Odor	Light odor

As cooking waste oil is available in a cheap price and also considered as the best option for the making of ethanol as compared to the vegetable oil costs. As the world annual consumption of petroleum and vegetable is athigh peak. In the last one decade, Global vegetable oil production was increased from 56 million tons[9].

It was found that Microalgae was considered having exceeded faster growth than the terrestrial forms of crops. The area yield of oil according to particular unit from algae is estimated to be in per year of gallons in per acre of land, which is considered to be as greater than the next best crop, palm oil.

Microrganism : Microalga : Schizochytrium sp. having oil content percentage of about 50–77% dry weight[10].

2.GASEOUS FORM:

Biosyngas:

Biosyngas is a combine form of gases consisting of carbon monoxide, carbon dioxide and hydrogen, that can be emerged from different sources which includes materials of renewable energy source like lignocellulose. Synthesis gas fermentation is done with the help of Acetogenic bacteria like *Acetobacterium woodii* and *Clostridium autoethanogenum*. These are evolved under group of anaerobic and acetogenic bacteria which are generally grows on hydrogen, carbon dioxide and carbon monoxide[12].

For the production of ethanol these bacteria can use syngas or synthesis gas as carbon and energy source. Cells of *Clostridium autoethanogenum* growing on hydrogen and carbon dioxide that produces not only acetate but also ethanol as the end product. In large scale industrial processes, the syngas and clostridium autoethanogenum is used for the conversion of syngas to acetate and ethanol in which many different pathways are being followed like ethanol, butanol or isoprene, starting with acetyl coA as a precursor[12].

3.2 BIOFUELS FROM AGRICULTURAL WASTES

As the wastes of agricultural are cost effective and renewabe that can be used for the emergence of fuel of bioethanol. Bioethanol having the capacity to use in the form of comaparative fuel for gasoline replacement. The production of bioethanol from The agricultural residues that are used for the production of ethanol includes wheat, sugar cane,barley and rice[13].Required form of pretreatment methods may exceed the concentrations of fermentable sugars . To make the whole process cost effective there is need of some new fermentation technologies for the Conversion of glucose to ethanol [14].

SUGARCANE : From sugarcane, ethanol production is in rapid expansion which is also leads to the reduction of carbon dioxide and thus considered as being a renewable fuel [14].

As due to the structural and chemical complexity of biomass it was an tedious or challenging work to convert biomass of lignocellulosic material to ethanol and makes them to use as feedstocks for its production [15].

WHEAT STRAW : With low commercial value, wheat straw is considered as an agricultural residue which are form of abundant resource. For ethanol production it is used as an attractive alternative. As the wheat straw could be used as clean, efficient and economically material for the production of bioethanol as well as for the high value-added products [16].

CASSAVA : The Government conducted the production of bioethanol from cassava. As from the energy analysis, the results determined that bioethanol produced from cassava has a negative energy value [17].

RICE STRAW : As it is an most abundant renewable resources used for the bioethanol production and used as lignocellulosic material for its production. It is having high content of celluloses that is hydrolyzed into fermentable sugar for the production [18].

CORN : Nowadays the majorily fuel ethanol that was produced from starch of corn , but the supply of grains for its production is insufficient to meet an great demand. As due to complexity in its structure, the Enzymatic hydrolysis of lignocellulosic biomass is done of corn that can provide comparative source of fermentable sugars [19].

3.3 BIOFUEL PRODUCTION FROM KITCHEN WASTES:

In this different processes are followed for producing ethanol from kitchen wastes. The process consists of various steps : preservation of the waste, saccharification of the sugars in the waste and ethanol fermentation from the saccharified liquid. To test the effect on ethanol production, both fermentation were carried out. The garbage which is produced

from the kitchen is used in open fermentation to a large extent. The kitchen wastes includes potato peels, kinnow mandarin, banana peels, mango peels and orange peels [6].

POTATO PEELS: Potato peel was considered as a zero value waste but the bioethanol from this waste has an large potential market and productivity of its ethanol production is in increased pace . For its fermentation *Saccharomyces Cerevisiae var.bayanus* is used and various hydrolysis steps of enzymes and acids are used in a number of batches [20].

KINNOW MANDARIN (CITRUS RETICULATA): With continuous saccharification and the fermentation ,the ethanol is produced from dried and hydrothermally pretreated Kinnow mandarin waste. Optimized parameters resulted in an high ethanol concentration [21].

BANANA PEELS: After continuous saccharification and fermentation the ethanol is extracted from grinded and dried form of banana peel after the hydrothermal sterilization. For ethanol production optimizes concentrations of cellulose, pectinase, temperature and time is determined with Central Composite Design (CCD) from banana peels. By simulataneous saccharification and fermentation some parameters should be considered like inoculums concentration, temperature, incubation period and agitation time on ethanol production from the kinnow waste and banana wastes [21].

