“BIOFUEL PRODUCTION FROM FOODWASTE”

A DISSERTATION

SUBMITTED IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE

OF

MASTERS OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by:

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2k19/MSCBIO/18

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DEPARTMENT OF BIOTECHNOLOGY

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CANDIDATE'S DECLARATION

I, Indu, 2k19/MSCBIO/18, student of M.Sc. (Biotechnology), hereby declare that the project dissertation titled "biofuel production from food waste" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Masters of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: Delhi **INDU**

Date: 28th May, 2021 **(2k19/MSCBIO/18)**

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CERTIFICATE

I hereby certify that the project dissertation titled “Biofuels production from food waste” which is submitted by Indu, Roll No 2k19/MSCBIO/18, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision.

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ABSTRACT

According to the FAO of UN, 33% of the production of food items all over the world for humans is wasted in the Food Supply Chain. In addition to this, with increasing population demand for fuel as energy source also increasing day by day and as we know fossil fuels are limited and their combustion leads to pollution and global warming by emission of greenhouse gases. The alternative for the above problems is to generate a sustainable energy source by food waste (FW) which decreases pollution, reduces our dependency on fossil fuels, and is easily replenished. Biofuels obtained from the fermentation of FW is a suitable option in front of us to meet the requirements of energy demand and environment safety issue. Bioethanol and biodiesel are abundantly produced over the world. Currently, in most countries, the wasted food is either filled in the land or incinerated along with the other municipal waste to recover the possible amount of energy. But the problem with these two efforts is that they are very costly and facing environmental problems. So, we can go with the other kinds of approaches to recover the energy from the food waste. As we know, the FW has an organic and nutrient-rich composition that is why we can use it as a good feedstock for biofuel production. we focus on the current techniques and procedures that are used around the world.

In 2010, biofuel production reaches up to 105 billion litres across the world. Biofuel production technology improving and increasing day by day. There are a few kinds of biofuels are manufactured around the world and this report including bioethanol, biogas, biohydrogen and biodiesel.

Bioethanol production includes high carbohydrate-rich feedstock like sugarcane residues and corn and food wastes from kitchens. Steps followed in bioethanol production are pretreatment, saccharification or hydrolysis, fermentation (mostly by

S. cerevisiae), and distillation of ethanol. Pretreatment is done to delignification of biomass and to separate cellulose parts from lignin and hemicellulose so enzymes can work efficiently in later stages of the process. Saccharification is done to break down cellulose into simpler sugars like glucose, galactose, ribose and xylose. Then sugars are fermented by S. cerevisiae to get acids, gases, and ethanol.

Biogas is produced through an anaerobic process. This can be done by 2 ways: single-stage anaerobic digestion which includes, hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Another way is two-stage anaerobic digestion in which the first stage yields hydrogen and VFAs and the second stage yields biogas and carbon dioxide. Biogas yield improved in second stage anaerobic digestion by 37%.

Biodiesel is a monoalkyl ester of unsaturated fats. It will reduce about 75% of the overall emissions of engines and acts as lubricants. Biodiesel production from oils and lipids is done by transesterification. Water and pigment residues in the oil sample can act as inhibitors in the process of transesterification.

ACKNOWLEDGEMENTS

I would like to thank all the individuals who guided and supported me in completing this project. Their continuous encouragement helped me in completing the project successfully.

I would like to thank Prof. Pravir Kumar, Head, Department of Biotechnology and Prof. Jai Gopal Sharma, Ex-Head, Department of Biotechnology for their motivation and attention.

I would like to thank my mentor Dr. Kriti Bhandari, Assistant Professor, Department of Biotechnology for giving me the opportunity to do this project. She was always present for guiding me even at odd times. She has been a constant source of encouragement, inspiration, and support.

I feel happy and content in expressing my deep sense of gratitude to all those who have helped me directly or indirectly in successfully completing this project.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

FW- Food Waste

FAO- Food and agricultural organisation

FSC- Food supply chain

FMD- Foot and Mouth Disease

UK- United Kingdom

HMF- Hydroxymethylfurfural

SHF- Separate Hydrolysis and Fermentation

SSF- Simultaneous Saccharification and fermentation

SSCF- Simultaneous Saccharification and Co-fermentation

CBF- Consolidated Bioprocessing

VFA- Volatile Fatty Acids

ASBR- Anaerobic Sequencing Batch Reactor

UASB- Upflow Anaerobic Sludge Reactor

SRT- Solid Retention Time

HRT- Hydraulic Retention Time

OLR- Organic Loading Rate

VS- Volatile Solids

VFA- Volatile Fatty Acids

HS-AcD- High Solids Anaerobic Co-digestion

R-TPAD- Recirculated Two Phase Anaerobic Digestion

PBR- Packed Bed Reactor

CSTR- Continuously Stirred Tank Reactor

FBR- Fluidized Bed Reactor

NL- Normalised Litres

DME- Dimethyl Ether

IFWS- Industrial Food Waste

FAME- Fatty Acid Methyl Ester

1 INTRODUCTION

FW refers to those materials or substances which are non-consumable for humans[1]. According to FAO, around 1.3 billion tonnes of FW go along with the FSC (Food Supply Chain). Due to the exponential growth of the human population, it is common to estimate that the amount of food waste will increase in the upcoming years.

