Waste peels as low-cost substrate for microalgal cultivation under a biorefinery approach

A Thesis

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

by

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DEDICATED

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MY PARENTS

SCHOOL OF ENERGY SCIENCE AND ENGINEERING

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STATEMENT

I do hereby declare that the content embodied in this thesis entitled "**Waste peels as low-cost substrate for microalgal cultivation under a biorefinery approach**" is the result of investigations carried out by me at the School of Energy Science and Engineering, Indian Institute of Technology Guwahati, Guwahati, India, under the guidance of **Prof. Kaustubha Mohanty** and **Prof. Debasish Das**.

In keeping with the general practice of reporting scientific observations, due acknowledgements have been made whenever the work described is based on the findings of other investigations.

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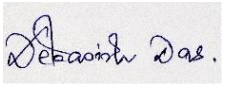
CERTIFICATE

This is to certify that **Ms. Barasa Malakar** has been working under our joint supervision since July 2014. We hereby forward her thesis entitled *"**Waste peels as low-cost substrate for microalgal cultivation under a biorefinery approach**"* to be submitted for the award degree of Doctor of Philosophy to IIT Guwahati. We certify that she has fulfilled all the requirements according to the rules of this institute and the investigations in her thesis have not been submitted elsewhere for a degree or diploma.



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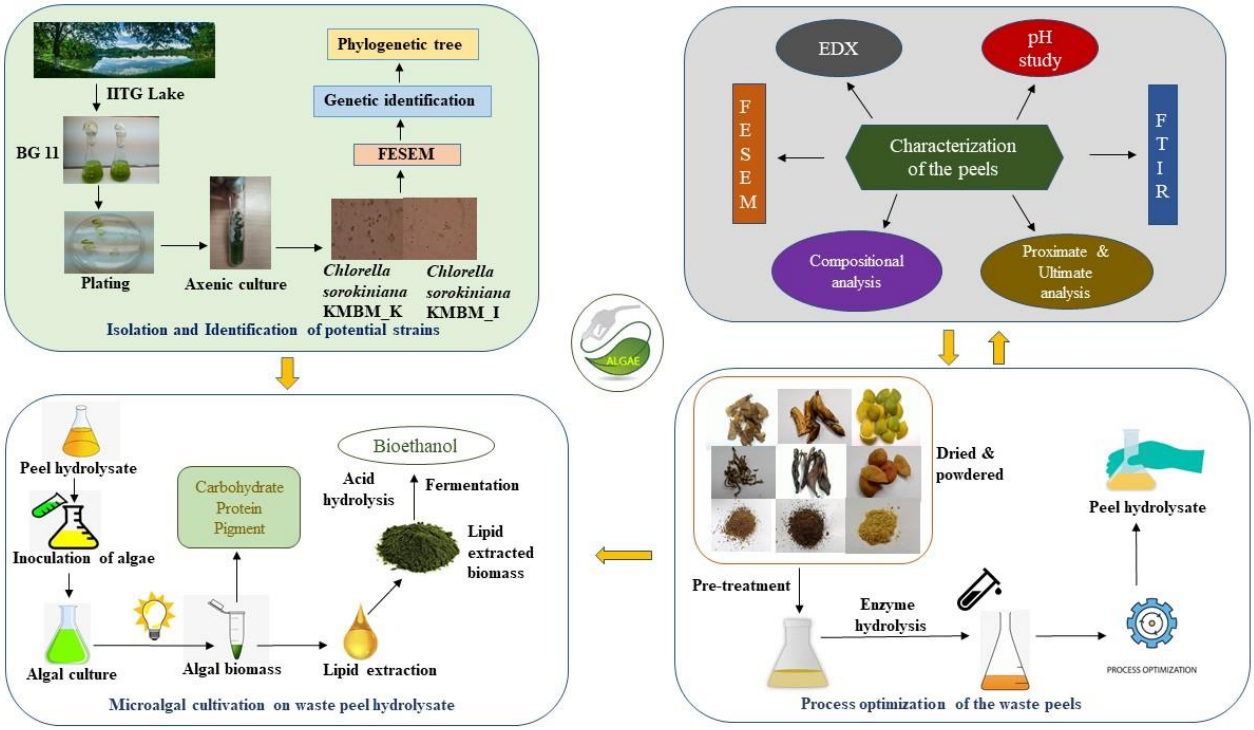
ABSTRACT



Continuous escalation of worldwide energy demand, depletion of conventional fuels and environmental concerns regarding the usage of fossil fuels has urged our globe to search for alternative resources for suitable replaceable fuels from alternative non-food resources which are economically viable, renewable and sustainable. For addressing this concern, the potent utilization of cheap and renewable biomass for the production of fuels and value-added bio products is deemed to play a key role. Biomass such as microalgae is a suitable candidate for that and has gathered more interest than land plants and crops because they have faster growth rate, higher photosynthetic efficacy, high oil content and sustainable biomass production. Oleaginous microbes like microalgae, fungi and bacteria has the ability to accumulate lipid. Microalgal biomass as a renewable feedstock has received substantial attention for the development of sustainable energy system that can replace depleting fossil fuels in the area of producing energy.

Due to their metabolic versatility, microalgae are treated as a potential and viable energy feedstock. Microalgae has emerged as an excellent resource in this regard but the excessive cost

of nutrients is a vital restriction for producing economically viable algal fuels. However, the commercial production of microalgal biomass have a major limitation because of the high cost involved in the process. The cost of growth medium is a major issue in the large-scale algal growth and commercial production of microalgal fuels. Substitution of the chemical growth medium with low cost organic biomass substrate could help in dealing the cost related problems associated with cultivation of microalgae and also in dealing with heaps of agricultural and food waste generated daily. These food and agricultural wastes are high source of nutrients and carbohydrates and hence can be used as the cultivation medium for microalgae.