MANGO PEELS :As in whole world production India ranks first in Mango production and is the most important tropical fruits. It is used for ethanol production because of having tropical fruit fibre [22].

ORANGE PEELS : For enhanced ethanol production orange peel is used as a feedstock and the two ethanologenic yeasts are used which is named as *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*, which is used for fermenting the sugar solution at 37 degree Celsius.The oil which is produced from the orange peel was used in the determination of effect on ethanol production [23].

Household food wastes were considered as major stocks of feed for ethanol production as it is cheap. A simultaneous saccharification and fermentation steps has been followed for reducing the viscosity of the substrate for better mixing and sugar content availability for the fermenting microorganism. As this step leads to increase in ethanol production and productivity which lead to an positive effect only [24].

3. MATERIALS AND METHODS

3.1 Preparation of substrate

The PPW or potato peel wastes were collected which were obtained from steamed cooked potato and were peeled and trimmed. These peels were collected from small food corners in lovely professional university in different batches. The collected peels were then washed so as to remove remaining pulp on the peels. Then these washed peels were dried in oven and these dried peels were then ground in a grinder and converted into small particles size [20].

3.2 MOISTURE ANALYSIS: Firstly the potato peels are washed and analysed its wet weight and then that peels are dried at 100 degree Celsius until its weight get constant and in this after 180 minutes of drying the peels weight comes into constant weight. The difference between wet weight and dry weight, the moisture content could be determined[25].

3.3 PRETREATMENT:

Acid hydrolysis

The HCL can be used to achieve acidic hydrolysis. The acidic hydrolysis is done to completely convert starch into simple reducing sugars. In this 0.5% HCl was added to ground potato peel (1g/1ml). It was then autoclaved at 121° Celsius, 15 psi for 15 min. finally the sample was cooled down and stored in dark bottle at 4°C till further use. This filtrate will serve as sample source of reducing sugar for further fermentation process. The pH of clear filtrate was adjusted to approximately 6.0-6.5 [25].

3.4 REDUCING SUGAR ESTIMATION:

For the estimation of reducing sugar, the Dinitrosalicylic acid method were done and for the preparation of its solutions the composition is given in table 4.

Dinitrosalicylic Acid Method

Table 4

Composition : (100ml)

DNSA salt	1g
Phenol	0.2g
Sodium Sulphite	0.05g
Sodium Hydroxide	1g

Pottasium sodium tartarate : 40%

Standard Glucose stock – 1mg/ml

Preparation :

The extract and standard stock glucose solution were pipetted in test tubes .The extract was pipette out from 0.1-0.5 and volume of 3ml and rest was make up with distilled water and on the other hand glucose from 0.2-0.6 is taken and rest 3ml makeup with distilled water.

Then in whole tubes 3ml DNSA were added and then kept it into boiling waterbath for 10min. Then after cooling down added 1ml of potassium sodium tartarate in the whole tubes.

Then estimate total reducing sugar at 540 nm with the help of analytical method of spectrophotometer[25].

3.5 Total Sugar Estimation:

For the estimation of total sugar the composition of solution is given in table 5.

Table5

Composition :

Glucose Stock : 1mg/ml

Anthrone Reagent : 0.1g in 76% sulphuric acid.

Preparation:

For the preparation of solution , the glucose solution were pipetted out 0.1ml-0.6ml in test tube and make up to 1ml by adding distilled water. Then added 5ml of anthrone in each tube and keep it in water bath for 10min.

Test tubes should be put on ice bath while adding anthrone reagent to avoid spurting [25].

3.6 Microorganism used :

The yeast *Saccharomyces Cerevisiae* were used for the fermentation of the hydrolyzed potato peel waste (PPW), and was maintained on the YEPD medium[25].

Microorganism : *Saccharomyces Cerevisiae*

Growth Medium	YEPD
Growth condition	Aerobic
Temperature	30° Celsius
Time	24 hour

Maintenance of culture:

The yeast culture were maintained by subculturing at YEPD medium at 30 degree Celsius for 24 hours and then can be stored at 4 degree Celsius for further use. The composition of YEPD medium is given in Table 6 for the growth of *Saccharomyces cerevisiae* [24].

Table 6

Composition of YEPD:

Yeast extract	(3.0g/l)
Peptone	(10.0 g /L),
Agar	(15.0 g /L)
Dextrose	(20.0g/L)
Distilled water	(1L)

The medium was sterilized in autoclave at 15psi for 15min.

3.7 Inoculum and inoculation : The yeast inoculum were prepared in YEPD broth. The inoculum then inoculated in production medium which were incubated in shaking incubator for 48 hour at 32 degree Celsius and the composition for the preparation of production medium is given in table 7[25].

Fermenting Medium : (250ml flask)

Table 7

Composition: (100ml)

Yeast extract	(0.1%)
Peptone	(10.0%)
KH ₂ PO ₄	(0.1%)
MgSO ₄ 7H ₂ O	(0.05%)
(NH ₄) ₂ SO ₄	(0.5%)
Sample	(20ml)
Distilled water.	(80ml)

The medium was set PH 5.0 and then sterilized at 15psi for 15 minutes.