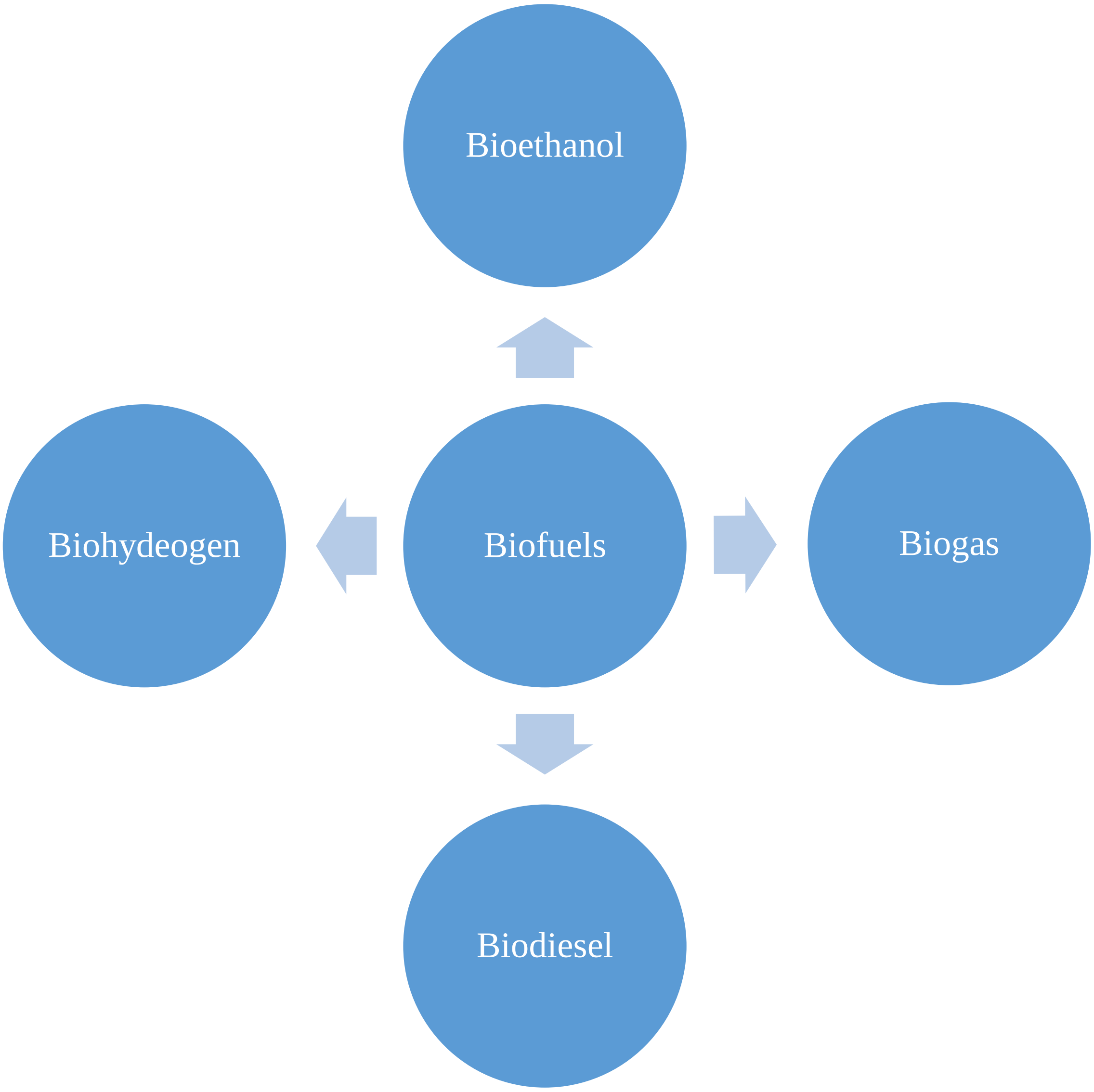

Figure 1: biofuels overview

In first-generation biofuels which majorly include bioethanol and biodiesel, most feedstock comes from edible crops like palm, sugarcane, corn, soybean, and rapeseed oil. But this system negatively affects the food supply chain and in addition to this, it also disturbed the environmental equilibrium so as an alternative to this, food waste is considered the best option available for biofuels generation. In second-generation feedstock comes Food waste and plant products like stems, dry leaves, wood chips, and lignocellulosic material. Since food waste composition varies concerning carbohydrate, fat, protein content so its treatment is facing difficulties for energy generation as it increases the cost of production and energy demand. Many suitable technologies and procedures have come to face the challenge by either reducing energy consumption or make maximum out of it to make it an economically viable and sustainable source. Using food waste as a feedstock, we can tackle two global problems simultaneously i.e. increasing energy demand and pollution. Even wastewater causes serious problems to the environment like wastewater contains oils in it which gets deposited on the surface of water bodies and make an anaerobic environment which proved fatal for the marine ecosystem. This wastewater can be used in the cultivation of microalgae for lipid extraction to produce biodiesel.

Traditionally, the treatment of food waste was done by composting landfilled or incinerated with municipal waste for energy generation[2]. But there are some limitations of incineration like this can cause air pollution and changes the chemical constituent of food waste. These limitations provide a force to find out the appropriate method to recover the energy[3].

Biofuels produced from food waste will lead to sustainable development, provides us affordable and clean energy sources and above all, it is renewable. Food waste mainly consists of lignin, proteins, lipids, polymers of carbohydrates which includes starch, cellulose, and hemicellulose, etc. in which the total protein in food waste is 3.9-9% while, sugar is 35.5-69%. Due to high sugar content availability, food waste can be used as a feedstock for the generation of methane, ethanol, biogas, and biodiesel[4], [5], [14], [6]–[13]. But carbohydrates present in the feedstock are less amenable to fermentation while oligosaccharides and monosaccharides are easily amenable to fermentation and we can get these by hydrolyzation of carbohydrates which breaks the glycosidic bond of the carbohydrates and release the polysaccharides as monosaccharides and oligosaccharides. The biofuel production using food waste as a feedstock is very efficient, less costly, and more environmentally friendly as compared to the other methods utilizing different feedstock[13]. There are different kinds of food waste valorization methods to produce different types of biofuels from food waste like biodiesel, biogas, ethanol, and hydrogen.

2 LITERATURE REVIEW

2.1 characteristics of food waste

The food goes into waste through the whole FSC, from harvesting to household consumption. There are different phases in the food supply chain which are different in different regions are shown in Fig.1. In low-income areas or underdeveloped regions, food waste is higher during agricultural production and harvesting. There are a variety of food items that go wasted around the world and it is very difficult to quantify every item. Therefore, FAO categorizes the different food waste into 8 different categories- Cereals, oil crops and pulses, fruits, meat, seafood, milk and eggs, starchy roots, and vegetables Fig.2.

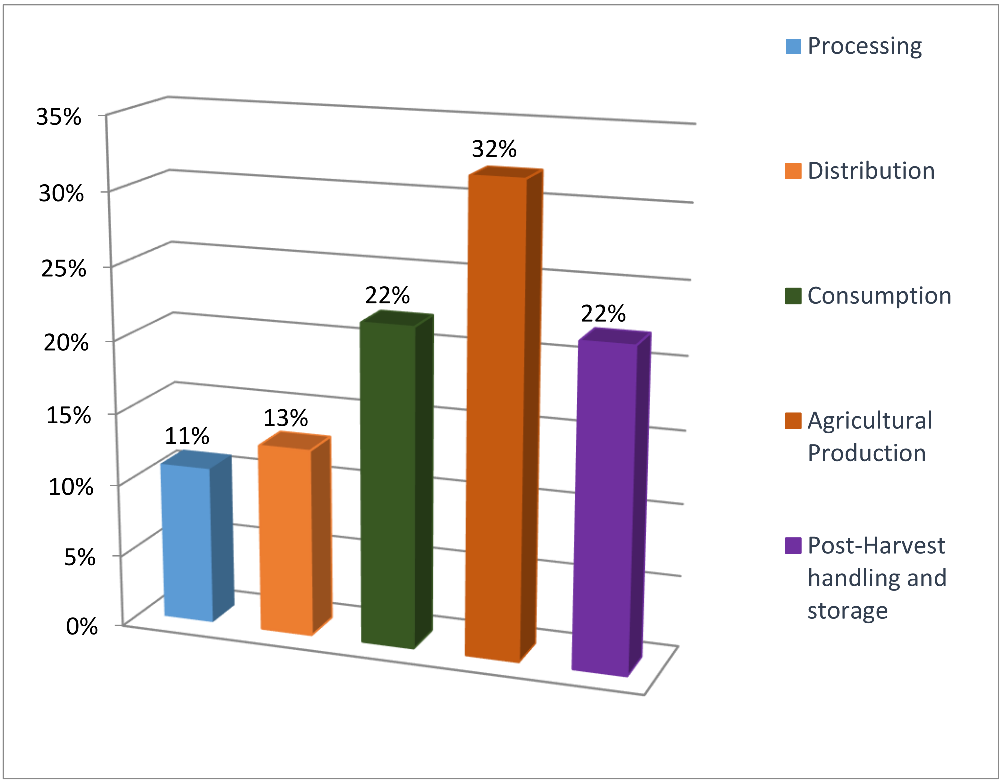

Figure 2. The relative food wastage throughout FSC around the world.

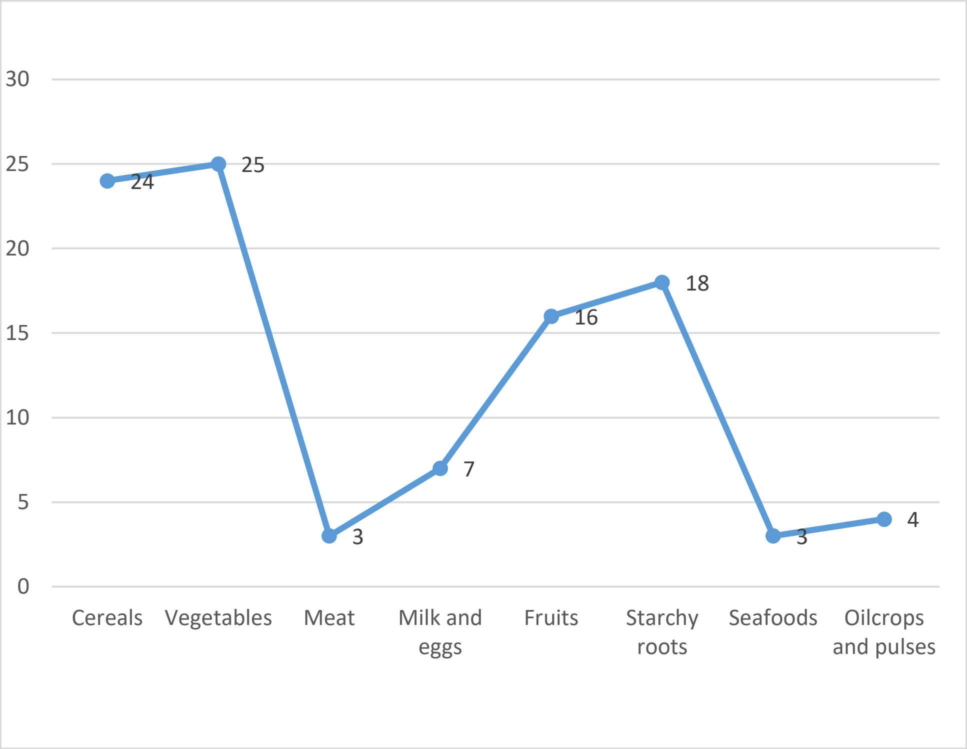

Figure 3. Contribution of different categories of food in wastage around the world

Figure 2 shows the contribution of different commodities in food wastage in which 85% of total food waste is contributed by food crops and the rest 15% from animal products. The contribution of commodities may vary in a different region, but the entire food waste majorly contains carbohydrates, proteins, and lipids.