The suitability of potato, banana and sweet lime peel hydrolysate was evaluated for microalgae cultivation as they are considered to be abundant source of carbon and, vital minerals and nutrients. Different pre-treatment processes were carried out for all the three peels and the best conditions yielding higher amount of glucose concentration were further hydrolyzed by enzyme. The pre-treated extract at 80 min in case of dilute acid hydrolysis with autoclaving conditions gave higher glucose concentration amongst all the different types of pre-treatment processes for both potato and sweet lime peels and hence considered for further analysis. For banana peels, at 40 min in case of autoclaving treatment followed by enzymatic hydrolysis, the pre-treated hydrolysate gave the higher concentration of glucose amongst the various treatments executed. Response surface methodology (RSM) was used to optimize the hydrolysis conditions to attain high glucose concentration. The three parameters chosen for the study were; time (h), temperature (^{o}C) and the rotation frequency or agitation speed of the incubator (revolutions per minute i.e. RPM). 46.17 ±0.77 g L^{-1}, 29.84 ± 0.57 g L^{-1} and 35.90 ±0.43 g L^{-1} of glucose yield were obtained for potato, banana and sweet lime peels respectively under optimum conditions. The waste peels were further characterized through proximate and ultimate analysis, compositional analysis, FESEM, EDX, FTIR and pH study.

This study demonstrated that these organic peel waste can act as an effective, eco-friendly, efficient and natural substrate for cost effective microalgal cultivation. Two indigenous microalgae strains *Chlorella sorokiniana* KMBM_I (Strain "I") and *Chlorella sorokiniana* KMBM_K (Strain "K") were tested for their growth and adaptability in the peel wastes. Growth kinetic parameters of the strains were analyzed in varying culture conditions. The present findings suggested that these may be suitable for cost effective medium for microalgal cultivation thus opening new

possibilities of making the most out of waste crop residues. In order to meet the energy demands, utilization of waste crop residues for microalgal biomass and lipid production is a positive and sustainable approach as an alternative to fossil fuels.

The excessive price of nutrients needed for microalgal cultivation is a vital constraint, although mass production is essential for attaining economically viable production of algal fuels and value-added products. Increasing energy demand has prompted the world to look for alternative oil resources. There is a crucial demand for sustainable and economically viable source of energy due to the reduction in conventional fossil fuel supplies. Peel wastes are zero value material rich in a lot of nutrients and elements, and could be utilized as microbial cultivation medium. These peel wastes after following some pre-treatment or hydrolysis, acts as an effective growth medium for oleaginous microbes. The biomass and lipid extracted can be further investigated for potential applications in biofuel, feed and food additives. A new insight can be obtained with this study as it integrates the concept of lipid extracted microalgal biomass residue utilization (LEMBR) approach along with waste disposal thereby serving in the management of these wastes. This study showed that these wastes can be used as a natural, cost effective, ecofriendly, efficient and affordable substrate for the cultivation of the microalgal isolates.

In this study, a considerable amount of de-oiled lipid extracted microalgal biomass residue was generated as a waste product. Valorization of these de-oiled microalgal residue into value-added products through microalgal biorefinery concept is possible to offset the biofuel production cost. Biorefineries follows the concept of zero waste production and are very energy efficient. Biorefineries aids in taking advantage of the huge potential of microalgae by recovering more value-added products from wastes.

For microalgal based bioethanol production, those microalgae which contain carbohydrates in high quantity specially glucose are considered to be the most favorable feedstock. The defatted biomass after lipid extraction was hydrolyzed and the obtained hydrolysate was fermented to generate bioethanol. This is a significant step in the production of sustainable biofuels through reuse and recycling of spent microalgal biomass.

This study highlights the potential of a bio-based refinery of two novel microalgal isolates in producing lipid, bioethanol and value-added products etc. The isolates demonstrated a remarkable amount of lipid, protein and carbohydrate. The strains also exhibited notable amount of pigments like chlorophyll-a, chlorophyll-b and carotenoids, thereby allowing their utilization in food, feed, cosmetic and pharmaceutical industries. The GCV for the strains "I" and "K" are 18.15 MJ Kg^{-1} and 20.30 MJ Kg^{-1} respectively and NCV for the strains are "I" and "K" are 16.57 MJ Kg^{-1} and 18.61 MJ Kg^{-1} respectively.

The spent biomass after extraction of lipids were reused and recycled for sustainable biofuel synthesis. Experimental results indicated that a waste-based refinery could lead to efficient production of value-added products from microalgae utilizing the organic wastes, in turn contributing to the establishment of a "green society". The highest biomass yield of 2.56±0.09 g L^{-1} and lipid content of 26.34±0.24 % was observed when the microalgal strain "K" was cultivated in the mixed peel extract of potato, banana and sweet lime. This lipid can be further processed to produce biodiesel while the spent defatted LEMBR were utilized to produce bioethanol of 7.16±0.43 g L^{-1}. The study demonstrated sustainable bioenergy production with simultaneous value added bioproducts generation from microalgae through waste peel valorization. This bioconversion facilitates the way for emergence and creation of algae based biorefinery through the production of biofuels and bioproducts. Further, this waste peels based microalgal biorefinery concept could aid in the establishment of "zero waste" strategies with proper scale up techniques. This study demonstrates the potential of indigenous native species of microalgae, for a biorefinery that generates lipids, bioethanol and various value added bioproducts.