The Required percent of inoculum is ten that was used for the fermentation of PPW.(C Kasavi.,etal, 2008).

The primary inoculums prepared in YPED broth were inoculated to production media that were prepared in 250ml flask at three units and incubated at 32 degree Celsius under shaking condition for 24 hours and 48 hours in figure 1 and one flask of production medium is taken without the inoculation and its sample of 5ml is taken into the test tube as t=0 and then it is stored for further use. After the incubation of two incubated medium for 24 hour and 48 hour it were taken in test tubes as t=24 and t=48 and these three samples were then centrifuged and the supernatant were then used for the estimation of reducing sugar after the incubation which were shown in fig2.

4. RESULTS AND DISCUSSIONS:

As potato peel waste (PPW) has a starch which is of high content but the fermentable form of reducing sugar was very low and there will be testing of potato peel wastes (PPW) is done to fermentable reducing sugars.

In YEPD medium the growth of *Saccharomyces Cerevisiae* is gradually increased.

RESULT 1: Following data was obtained after assaying samples and standard stock for reducing sugars and the graph was plotted in fig 1:

Glucose concentration (mg/ml)	Absorbance	Sample concentration (mg/ml)	Absorbance
0.1	0.045	0.2	0.032
0.2	0.056	0.4	0.074
0.3	0.116	0.6	0.127
0.4	0.219		
0.5	0.367		

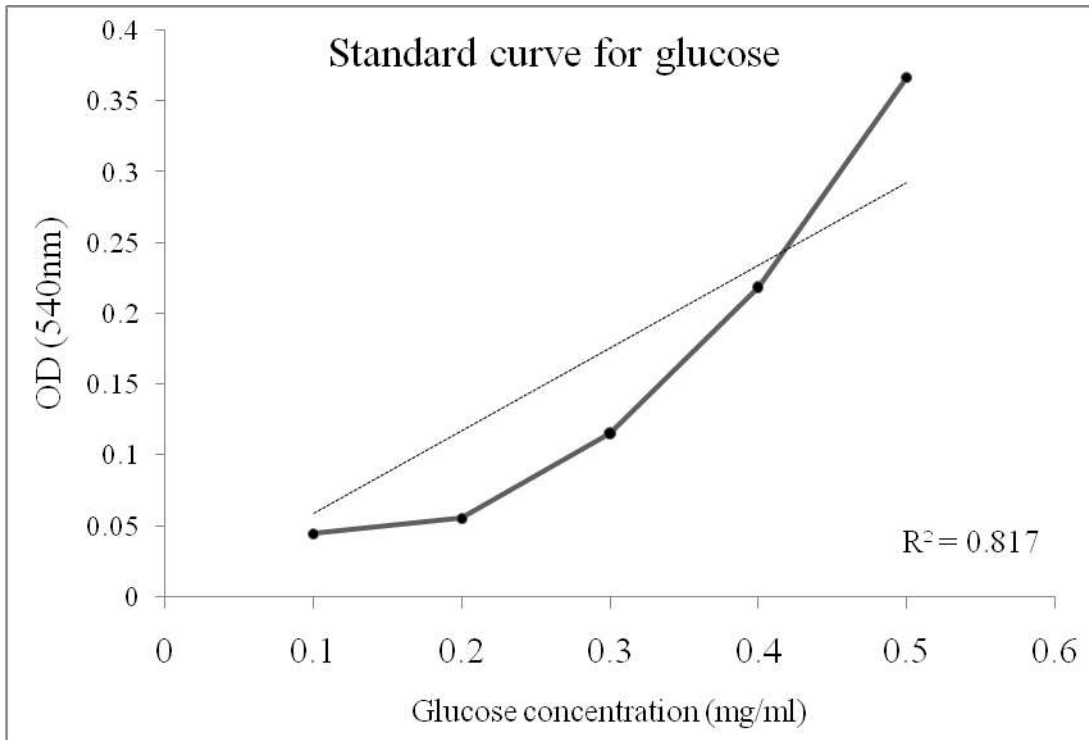


Fig. 1: Standard calibration curve for glucose obtained to determine concentration of reducing sugars in the pretreated and fermented samples.

RESULT2: The result of production media inoculated with *Saccharomyces Cerevisiae* after incubation for 24 hour and 48 hour which was shown in fig2.



(a)



(b)

Fig.2: Media inoculated in YEPD media after incubation for (a) 24hours and (b) 48 hours



Fig 3: Samples were centrifuged and used for sugar estimation

CONCLUSION

Our results revealed that PPW contains some amount of reducing sugar which can be harnessed for fermentation process and biofuel production. This work will further be continued to perform fermentation and optimize the conditions required for fermentation.

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