2.2 Several methods for food waste management

Management of food waste is especially important and includes collection, distribution, recycling, processing, and discarding of food waste items. The waste hierarchy in various countries has been utilized in the following manner[14]:

- Prevention of food waste.
- Recovering food to feed hungry people.
- Providing food for livestock e.g., poultry.
- Composting of food waste and use it to improve the fertility of the soil.
- Landfilling or incineration.

The general principle of waste management is the prioritization of various available options of waste management based on their environmental capability. The utmost priority in the waste management procedure is to prevent food from wasting and with proper hygenic parameters, food is provided to hungry people. But this includes some social, ethical, and sanitary issues and a major part of food is not always edible. Due to these issues, the collection and transportation of food waste for consumption is difficult. Because of the problem in the prevention and recovery of food waste for consumption, the wasted food has been given to livestock. But feeding waste food that contains meat can cause FMD. To reduce the FMD legislation in the UK and European Union prohibits the food waste given to feed poultry animals.

The next step in the waste management hierarchy is composting food waste. Composting can biologically convert the food waste to stable compost. Compost is a soil-like product that increases the fertility of the soil. Composting takes a long period and in addition to that maintenance of certain conditions should be checked. The environment-friendly compost can be produced under ideal conditions which can also reduce its mass and volume by 40%. This method does not produce any other product.

A major part of the food waste is processed through landfilling and incineration. Landfilling is a traditional method of waste management and has various advantages such as a reduction in land loss. Each tonne of landfilled food waste contains methane and carbon dioxide in a ratio of 60:40. But this approach has been stopped due to some drawbacks such as potentially harmful chemicals is produced into soil and ground. Incineration of food waste is the burning of food waste which may change the chemical constituents of food waste and can severly damage the air quality and some part of it is recovered as energy.

3 BIOFUELS PRODUCTION

3.1 BIOETHANOL PRODUCTION

Because of wide industrial applications, the demand for ethanol has been increased globally. Majorly, it is used to produce ethylene and has a demand of 140 million tonnes per year, which is further utilized to produce polyethylene and plastics. As the demand for bioethanol is extremely high, so we need to find a feedstock that delivers the ethanol at a low cost. Though bioethanol has lower energy density over currently available fossil fuels, its production characteristics like its lower production cost, superior production rate, less pollution-causing qualities, and renewability make it superior [15], [16]. Generally, cellulose is used to produce bioethanol, and some starch-rich crops like rice, potato, and sugarcane are also used to produce bioethanol. Hydrolysis of food waste that contains cellulose in large quantity is difficult which in turn makes it difficult to use cellulosic feedstocks, while glucose can easily be obtained from the starch by using commercially available enzymes and then fermented with the help of *S. cerevisiae* to produce ethanol. Wastes like lignocellulosic, food and municipal waste can also be used as an alternative feedstock for ethanol production[17], [18].

Ethanol is also used as fuel for vehicles by mixing it with gasoline. Ethanol is used in different concentrations with gasoline but generally, E10 is used for vehicles which mean a mixture of 10% ethanol and 90% gasoline can be used to reduce air pollution by oxygenating the fuel and there is no requirement of engine modification when using E10 as fuel. Different concentration of ethanol in gasoline are used as fuel for vehicles such as E10, E15, E20, E25, E70, E75, E85, E95 and E100 [19].

Ethanol production from food waste is done in different steps so we can divide the process into the following sections:

3.1.1 Pre-treatment

Harsh pre-treatments are required before enzymatic hydrolysis. Autoclaving of food waste must be performed before fermentation to improve purity and yield. But it has few drawbacks e.g. heat may partially degrade the sugars and other components of food waste and side reactions also take place due to these reasons, the number of sugars and amino acids is decreased[5]. The pre-treatment of fresh and wet food waste is not necessary as fresh and wet food wastes are found to be more effective than dried food waste which has to be rewetted for the process because of reduction in active surface area for enzymes to work, of the dried substrate, which results in reduction of reaction efficiency. Therefore, the feedstock of food waste without drying, pre-treatment is much preferable for the production of bioethanol but only until the contamination of microbial communities is manageable[20].

In the pretreatment step, the cellulose part is separated from the lignin and hemicellulose part of FW so that enzymes work efficiently. Acids are used in pretreatment for delignification of the biomass. Concentrated acids with high temperature and high pressure can make fermentation inhibitory molecules like furfural, Hydroxymethylfurfural (HMF) and acetic acid. So, to avoid this complication, the use of diluted acids is recommended and pH adjustment is necessary before going to hydrolysis in case of acid treatment. Another way of pretreatment is using ionic liquid or organic solvents like methanol, acetone or ethanol as a common practice. Some physical techniques that are energy demanding processes are also used to reduce the size of food waste including grinding, milling and sonication. Environmentally safe and less energy-consuming biological degradation method is available which used microbes but takes a lot of time making it unfit for industrial use.

3.1.2 Saccharification

This step is also known as hydrolysis in which cellulose is a breakdown to get simpler sugars like glucose, xylose, ribose, and galactose. As we know, yeast cells are not capable of fermenting starch or cellulosic fractions and due to this, the efficiency of conversion of food waste depends on the number of simple sugar molecules present in the feedstock which is formed during sugar saccharification[21]. If we take a mixture of enzymes for example α-amylase, β-amylase, and glucoamylase from the different sources then this will show high effectiveness for the substrate having high molecular weight. A saccharifying enzyme such as Pullulanase is capable of catalysing the hydrolysis of the branched polysaccharides[22]. It specifically acts on the α-1,6-glucosidic linkages of carbohydrates and this results in releasing of the linear oligosaccharides. Small fermentable sugars can be produced from the saccharification process.

By utilizing α-amylase (120U/g), cellulase (8FPU/g), β-glucosidase (50U/g) and glucoamylase (120U/g), the highest concentration of glucose is obtained of about 65g [23]. A study of Hong and Yoon [24] obtained yield of 60g RS/100g food waste while utilizing α-amylase, protease and glucoamylase.

Hydrolysis is done by enzymes whether industrially manufactured or in situ produced ones. Commercially available enzymes are expensive and inefficient for complete hydrolysis so a mixture of enzymes produced in-situ is considered as best option to get maximum output with less expenditure. Since different kinds of FW consist of different amounts of content of carbohydrate so according to type of FW, the enzyme mixture also differs. Mostly used enzymes are amylase, protease, cellulose, lipase and pectinase in different concentrations and compositions.

In situ enzymes are produced by microbes. Commonly used bacterial and fungal strains for cellulase production is clostridium, *Thermomonospora, Tricoderma and Aspergillus*. *Tricoderma* produces highest amount of cellulose. Three types of cellulase used in hydrolysis is exoglucanases, endoglucanases and β-glucosidases. In-situ Fungal mash is another ultra-fast method of hydrolysis contains different kind of enzymes.

3.1.3 Fermentation

Simple sugars are fermented i.e. converted into acids, gases and ethanol by yeast and bacteria. *S. cerevisiae* (baker’s yeast) is used majorly for this purpose. other than this *Pichia stipites, Kluyveromyces fagilis, and Candida shehatae* are also reported as effective yeast strains to produce ethanol from different sugars. Different processes used for fermentation include separate hydrolysis and fermentation (SHF), simultaneous saccharification and co-fermentation (SSCF), simultaneous saccharification and fermentation (SSF), and consolidated bioprocessing (CBP). Each process has its benefits and applications.