Keywords: Biomass; Biorefinery; Hydrolysis; Microalgae; Peel; Pre-treatment.

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Symbols and Units

g L^{-1}	Gram per Litre
mg L^{-1}	Milligram per Litre
ºC	Degree Celsius
H	Hour
mL	Millilitre
w/w	Weight by weight
w/v	Weight by volume
v/v	Volume by volume
MJ Kg^{-1}	Megajoules per Kg
mL L^{-1}	Millilitre per Litre
psi	Pounds per square inch
d^{-1}	Per day
nm	Nanometer
g L^{-1} d^{-1}	Gram per Litre per day
mg L^{-1} d^{-1}	Milligram per Litre per day
mg g^{-1}, mg/g	Milligram per Gram
ppm	Parts per Million
G	Gram
mg	Milligram
µg	Microgram
µg mL^{-1}	Microgram per Millilitre
mg mL^{-1}	Milligram per Millilitre

wt %	Weight percentage/percent
ton ha^{-1} y^{-1}	Tons per Hectare per year
min	Minute
mg per 100 g	Milligram per 100 Gram
µg per 100 g	Microgram per 100 Gram

LIST OF ABBREVIATIONS

A	Autoclaving treatment
A_S	Ash
ANOVA	Analysis of variance
B	Banana peel
BLAST	Basic local alignment search tool
BPP	Banana peel powder
B+SL	Banana+Sweet lime peel
C	Control (untreated)
CCD	Central composite design
Chl	Chlorophyll
Chl-a	Chlorophyll-a
Chl-b	Chlorophyll-b
Chlorella sorokiniana-I	*Chlorella sorokiniana* KMBM_I, Strain "I"
Chlorella sorokiniana-K	*Chlorella sorokiniana* KMBM_K, Strain "K"
CHNS(O) Elemental Analyzer	Carbon Hydrogen Nitrogen Sulphur (Oxygen) Elemental Analyzer
CO_2	Carbon dioxide
DA	Dilute acid hydrolysis
DA+A	Dilute acid hydrolysis and Autoclaving treatment
DA+H	Dilute acid hydrolysis and Hot water treatment
DCW	Dry cell weight
DHA	Docosahexaenoic acid
DSP	Downstream processing
EDTA	Ethylenediamine tetraacetic acid

EDX	Energy dispersive x-ray spectroscopy
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl ester
FC	Fixed carbon
FESEM	Field emission scanning electron microscope
FTIR	Fourier transform infrared spectroscopy
GCV	Gross calorific value
GHG	Greenhouse gas
GRAS	Generally recognized safe microorganism
H	Hot water treatment
HHV	Higher heating value
HPLC	High performance liquid chromatography
H_2SO_4	Sulphuric acid
K	Divisions per day
L	Lime treatment
L+A	Lime treatment and Autoclaving treatment
L_C	Lipid content
LEMBR	Lipid extracted microalgal biomass residue
L+H	Lime treatment and Hot water treatment
LHV	Lower heating value
M	Moisture content
MEGA	Molecular Evolutionary Genetics Analysis
NCBI	National center for biotechnology information
NCV	Net calorific value
NREL	National Renewable Energy Laboratory

O_2	Oxygen
OD	Optical density
P	Potato peel
P_B	Biomass productivity
P+B	Potato+Banana peel
P+B+SL	Potato+Banana+Sweet lime peel
PBS	Phosphate buffer saline
P-I	Strain “I” cultivated in potato peel
P-K	Strain “K” cultivated in potato peel
P_L	Lipid productivity
PPP	Potato peel powder
PUFA	Polyunsaturated fatty acid
PVC	Poly vinyl chloride
rRNA	Ribosomal RNA
R^2	Coefficient of determination
RPM	Revolutions per minute
RSM	Response surface methodology
SD	Standard deviation
SL	Sweet lime peel
SL-I	Strain “I” cultivated in potato peel
SL-K	Strain “K” cultivated in potato peel
SL+P	Sweet lime+Potato peel
SLPP	Sweet lime peel powder
sp.	Species
Strain “I”	*Chlorella sorokiniana* KMBM_I, *Chlorella sorokiniana*-I

Strain “K”	*Chlorella sorokiniana* KMBM_K, *Chlorella sorokiniana*-K
T_D	Cell doubling time
USP	Upstream processing
UV-vis	Ultraviolet visible
VM	Volatile matter
μ	Specific Growth rate

CHAPTER 1

INTRODUCTION

1.1. Global energy crisis and the requirement for renewable and sustainable energy

Energy is of utmost importance in today's world because it is directly proportional to the development of any nation. The need for energy for daily usage of humankind is escalating quickly. Mankind majorly depends on fossil fuels for their energy requirement. However, the consumption of fossil fuels in the long run is not sustainable as the available resources are limited. Energy sources are broadly classified as renewable and non-renewable (Fig 1.1) (Behera, 2015).

Fossil fuels emit harmful gases such as nitrogen monoxide, carbon monoxide, sulphur dioxide, nitrogen dioxide, etc. in huge amounts when burnt, giving rise to air pollution, climate change, glacier melting, and global warming. The importance of renewable sources cannot be underestimated as it is known that non-renewable sources will eventually deplete as they are not sustainable. Fast population growth, excess industrialization, and enhanced use of fossil fuels have paved the way for global warming and the world energy crisis (Rupprecht, 2009). The two crucial elements contributing to the global energy crisis are escalating population growth and rapid industrialization (Jambo et al., 2016). Global energy demand is escalating each year due to rapid urbanization. Conventional fossil fuels such as coal and petroleum are the primary options of energy sources for transport-related applications. Hence, it is necessary to explore environment-friendly and sustainable renewable energies to satisfy fossil fuel consumption. The global energy security and environmental issues can be handled by renewable energy. Reduction of greenhouse gases emission and cleaner power generation are few important attributes of renewable energy (Milano et al., 2016). The everyday rising price of petroleum and diesel, the gradual depletion of fossil fuels and the rise in global warming has forced humanity to look out for green, renewable, and sustainable energy sources.

Renewable energy sources (Fig 1.2) include solar, biomass wind, geothermal, tidal and hydroelectricity, while non-renewable energy sources include natural fossil fuel resources like coal, crude oil, and natural gas (Goldemberg and Guardabassi, 2009; Dragone et al., 2010; Rajkumar et al., 2014).

The global energy demand is expected to enhance by 37% by 2040, according to International Energy Report 2014. The two most crucial areas with the highest energy demand among all the other sectors in India are the industrial and transportation sectors. As per the GOI

Energy Statistics 2013 (Ministry of Statistics and Program Implementation, GOI), there has been huge magnification in consumption of vital energy resources such as crude oil, coal, and petroleum products in the last decades. India is dependent on foreign crude oil, and 70% of total crude oil demand is met through imports. Hence, ensuring India's future energy requirement, long-term energy security, and sustainable energy resource development is vital. The tremendous gap between demand and supply of India's energy sector could be met through energy conservation by developing alternate energy resources from biofuels. In this context, the Government of India permitted the mandatory blending of diesel, gasoline, and petrol with biofuels to put emphasis on biofuels as an alternate energy resource. Bioenergy has India's third-largest potential for power generation after wind and energy resources. Being one of the fastest-growing economies, India is the fourth largest energy consumer in the world after the USA, China, and Japan (Joshi et al., 2017).

The energy derived in solid, liquid, and gaseous forms from biological feedstock is termed as Bioenrgy.13% of the total global energy consumption is contributed by renewable energy, out of which bioenergy accounts for 10% approximately (IEA, 2010). This comprises biofuels such as biodiesel and bioethanol for transportation, biogas such as biohydrogen and biomethane, and electricity from raw biological materials (IEA, 2013).

International Energy Agency (IEA) has declared that energy from combustible waste sources has a higher prospective as an alternative fuel in comparison to other renewable sources of energy (IEA, 2010). Oil reserves are non-renewable and not abundantly available. Reduction in crude oil dependence is critical to its replacement concerning renewable energy resources. The International Energy Agency (IEA) reported that the 2020 primary energy demand could reduce oil, coal, and natural gas by 9%, 8%, and 2%, respectively. Bioenergy can be fabricated from biomass as biofuels (liquid, solid or gaseous), heat, or power through biochemical or thermochemical processes. It is estimated that the market size for liquid biofuels could grow significantly up to 27% by 2050, although the current rate is 3% of the global transport energy (Clauser et al., 2021). 14% of the total world energy demand is considered to be supplied from renewable energy sources (UNDP, 2000). Renewable energy sources are an excellent source for mitigating greenhouse gas emissions, reducing global climate change and global warming (Panwar 2011). Biomass contributes to around 14% of energy consumption and is the fourth largest energy

globally after coal, petroleum, and natural gas. Hence, biofuel generation from combustible sources is acknowledged as an alternative fuel for sustainable energy development (Pittman et al., 2011). In this prospect, Microalgae has gathered considerable interest from scientists. (Medipally, 2015)

More than 300,000 microalgal species are found in nature, and its biodiversity is significantly higher than that of crops/plants (Kandasamy et al., 2022). Microalgae can lessen atmospheric CO_2 emission globally by 50%. Microalgal lipid content is 20-50% approximately of its dry weight with a probability of reaching up to 80% (Spolaore et al., 2006). Also, microalgal production needed no pesticide or herbicide.

Microalgae are capable of manufacturing algal oil of 58,700 L ha^{-1}, which in turn can fabricate biodiesel of 121,104 L ha^{-1}. The microalga *B. braunii* can accumulate up to 80% of bio-oil in its microalgal biomass (Khan et al., 2018). Microalgal harvesting could take up 20-30% of the total production cost of biomass (Mohammadjavad et al., 2019). Microalgal biomass is significantly enriched in lipids (4.5-7.5 ton ha^{-1} y^{-1}) compared to jatropha (4.1 ton ha^{-1} y^{-1}), rapeseed (0.7 ton ha^{-1} y^{-1}) and soybean (0.4 ton ha^{-1} y^{-1}) (Raheem et al., 2018). Microalgal biomass can produce a variety of biofuels such as bioethanol, biodiesel and bio gasoline etc. through conversion processes such as transesterification, pyrolysis liquefaction, gasification, anaerobic digestion and fermentation (Milano et al., 2016).

Microalgae have the ability to synthesize bioactive compounds such as antioxidants, carotenoids and value-added organic molecules used in food, cosmetics pharmaceuticals and feed industry in addition to the energy content of biomass of microalgae. Carotenoids such as astaxanthin, zeaxanthin and canthaxanthin are considered free radical scavengers, preventives of cancer and protectants against chronic and acute health problems. These applications are in addition to its primal energy purposes of microalgae. The microalgal biorefinery approach includes producing a wide range of biofuels and value-added products through various cost-effective and sustainable technologies. After oil extraction and valuable pigments, the spent microalgal biomass could be used as a substrate to produce biohydrogen and bioethanol through fermentation. This type of conversion plays a dual role in sustainable and renewable bioenergy production of the

microalgal biofuel industry (Nobre et al., 2013). High biomass production is the primary landmark for commercializing algal biofuel (Mallick et al., 2016).