In SSF (Simultaneous Saccharification and Fermentation), the same unit is used for carrying out hydrolysis and fermentation. The advantage of using SSF includes a low cost of investment and no inhibitory products like glucose as it is consumed simultaneously by microbes which in turn produce ethanol, results in an increasing rate of hydrolysis and ethanol concentration. 35 °C is considered optimal temperature for yeast and hydrolysis is carried out at 50 °C. SSF is used for the valorization of sugarcane residues or bagasse, the waste product of the sugar industry yield bioethanol, a concentration of about 4.88 g/L under optimum conditions [25].

In SSCF, the same compartment is used for hydrolysis and fermentation along with co-fermentation of 5 carbon sugars by genetically modified strains of s. cerevisiae that can also ferment xylose. This is suitable for xylose fermentation, but the yield is about 35% reduced as compared to the maximum yield.

CBP is just a one-step process i.e., production of enzymes, saccharification, and fermentation all processes take place simultaneously in the same compartment. The microbe used in this process is cellulase-producing *Clostridium thermocellum.*

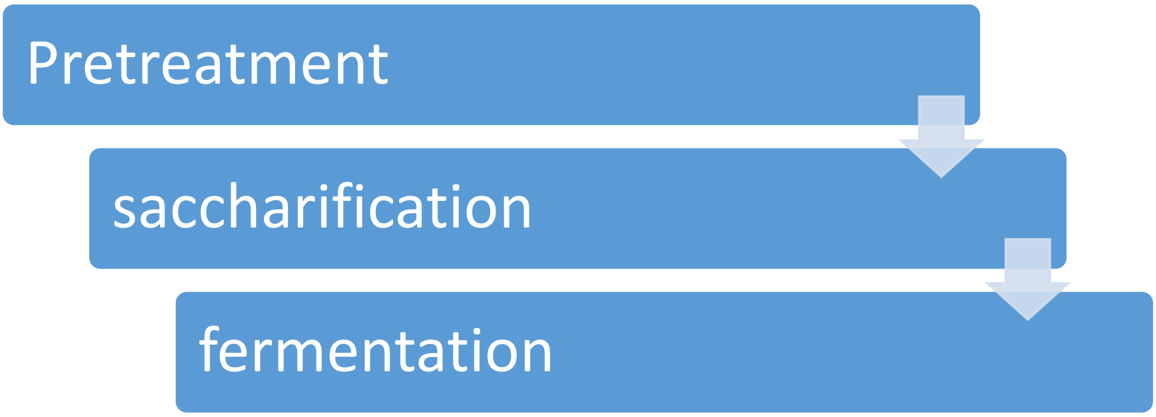

Figure4: methodology of bioethanol production

3.1.4 Configurations

By raising the concentration of enzymes and temperature at the various solid loads, speed, hydrolysis times, and agitation, we can achieve the highest glucose yield. High glucose concentration can repress the catabolite of enzymes [26]. Therefore, for obtaining the high ethanol yield from food waste, SSF and fed-batch methods are developed[3], [26].

Fed-batch fermentation is generally utilized for production of high concentration of reducing carbohydrates and then this can be further fermented to get ethanol[27]. Contrasted with batch culture, Yan and Yao[28] found that hydrolysis and resulting ethanol production were both improved altogether utilizing fed-batch mode, for example, the yield of glucose bioconversion is 92% of its hypothetical value.

SSF can also be used as an alternative and can reduce the risk of catabolite repression of the enzyme. This method involves saccharification and fermentation as a single-step process. This single step process keeps the glucose concentration at the lowest level to reduce inhibition of enzymatic hydrolysis[29]. This procedure is performed in a solitary tank, with lower amounts of energy consumption, higher ethanol yield, in shorter period and utilizing fewer enzymes[27]. For performing successful SSF, optimization of fermentation conditions is very crucial procedure because fermentation microorganisms and enzymes may have distinctive ideal and temperatures and pH. In an investigation by Hong and Yoon[24], after 48-h of fermentation around 60 g RS and 36 g ethanol were produced from 100 g of food waste. koike [9] additionally revealed ethanol generation from non-diluted food waste (trash) in a continuous simultaneous saccharification and fermentation process and got an ethanol efficiency of 17.7 g/L h. Mama et al. [3] examined in the SSF procedure utilizing kitchen trash by *Zymomonas mobilis* which is an acid tolerant strain, without sterilization. 0.49 g/g ethanol/sugar was gotten in 14 h which gives 10.08 g/L h of ethanol yield.

3.1.5 Other strategies to improve ethanol yield.

different strategies have been explored to increase the productivity like cell recycle through sedimentation and use of strains which can highly tolerate ethanol [30] Improvement in the ethanol tolerance has also reported [31], when recombining the good bioethanol yielding microbial strains and gene responsible for amylase production, but the stability of recombinant gene is not proven yet. To improve execution of continuous fermentation, cell recycling found to be very effective[32].

Hydrolysis is considered most expensive step in bioethanol production so we can reduce cost and effectiveness of process by immobilization of enzymes[33]. Magnetic Nanoparticles are used as support material for enzymes to form nanobiocatalyst. These enzymes after the process recovered easily and reused later.

Fusarium oxysporum F3 a fungus can produce multienzymes on different solid residues. These enzymes and this fungus is used for saccharification of FW and further for bioethanol production either alone or with S. cerevisiae. Fungus *F. oxysporum* alone yields 16.3 g/L while in combination with *S. cerevisiae* yield reached to 20.6 g/L and if glucoamylase is added with these two, yield goes up to 30.3 g/L [34].

3.2 BIOHYDROGEN PRODUCTION

Hydrogen gas is utilized as a compressed gas and because of its non-polluting burning and high energy productivity (142.35kJ/g), it is one of the most crucial fuel sources. The developing strategies for sustainable biohydrogen production have been investigated. Different techniques have been created to generate hydrogen gas from FW and the Hydrogen gas productivity utilizing different methodologies ranges from 0.9 mol H_2/mol hexose to 8.35mol H_2/mol hexose. Certain different elements may affect hydrogen gas production like pre-treatments, process configuration, fermentation stage, and composition of food waste.

3.2.1 Substrate composition

The potential of hydrogen gas production was reported 20 times higher from the food waste that has high carbohydrate content as compared to the food waste that contains fat or protein in high amounts. The C/N (carbon/nitrogen) ratio was reported as an important parameter in hydrogen production. It shows the carbohydrate and protein content of food waste. Kim [35] communicate the hydrogen yield of about 0.5 mol H2/mol hexose at the Carbon to nitrogen ratio lower than 20 and while at the higher Carbon to nitrogen ratio, the hydrogen yield drops and this just happened because of the higher yield of lactate, propionate and valerate. When the C/N ratio was stabilized with the alkaline shock, the hydrogen gas productivity reached up to 0.9mol H_2/mol hexose.

3.2.2 Pretreatment

The pretreatment procedure is consistently essential during the bioconversion of food waste into biofuel. The pretreatment approach can change the physical properties of the food waste, improves item productivity and purity of food waste, and prevent rot and microbial contamination. C and N sources in food waste are in the form of polymer structures for example lignocellulosic compounds, starch, and proteins. For normal procedures, these oughts to be separated into simple and fermentable sugar and VFA (volatile fatty acids) by proper pretreatment. There are different pretreatment procedures for the different substrates.

Food waste can also utilized as a source of hydrogen-producing microflora. Kim and Shin [36] has applied several pretreatment procedures for the selection of microflora to produce hydrogen. As we know, Hydrogen-producing bacteria are dominant in pretreated Food waste, while in untreated food waste most abundant species are Lactic acid bacteria. Heat treatment was found effective to suppress lactate production and increase the production of H2/butyrate. But implementation of this technique in large-scale hydrogen production has the disadvantage that heat treatment elevates the cost of the procedure. Luo [37] examined the different pretreatments of inoculums, and finally come to an end that pre-treatment is not a crucial step in hydrogen production and it just has short-term effects.