Agricultural, aquatic, and forest residues are significant feedstocks for producing various biofuels. However, the exhaustion of these existing biomass without proper alternatives has resulted in enormous biomass scarcity and various emerging environmental crises such as biodiversity loss and deforestation. (Goldemberg, 2007, Li et al., 2008, Saqib et al., 2013). Hence, researchers have shifted their interest and focus on algal biomass as the renewable and alternative feedstock for biofuel production. (Demirbas, 2007). Actually, it is a difficult challenge to find an alternative resource that can replace fossil fuels. As algal biofuels have several advantages, they have been considered the best resource to replace non-sustainable and non-renewable fossil fuels. International Energy Agency (IEA) data reported that at the end of 2019, the global emission of CO_2 was 33GT. Microalgae use atmospheric CO_2 for photosynthesis during their growth and sequester around 80% of CO_2 from the cultivation medium (Hossain et al., 2022). Large-scale microalgal cultivation is required to commercialize microalgal biofuels through high biomass and lipid content (Peng et al., 2020). Developing an economic microalgal cultivation system is still an issue in the large-scale commercial production of biofuels derived from microalgae (Ahmad et al., 2022).

In order to amplify India's energy security, address environmental concerns, minimize fuel import dependency and improve the domestic sector, the Government of India has been promoting the "Ethanol Blended Petrol" Program (EBP). The Government of India 2018 notified a "National Policy on Biofuels" and targeted 20% ethanol blending in petrol by 2030. In June 2021, the Hon'ble Prime Minister established a clear pathway for obtaining 20% ethanol blending through "Roadmap for Ethanol Blending in India 2020-2025". This roadmap also cited a 10% ethanol blending milestone to be obtained by November 2022. The EBP program is on track to get the 20% ethanol blending target by 2025-2026 with all the initiatives taken by the Government (https://pib.gov.in/PressReleaseIframePage.aspx?PRID=1831289).

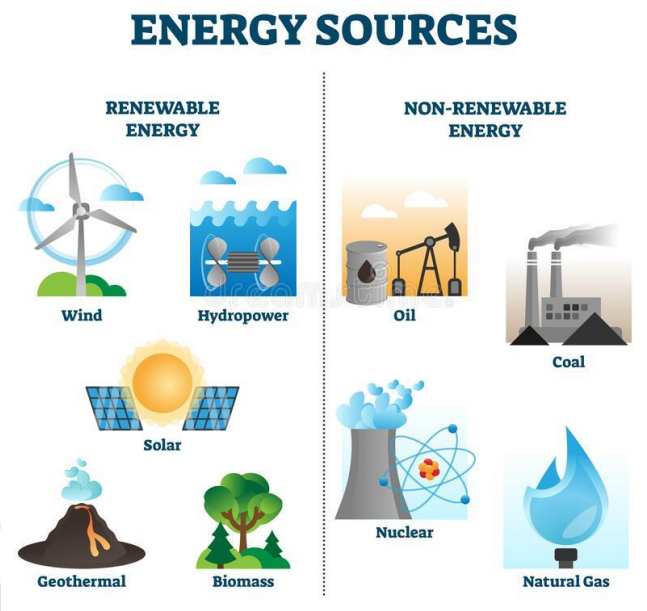


Fig 1.1. Types of Energy sources

(Source: https://www.dreamstime.com/illustration/renewable-energy.html)

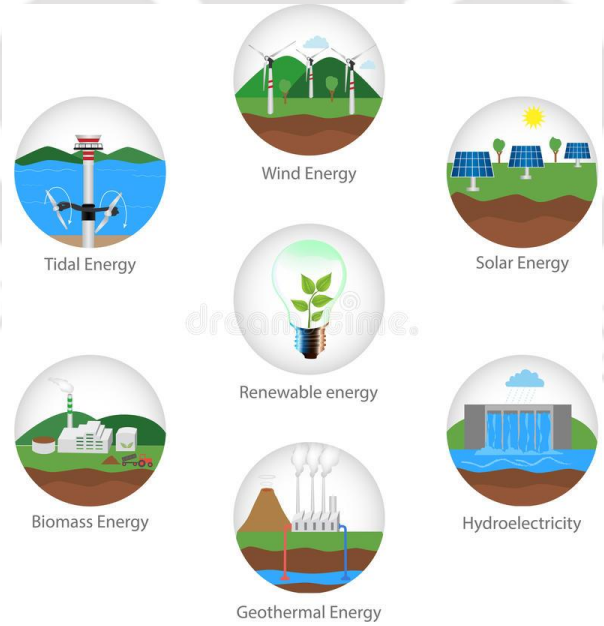


Fig 1.2. Types of Renewable energy sources

(Source: https://www.dreamstime.com/illustration/renewable-energy.html)

1.2. Biofuels

Fossil fuels are made up of mainly hydrocarbons and are gradually depleting as they are non-renewable. EIA (Energy Information Administration) estimated that the worldwide fossil fuel reserves would be depleted in less than 50 years. Fossil fuels harm the environment by contributing to global warming, releasing huge amounts of greenhouse gases while burning. Biofuels are renewable energy resources that can act as an alternative to fossil fuels. Biofuels are mainly derived from crop plants and the biomass from these plants are known as feedstock in biofuel generation. It can be concluded that any material used for biofuel production is known as feedstock (https://letstalkscience.ca/educational-resources/stem-in-context/biofuels-alternative-energy). Biofuels represent the most vital bioenergy production segment worldwide (Ho et al., 2014).

Biofuels are mainly classified into four categories depending on the raw material. Table 1.1. describes in detail the advantages and disadvantages of the different generations of biofuels.

First-generation biofuels are mainly made from consumable food crops rich in sugar, starch and vegetable oils, using conventional technology. Common biofuels of this generation include biogas, biodiesel, bioalcohols and bioethers. Crops such as sugarcane, sorghum, sugarbeet, maize, corn, rapeseed, soybean, canola, and sunflower were highly used for first generation biofuels. But since these biofuels clashed with the normal food system, it was not favoured worldwide.

Second-generation biofuels- Since the first generation received backlash due to using food crops, hence the second generation focused on non-food crops. Non-food crops like Jatropha, Pongamia, grass and lignocellulosic waste feedstock like wood or forest residue, rice husk and straw, wheat and corn stalks, sugarcane bagasse, etc. are used in this generation. These fuels are mainly composed of the cellulose in the cell wall of these non-edible feedstocks and are also known as cellulosic biofuels. Biohydrogen, bioethanol, and biomethanol are common biofuels of this generation.

Third-generation biofuels- Microbes like algae are used in this generation to produce biofuels like biodiesel, bioethanol, biobutanol, etc. Algae can create energy through photosynthesis, which

is accepted as a low-cost and high-energy source for generating biofuels. This has approximately ten times higher yield than second-generation biofuels.

Fourth-generation biofuels- Genetically modified microorganisms like algae, cyanobacteria, etc. and bio-engineered plants, through biotechnology and genetic engineering development, are used to produce fourth-generation biofuels. This is the most recent addition to the list of generations of biofuel. (vikaspedia.in/energy/energy-production/bio-energy/biofuels).

Table 1.1. Biofuel generations with their pros and cons (Source: Aro, 2016; Kagan, 2010; extension.okstate.edu).

Biofuel generation	Feedstock/ Resource	Pros	Cons
First	Agricultural crops	❖ Familiar feedstock ❖ Reduced greenhouse gas emissions compared to fossil fuels	❖ Food versus Fuel controversy ❖ Seasonal outputs and geographical limitations
Second	Non-food energy crops	❖ Less debate due to non-food feedstock ❖ Surplus supply of feedstock	❖ May compete with food crops for land ❖ Technological breakthrough required
Third	Microalgae	❖ Most promising feedstock as an array of product generation is possible ❖ High yield and productivity ❖ No dependence on particular season	❖ Technological development needed ❖ High cultivation cost ❖ Research is still in developing stage
Fourth	Genetically modified algae	❖ Least negative environmental impact ❖ Less controversial ❖ Multiple product generation is possible	❖ Early research stage ❖ Long processing time ❖ High capital cost

1.3. Microalgae as an emerging renewable resource for biofuel production

Microalgae are photosynthetic creatures that grow in various aquatic environments, including water bodies like ponds, lakes, rivers, and even wastewater. They are seen to tolerate a wide range of growth conditions like temperature, pH, salinity, and light intensity. Microalgae has diverse applications, including health or nutrient supplements, cosmetics or biofuels, or even bioremediation in wastewater treatment. Concerning renewability, sustainability, cost-effectiveness, and environmental friendliness, microalgae replace fossil fuels as biofuels. Microalgae, the third-generation biofuel production feedstock, has been considered the best alternative to fossil fuels because it avoids the limitations of first- and second-generation biofuels. The key advantage is its short harvesting cycle, which is more feasible than conventional and seasonal crops with a harvesting cycle of once or twice per year (Behera et al., 2015).

Microalgae are renewable, economical, and sustainable tiny factories for biofuel production. It has gathered extensive research for its industrial applications; however, its upgradation from small to pilot scale and finally to commercial scale is feasible and viable by tackling its limitations and challenges. Hence, to produce biofuels and other value-added bioproducts from microalgae, a new emerging biorefinery approach has been developed with zero waste concept. Due to this, the industrial cultivation of microalgae has dramatically increased in the last few years. The main reason is that algae can be utilized as a sustainable and renewable feedstock (Khan et al., 2018).

Microalgae can produce up to 20 times more bio-oil than traditional crops. The current microalgal bio-oil production rate is 10,000 L ha^{-1} y^{-1}, much higher than other sources such as jatropha, sunflower palm, and soybean crops. The microalgal growth rate is high, and the harvesting cycle is short. Genetic engineering can also be applied to microalgae to increase lipid productivity. Dried microalgal biomass feedstock can be converted to biofuels and other valuable products. Conversion of biomass to biofuels and other value-added products is a complex, expensive, and technologically challenging process. To convert microalgal biomass's lipid, protein and carbohydrate fractions into solid, liquid, gaseous biofuels and other value-added products, complex techniques are required. Different chemical conversion techniques such as fermentation, anaerobic digestion, liquefaction, pyrolysis, gasification and transesterification are applied to

microalgal biomass to produce various biofuels such as hydrogen, syngas, methane, diesel, acetone, butanol and charcoal. Microalgal biomass can generate an array of valuable products such as nutritional and pharmaceutical supplements, organic fertilizers, animal and fish feed, pigments, proteins, and diverse range of biofuels like biodiesel, bioethanol, biohydrogen, and biogas. It can also mitigate pollution and effectively participates in waste water treatment. Microalgae produces various molecular compounds such as lipids, carbohydrates, protein, pigments, antioxidants, carotenoids, enzymes, sterols, peptides and polymers. The microalgal lipids and carbohydrates are converted to various biofuels whereas the proteins, carotenoids, and pigments can be transformed into cosmetics, biofertilizers, animal feed, and nutritional supplements. It is seen that microalga *Nannochloropsis granulate* is more productive for oil/lipid with a maximum 48% dry weight, whereas *Spirulina maxima* are known to be best for protein production (Chowdhury and Loganathan, 2019).