Microorganisms	**Rate of H2 production (mL/h)**
Gloeocapsa alpicola	25
Anabaena variabilis	20
Rhodobacter capsulatus ST410	16
Anabaena variabilisATCC29413	13
Anabaena PCC7 120	14.9

TABLE1: list of some high H2 producing strains with rate of H2 production

3.2.3 Process configurations

Numerous fermentation systems have been available for hydrogen manufacturing such as batch, semi-continuous, continuous, one or multiple stages [38]. ASBR and UASB are the reactors that have been examined to provide high hydrogen generation rates because of the having high biomass concentrations [36]. In these processes, the composition, metabolic pathway, microbial size and substrate uptake efficiency can be determined by SRT. Shorter the solid retention time the lower will be the substrate uptake efficiency which results in the reduction of overall process efficiency, while the productivity and technical feasibility of hydrogen production would enhance after achieving the optimal SRT at low HRT (Hydraulic Retention Time). We need to increase the retention time to get better efficiency. Kim et al.[36] examined the effect of SRT and HRT and it was found that he obtained the max. hydrogen yield of 80.9mL H_2/g with SRT of 126h and 33h of HRT. Wang and Zhao [39] reached to the hydrogen yield of 65mL H_2/g vs at SRT of 160 d which is a very long time and is done in a two-stage process.

However, OLR is a component that has its own effect on the food waste conversion to hydrogen. In some studies, the lower hydrogen productivity was seen at higher OLRs or vice versa. There would be an optimum OLR for maximum hydrogen yield [39]. Wang and Zhao reported the steady hydrogen yield at lower OLR. Another important parameter is pH and the optimum pH reported for hydrogen generation from organic waste range from 4.5 to 6.5 [40]. The rackup of fermentation products, i.e., CO_2, inhibits the microbial growth after increasing the acidity. Such fermentation products can be expelled out from the fermentation channel by basic gas sparging and agitation. Frequently, pH can also be controlled by introduction of inoculum recycling [35]–[38], [40], [41]. Another method for pH control is sludge recirculation which is an economically favourable method as compared to introduction of alkali. Recirculating high-alkalinity sludge is most favourable approach to maintain the long-term firmness of continuous two-stage process.

The productivity of hydrogen gas is generally low because most of the feedstock ended up as organic acids. Hydrogen gas production needs to be combined with methane and organic acid production processes. Kyazze et al. [40] examined the improvement in efficiency of hydrogen generation by using a two-stage hydrogen-methane production approach. Photo fermentation is a technique that has been alternately used for the conversion of organic acids to hydrogen gas.

3.3 BIOGAS PRODUCTION

Biogas, fundamentally methane, is a sustainable power source that has been utilized by people for an exceedingly long time. Methane is also called GHG (Green House Gas) which outflows from the food waste that is scraped in landfills

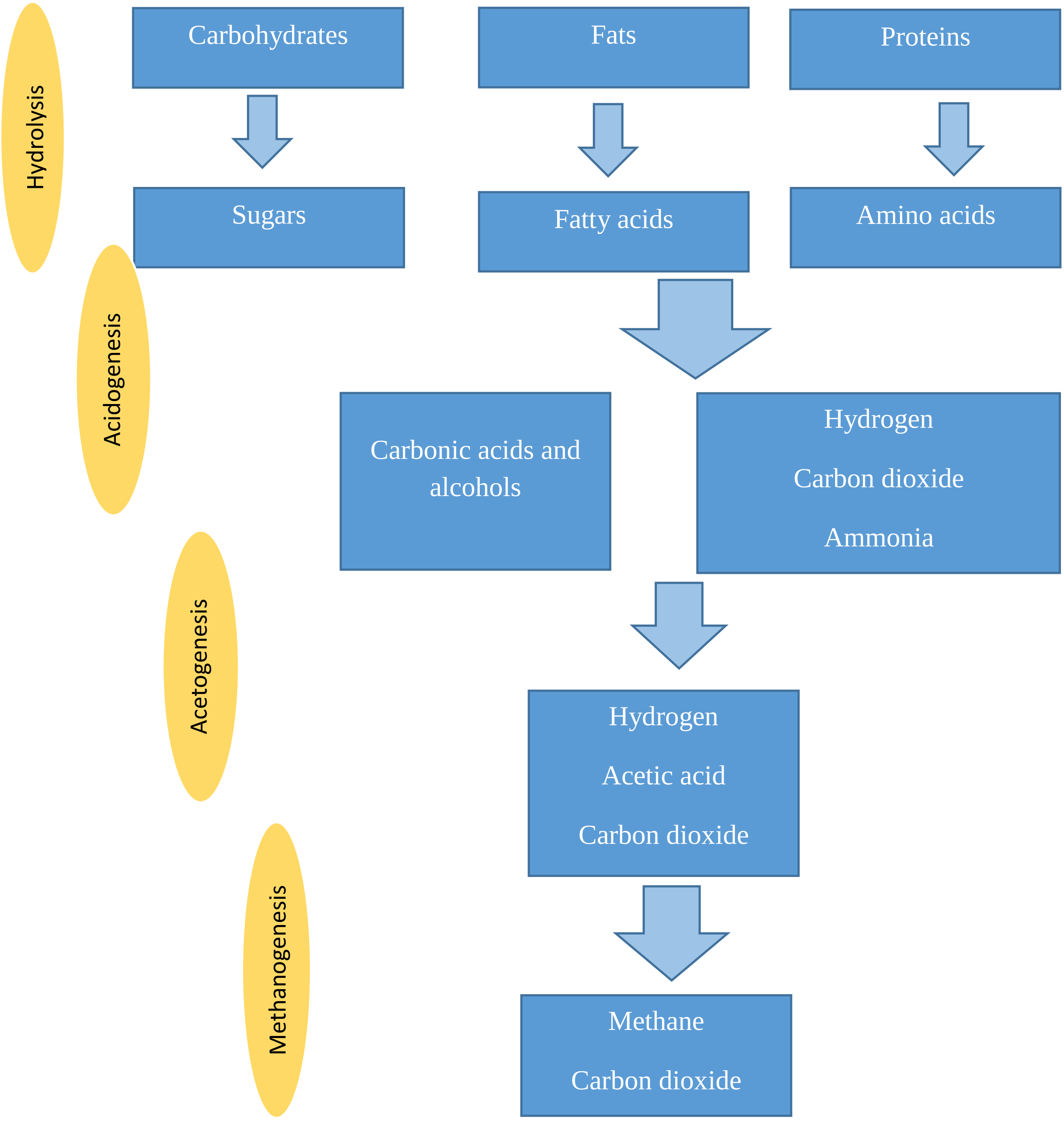

Figure 5: overview of biogas production

Bioconversion of food waste into methane under controlled conditions decreases the Green House Gas effect from food waste, yet additionally, it also recovers the accessible energy from the food waste.

The manufacturing of biogas is done through an anaerobic process and it is an admissible solution for the treatment of waste because it is cost-efficient, and waste is utilized as a feedstock. Anaerobic digestion is a chain of processes in which in the absence of oxygen, microbes break down compostable material. Biogas can be used for industrial as well as domestic energy purposes. The digestion or degradation process begins when bacterial species added to the waste to hydrolyze it and convert complex inorganic polymers into simpler sugars and other simpler units so that they can be accessible by other microorganisms. Then Acidogenic bacteria are added into the mixture which converts the sugars and aminoacids into CO2, H2, ammonia, and organic acids. Then comes the picture of the role of Acetogenic bacteria which transform these organic acids into acetic acid, accompanying additional ammonia, H2, and CO2. In the ending, methanogens transform these products into CO2 and biogas.

Lee et al. [42] investigated the single-stage anaerobic digestion utilizing a 5-L continuous digester and achieved the methane yield of 440mL/g VS (Volatile solids). A volatile solid is the combustible portion of total solids. The major factors that affect the performance of anaerobic digestion are process configuration and characteristics of feedstock [43]. Many physical and chemical properties of the food waste can influence the biogas production and stability of the process like particle size, nutrient contents, moisture, and volatile solid.