1.4. General introduction to microalgae

1.4.1. Characteristics and characterization of microalgae

Algae are photosynthetic prokaryotic or eukaryotic organisms that usually dwell in different water bodies.

Based on their pigment content, algae are divided into the following types (Malakar and Mohanty, 2021):

- Chlorophyceae, Green algae
- Rhodophyceae, Red algae
- Phaeophyceae, Brown algae

Chlorophyceae- The grass-green color of these algae is due to the presence of a photosynthetic pigment known as chlorophyll, which can capture light energy and produce food. A few Green algal species are *U. lactuca*, *C. vulgaris*, and *D. salina* (Joshi et al., 2018).

Rhodophyceae- Along with the photosynthetic pigment chlorophyll, the red algae possess a red protein pigment known as phycoerythrin. Due to its light-harvesting property imparts color to red algae, where the red light is reflected, and the blue light is absorbed (Rossano et al. 2003). The protein establishes a covalent bond with phycobilins containing chromatophores, enabling these

algal species to do photosynthesis. A few red algal species are Irish moss, *Porphyra* sp., and *Gracilaria* sp.

Phaeophyceae- The chloroplast of brown algae contains a supplementary pigment called fucoxanthin in addition to chlorophyll. (Shimoda et al. 2010). They are mainly found in marine habitats. A few brown algal species are *Laminaria digitata*, *Postelsia palmaeformis* and *Isochrysis* sp.

Based on their size, algae are classified into:

a) Macroalgae
b) Microalgae

Macroalgae are macroscopic, multicellular, and eukaryotic organisms. Known as seaweeds, they are one of the marine ecosystem's most ecologically and economically important living assets. They are a significant natural source of nutrients, proteins, amino acids, minerals, pigments, and trace elements. The red seaweed Porphyra umbilicalis, commonly known as Nori, the long brown seaweed *Macrocystis pyrifera,* widely known as kelp, and the green seaweed *Ulva lactuca,* generally known as sea lettuce, are some famous macroalgae (Christaki et al. 2013).

Microalgae are microscopic, unicellular, and prokaryotic organisms with a size range of 1 to 50 μm. They are rich in phosphorous, iron, beta-carotene, vitamins, folic acid, calcium, biotin, and pantothenic acid. Mostly they are phototrophic but can grow mixotrophically and heterotrophically (Fabregas and Herrero 1990). The chemical composition of microalgae makes them a highly significant bio-sustainable component for numerous valuable applications. (Pimentel et al. 2018). Microalgae can survive in all habitats. They have higher productivity, multiply quickly, and survive harsh environmental conditions. They are rich in valuable elements such as lipids, carbohydrates, protein, antioxidants, and pigments like chlorophyll and carotenoids. The green algal family Chlorophyceae such as *Chlorella vulgaris, Hematococcus pluvialis* and *Dunaliella salina* are a few of the species used commercially for biofuel production (Christaki et al. 2013).

Microalgae grows faster than macroalgae and also contains more lipids than macroalgae. (Lee et al., 2014). Microalgae is a rich source of oil content compared to other conventional resources (Table 1.2.).

Table 1.2. The oil yield of different biofuel feedstocks (Source: Mata et al., 2010).

Feedstock	Oil content (% oil by weight in biomass)
Corn/Maize	44
Soyabean	18
Castor	48
Canola/Rapeseed	41
Sunflower	40
Jatropha	28
Microalgae	30-70

1.4.2. Bottlenecks associated with microalgal biofuel technology

Microalgal biofuel production involves two significant phases: upstream and downstream process. The upstream process includes various cultivation strategies and techniques, whereas the downstream process incorporates harvesting technologies and sustainable biofuel production. Biofuel from microalgae is considered commercially viable because of lower greenhouse gas emissions, non-requirement of extra land for production, and improved air quality by absorbing atmospheric CO_2. Nevertheless, there are a few limitations in microalgal biofuel processing, including lower biomass and lipid productivity, small cells making harvesting of microalgae a

costly process, and a considerable amount of water required for cultivation. But these constraints can be overcome by technology improvement and genetic engineering for higher biomass yield and lipid content (Medipally, 2015). To increase microalgal productivity, culturing technologies should be improved (Tan et al., 2020).

"Algae-for-fuel" has gathered huge interest in the last few years. Upstream possessing of microalgal cultivation is considered the baseline in microalgae research. The upstream processes directly influence the quality and quantity of the microalgal biomass and are technically and economically significant. Isolation and screening of oleaginous microalgae through strain selection, bioreactor design, preparation of cultivation medium and environmental factors adjustment are essential steps in upstream processing (Mallick et al., 2016; Daneshvar et al., 20121). Generally, downstream processing of microalgae cultivation is divided into cell harvesting, drying, and biomass processing through oil extraction, conversion (transesterification), refining of the crude product, and the overall cost of production. Cell harvesting separates the growth medium from the biomass when microalgae reach the stationary growth phase. The biomass concentration depends on the algal strain, reactor type and cultivation conditions. Harvesting of microalgae is a complex process as its handing requires a great deal of water making it a water-intensive process. The tiny size of the microalgal cells and the dilute culture biomass concentration also add to this challenge. The capital and operational cost of harvesting techniques are also towards the higher side, around 50% of the total production cost. However, microalgae to biofuel or bioproduct conversion are still at the developmental stage. Rather than the technological aspect, process economics appears to a major hindrance for the commercial utilization of microalgal biomass (https://extension.okstate.edu/fact-sheets/downstream-processing-of-algal cultures.html).