3.3.1 Single-stage anaerobic digestion

This process is mostly utilized for municipal solid waste treatment. This method includes four reactions: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. These reactions carried out simultaneously in an isolated reactor. This design has less investment cost [44] and the chances of failures are also less in this system. This system could be wet or dry [45]; wet anaerobic digestion provides higher methane productivity in comparison to dry anaerobic digestion [46]. El-Mashad et al. [47]

investigated that the digester was not durable because of the volatile fatty acids inflation and low pH, this results in low biogas productivity.

Co-digestion of food waste items, yard waste and activated sludge simultaneously under anaerobic conditions in semi continuous biodigester is called as HS-AcD (high solids anaerobic co digestion). These substrates when mixed with inoculum and crushed oyster shells and $NaHCO_3$ in the ratio of 1 (S/I) give highest yields of methane i.e., 183 ml CH_4/g VS.

Along with food waste if drinking water treatment sludge is mixed in small amounts then it not only increases the yield of methane but also shorten the lag phase and retention time hence save about 4 days. It is experimentally calculated that 6 mg/kg DWTS addition into food waste increase the yield for about 58% and yield becomes 522 N ml/g VS in comparison to controlled digester [48].

3.3.2 Two-stage anaerobic digestion

Biogas is produced utilizing a two-stage anaerobic digestion process and it has been used to produce both Hydrogen and Methane [49]. In the first stage, the system is enriched with fast flourishing acidogens and hydrogen producing microbes to produce hydrogen and VFAs and then methanogens and slow flourishing acetogens are accumulate in the second stage, they convert the VFAs to biogas and CO2. Much research had compared the single and double stage anaerobic process and found two stage anaerobic digestion to be more durable. Massanet-Nicolau et al. [50] have also compared both systems and found that biogas yield improved in the two-stage anaerobic digestion by 37%. Two stage anaerobic digestion has greater potential for recovering the energy and this has also been proved by Lee and Chung [51].

In addition to food waste when paper waste is co digested, total energy yield increases [52]. This is done by recirculated two phase anaerobic digestion (R-TPAD) in which concentration of paper waste is increased up to 50 % with FW resulted into increase in biohydrogen yield from 50 to 79 NL-H_2/Kg and a slight decrease in biomethane yield from 426 to 329 NL-CH_4/Kg but overall energy yield is increased with increased in population of hydrogen producing bacteria i.e., *Caproiciproducens and Thermoanaerobacterium.*

Garden waste and food waste are co fermented in 2 stages to get higher yield of biomethane and biohydrogen in 90:10%. 2 processes of hyperthermophillic dark fermentation and mesophilic anaerobic digestion is coupled together for co fermentation as a result highest hydrogen yield is 46 L kg-1 in first stage and in second stage by end products of first stage biomethane yield of 682 L kg-1 is obtained [53]. These yields are found higher than individual substrate treatments. This co fermentation save time and energy because individual substrate treatment leads to lag phase after certain amount of biogas production which is not the case in co fermentation.

3.3.4 Reactor configurations

There are various reactors configuration that have been developed for biogas production. One of them is Packed bed reactors (PBR) which is developed to obtain high loading, immobilize microbial consortia and stabilize methanogenesis [54]. Parawira et al. [55] examined the performances of two dissimilar reactor configurations, one of which consisting a solid-bed reactor for acidification joined to an UASB reactor and other consists of a solid-bed reactor joined to a PBR. But there is a limitation of PBR over UASB as it degrades the organic materials rapidly than UASB, while the methane productivity (390 mL/g VS) was equal in both systems. UASB has various advantages amid the high-rate anaerobic reactors, it is widely used for treatment of several organic wastes. It immobilizes anaerobic bacteria by granulation which in turn gives higher microbial activity and good settling feature [56]. UASB reactor was efficient as it has high methane production of 0.912 L/g COD, this is because of accumulation of low volatile fatty acid under managable temperature and pH. A maximum biogas productivity of 1.37 L/g COD was obtained at a temperature 55°C, OLR 12.5g and high retention time of 4 days. CSTR and FBR were also examined for methane production [54] and the productivity of 670 normalized litres (NL) biogas/kg VS with the CSTR and 550 NL biogas/kg VS with the FBR were obtained by utilizing methane at a concentration of roughly 60% for both reactor systems. But the FBR reactor system is much more stable than CSTR.

In short, we can say that two-stage process could achieve much greater methane production and it is lowly susceptible to alteration in OLR than a single stage process. Koike et al. reported the highest biogas productivity of 850 L/g VS from the two-stage anaerobic digestion of food waste [9]. This process can convert the 85% of total food waste energy into ethanol and methane.

3.4 BIODIESEL PRODUCTION

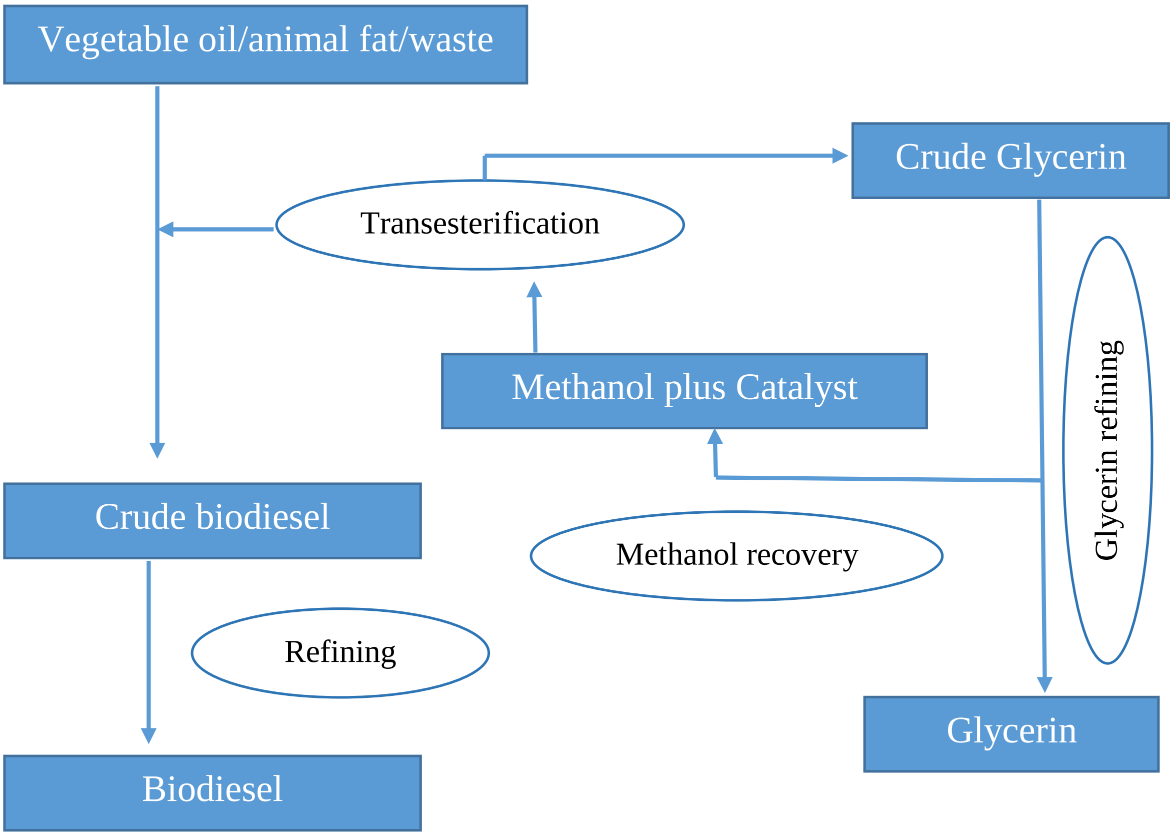

Figure 6. overview of biodiesel production process.

Biodiesel is a monoalkyl ester of unsaturated fats and this can be produced from various food waste. biodiesel is mostly obtained from vegetable oils and animal fat. biodiesel can be used directly without any temper in the existing designs of engines alone or in the form of mixture with petroleum. Use of biodiesel will reduce about 75% of overall emissions of engines and act as lubricant for the vehicle which increases the durability of the vehicle. There are numerous techniques for production of biodiesel from food waste including direct transesterification utilizing alkaline or acid catalysts or it can be produced by transesterification of microbial oils generated by several oleaginous microorganisms [9], [57]–[59]. Yeast strains can also produce microbial oils and this oil can also be used as a substitute for plants oils because this oil has alike fatty acid compositions. This oil is used as a primal matter for biodiesel production [60]. Algae is another well suited option for biofuel production due to its low cost and higher availability, it possesses higher potential for the production of biofuels. Pleissner et al. [61] have revealed that the food waste hydrolyzate has the potential to be used as culture medium and nutrient medium in cultivation of microalgae for the manufacturing of biodiesel. The food waste hydrolyzate was prepared by utilising *A. awamori* and *A. oryzae* and then the prepared hydrolyzate used as culture medium for the flourishing of microalgae *Schizochytrium mangrovei* and *Chlorella pyrenoidosa*. The resulting biomass production is 10-20 g because the microorganisms grow well on food waste hydrolyzate.

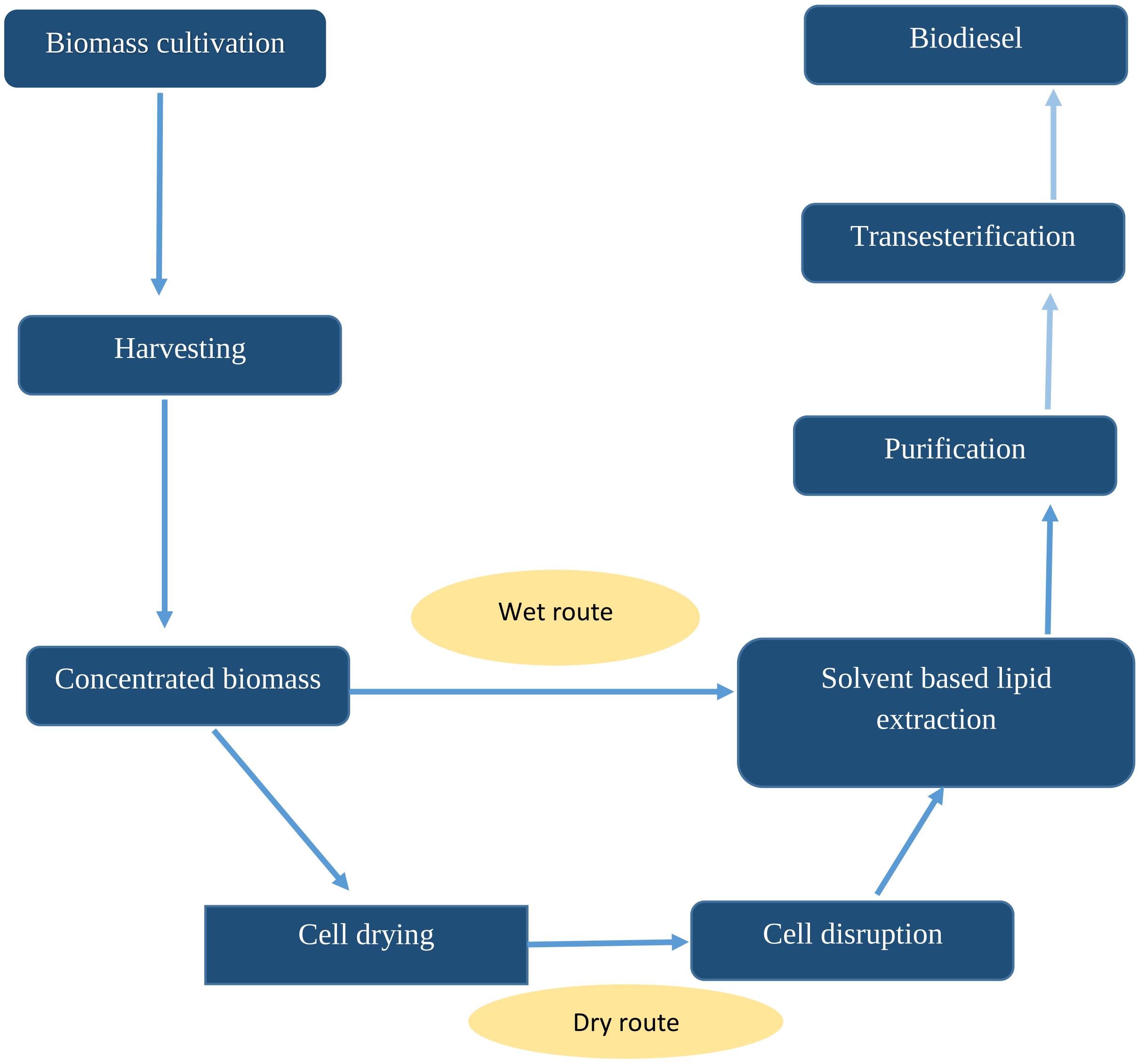

Figure7. Biodiesel production from microalgae through Dry and Wet route

Another suitable method of transesterification for biodiesel production includes use of auxiliary energies i.e., ultrasound (US), microwave, radiofrequencies for saving energy and reaction time and in addition to this, their use can save reaction inputs i.e., methanol to oil molar ratio. This is conducted on solid food waste oil as feedstock which contains high amount of oleic, palmitic, and linolenic acid. First these free fatty acids are pretreated by acid catalysed esterification followed by transesterification by conventional and ultrasound assisted reaction. In conventional method where heating and stirring is used for emulsification, which is slow and energy consuming, US assisted reaction include cavitation which generate gaseous microbubbles and their implosion because of higher outer pressure than inner lead to physical and chemical reactions to emulsify the triglycerides and methanol at higher speed. US assisted reaction save up to 40 minutes per reaction as compared to conventional method.

Biodiesel can be obtained in satisfactory amounts from edible and non-edible vegetable oils which is found highly suitable for diesel engines. This feedstock contains soybean oil and oils derived from baobab seed and Firmiana platanifolia (Chinese parasol tree). In addition to that algae are considered now a great source. To produce biodiesel, we require oil content which can be derived from industrial food waste with the help of DME (liquefied dimethyl ether). Extracted oil from IFWs (industrial food waste) is then transform into their fatty acid methyl esters and then into biodiesel. Feed stock found in IFWs is coffee grounds, soybean and rapeseed cake which yield oil content 16.8%, 0.9% and 2.6% respectively using DME. DME is energy saving and environment friendly solvent which is found a suitable alternative to conventionally used solvents for oil extraction like hexane.

Microalgal strains	**Lipid content (% dry weight)**
Schizochytrium sp.	50-77
Nitzschia sp.	45-47
Nannochloropsis sp.	31-68
Hormidium sp.	38
Botryococcus braunii	25-75
Achnanihes sp.	44.5
Isochrysis sp.	25-33

Table 2: list of some high lipid yielding microalgae with their lipid content

To produce lipid rich biomass in a culture which has carbon in limited amount, Papanikolaou [59] examined the capacities of five *Aspergillus sp.* and *Penicillium expansum* and achieved the lipid yield of 0.64 g/g dry cell weight and the biodiesel productivity of 0.74 g/g was prevail by Aspergillus sp. ATHUM 3482. The productivity of biodiesel from the food waste is relatively low and transesterfication is also required to produce biodiesel. The food waste also contains water as an additional obstacle in transesterification because this is inhibitory in this process. Another obstacle in transesterification is pigment, the separated crude oil obtained from food waste has obtained colour due to the pigment. This pigment decreases the efficiency of transesterification reaction so this pigment has to be removed from feedstock for further processing [62].

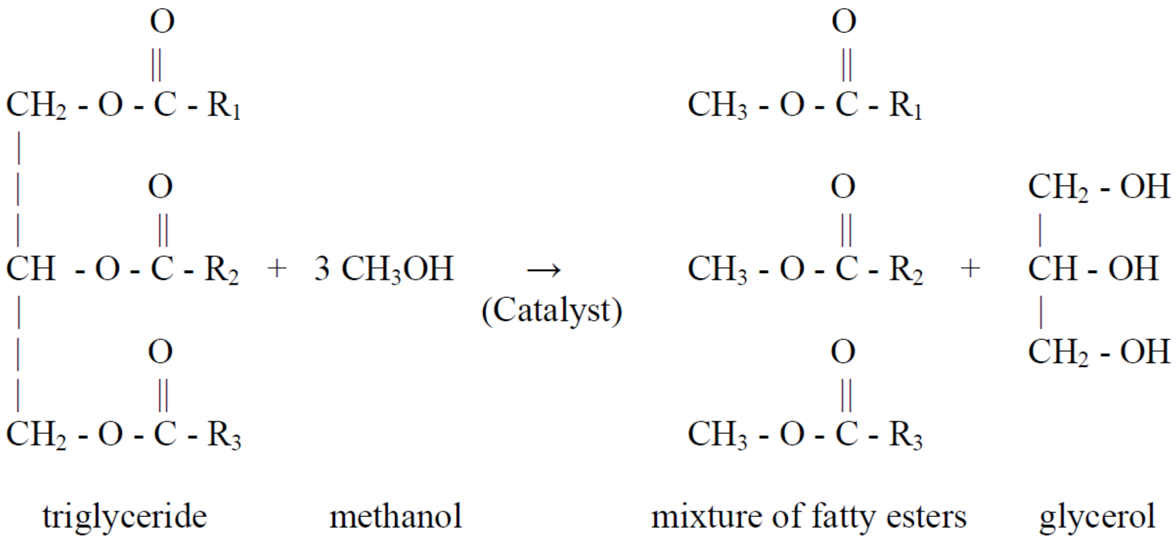

Figure 8. overview of the transesterification process

The greatest lipid yield of 0.74 g/g oil that was acquired from waste cooking oils and with a transesterification yield of 0.95 FAME/g lipids, the FAME (fatty acid methyl ester) profile directly affect the biodiesel properties [63] and it very well may be assessed that 86.5, 201.9 and 647 kT (kilotons) of biodiesel can be manufactured every year in South East Asia, Asia and on the planet, respectively. This can conceivably produce $24.5*10^6$ GJ energy every year universally.

4 DISCUSSION AND CONCLUSION

Food waste is presently the most promising raw material for biofuel generation to furnish a renewable and sustainable source of energy. It not only provides energy but using food waste for energy generation solve the problem of environmental pollution such as land, water, and air pollution. Food waste consists mainly of carbohydrate portions in large amounts and through fermentation we can convert it into bioethanol and biohydrogen. Biofuels production can be influenced by several parameters which include the availability of feedstock, pretreatment methods, temperature, catalysts, reaction configurations, etc.

In all the processes, step of the breakdown of the substrate is included which can be done either mechanically or biologically. Mechanical disruption includes energy consumption which results in a higher cost of production while biological breakdown includes microbes and their enzymes which are cheap but time-consuming at the same time. So the decision of method for the same is a choice and sometimes depends on environmental conditions.

This can be concluded that the production of biofuel like ethanol, hydrogen, biogas, and biodiesel from waste is economically feasible, but few hassles of collection and transport of food waste and appropriate method of conversion should be considered. The feedstock required for such production i.e., food waste should be of low or no cost and with environmental benefits. Considering the low to nil carbon emission by biofuels, it can be seen and approached as the future fuel technology. Biofuels are generally completely combustible as compared to standard petroleum fuels so no harmful effect to the environment and problem of global warming can be tackled in near future. Further, the regulation and cost of production could be upgraded by in-depth research and studies. We can say that it is high time to implement the future fuel technology which is sustainable and renewable.

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PUBLICATION DETAILS

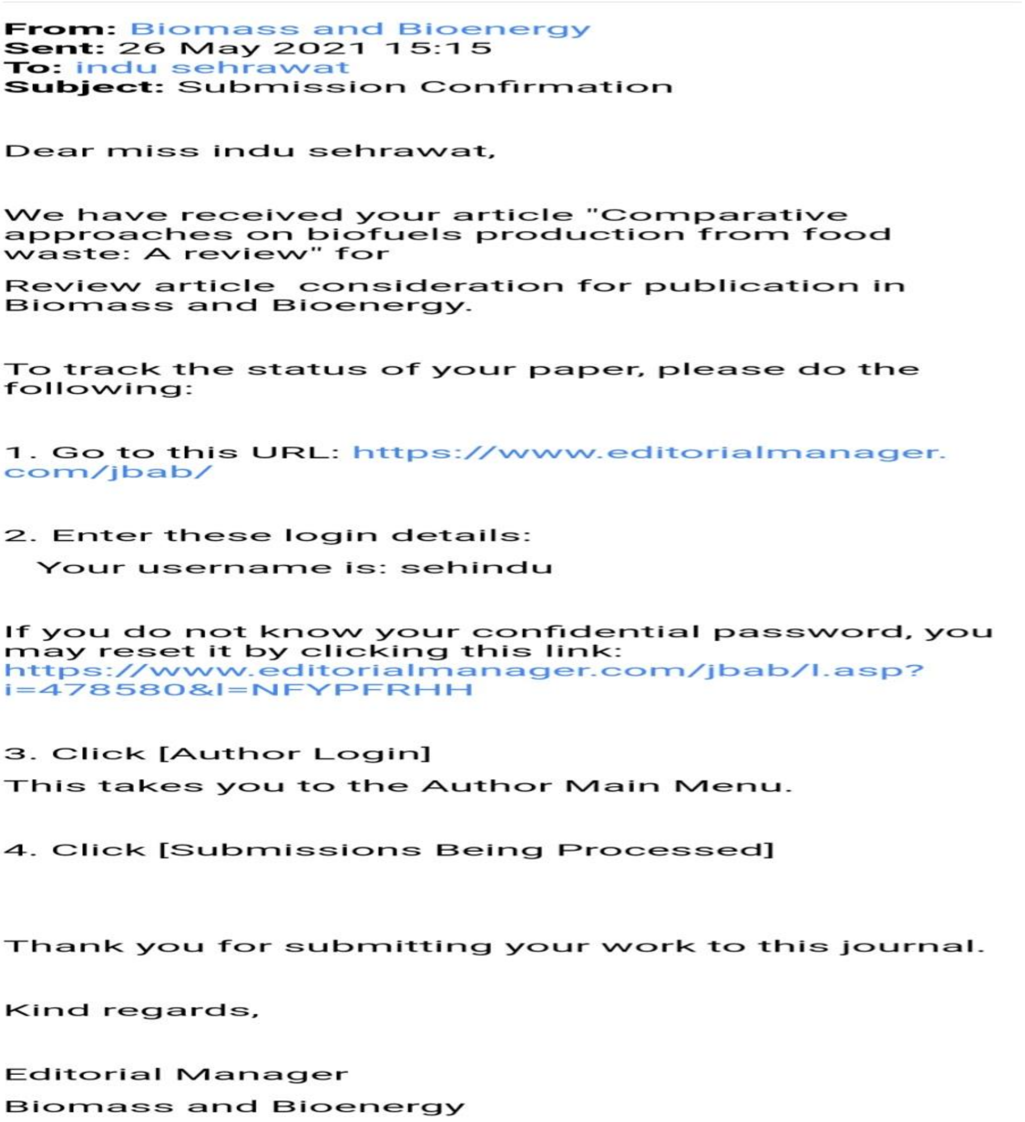
Title of paper: comparative approaches on biofuels production from food waste: A review

Author name: Indu, Dr. Kriti Bhandari, Aayaz Mohsin

Name of journal: Biomass and Bioenergy

Status of paper: Communicated

Date of paper Communication: 26th may, 2021



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I hereby certify that the work which is presented in the research work entitled “biofuel production from food waste” in fulfilment of the requirement for the award of Degree of Masters in Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during a period from 7th Jan 2021 to 28th May 2021, under the supervision of Dr. Kriti Bhandari.

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Title of paper: Comparative approaches on biofuel production from food waste: A review

Author’s name: Indu, Dr. Kriti Bhandari, Aayaz Mohsin

Name of Journal: Biomass and Bioenergy

Status of paper: Communicated

Date of paper communicated: 26th May, 2021

Date of paper acceptance: NA

Date of paper publication: NA

Indu

Indu

2k19/MSCBIO/18

SUPERVISOR CERTIFICATE

To the best of my knowledge, the above work has not been submitted in part or full for any degree or diploma to this University or elsewhere. I further certify that the publication and indexing information given by students is correct.

Kriti Bhandari

Place: Delhi

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Date: 28th May, 21