There are many advantages of microalgae as a feedstock for biofuel production. Some of them are listed below (Spolaore et al., 2006; Dismukes et al., 2008; Dragone et al., 2010):

(a) Microalgae grow throughout the year; hence, no specific seasonal requirement exists.

(b) Herbicides and pesticides are not needed for microalgal cultivation.

(c) Oil content is higher than other conventional crops.

(d) Can grow in freshwater, brackish water, seawater, and wastewater as well.

(e) Ability to grow under harsh conditions and stress.

(f) Tolerance to higher carbon dioxide content.

However, there are a few disadvantages as well in microalgal cultivation ((Demirbas, 2010; Ho et al., 2011). They are:

(a) Higher cultivation cost compared to conventional crops.

(b) High energy input is required during harvesting, which is approximately 30% of the total production cost.

(c) Microalgal cultivation is a water-intensive process.

(d) low biomass production, which acts as a hurdle for its industrial production.

Therefore, to develop an economical, cost effective and energy efficient process compared to other biofuels, low-energy harvesting techniques must be developed, which in turn can generate high oil productivity. This can be achieved through genetic modification, which is considered to be the future of algal biofuels. Advances in photobioreactor designs and using biorefinery concept leading to "zero waste concept" could further reduce the microalgal biofuel production cost (Behera et al., 2015).

1.5. General introduction to the waste peels used in this study

Fruit and vegetable peels are generated in considerable amounts in industries, hostels, juice shops, and households. They are mostly dumped along with other wastes without segregation making them unfit for further use. Vegetables and fruits peak the most consumed food globally. Citrus fruit waste constitutes around 50% of the wet fruit weight after its use (Russo et al., 2021). Each year Citrus fruit processing industry creates 10 million MT of waste globally (Suri et al., 2022). Approximately 70 to 140 thousand tons of potato peels and 36 million tons of banana peels are produced annually. Disposal of waste peels creates a huge environment issue as they contain high moisture content, are highly prone to microbial decay, and generate displeasing odour-

producing gases which hamper the natural balance of the air in the environment (Martinez-Fernandez et al., 2021; Zaini et al., 2022).

India is a major producer of potatoes, sweet limes, and different varieties of bananas. Potato (*Solanum tuberosum*) is accepted as the staple food and is a main carbohydrate source in many parts of the world and an integral part of much of the world's food supply. Sweet lime (mosambi or musubi, *Citrus limetta*) is a juicy fruit that is a leading juice substrate in all the juice-based industries. A favorite among juice lovers, sweet lime is a rich source of vitamins and minerals. Banana (*Musa* sp.) is a versatile food. There are wide varieties of banana species, and can be eaten raw as a fruit when ripe and cooked as a vegetable when unripe. Various food products and beverages such as banana chips, banana muffins and cakes, banana wafers, banana milkshakes, banana beer and wine are widely available worldwide. Banana puree and powder are used in baby food products as it is loaded with vitamins and minerals. Potato dominates the chips-producing industries like sweet lime dominates the juice and jam-jelly-making industries.

Potatoes, bananas, and sweet lime peels are agricultural waste products. The solid peel, discarded in the industries, is zero value by-product, collected in large amounts depending on the peeling in these industries after their processing. Mostly abrasion peeling and steam peeling are employed in these food processing industries (Schieber and Saldana, 2009).

Fruits and vegetable peels are an abundant source of organic carbons, amino acids, vitamins, and numerous macro and micro nutrients. Sweet lime peels contain 35.25% carbohydrates and 9.7% protein. They include 121.2 of Ca, 142.1 of K, 37.7 of Na, 19.7 of Mg mg per 100 g. Also, they contain 943.4 of Fe, 72.7 of Cu, 200.3 of Mn, 151.4 of Zn µg per 100 g (Barros et al., 2014). Potato peel contains 68.7% carbohydrates, 1.3% nitrogen (Arapoglou et al., 2010)) and 17.1% protein. They contain 24.80 ppm of Cu, 22.80 ppm of Zn, and 178 ppm of Fe. Also, they contain 0.041% Na, 0.156% Ca, 3.090% K, 1.80% P (Jekayinfa et al., 2015). The crude composition of potato peel and sweet lime peel has been reported as: 55.25% cellulose, 11.71% hemicelluloses, 14.24% lignin (Lenihan et al., 2010), and 18.3% cellulose, 26.2% hemicelluloses, 8.9% lignin (Kaliyan et al., 2016) for potato and sweet lime peel respectively. The composition of the banana peels is reported as: 41.38 % hemicellulose, 9.9 % cellulose, and 8.9 % lignin (Kabenge et al., 2018), 68.31 ± 0.83 % carbohydrate content, 7.57 ± 0.30 % protein content (Ahmed et al.,

2016), 56.24 ± 0.01 mg/g Calcium, 0.92 ± 0.22 mg/g Iron, 69.05 ± 0.42 mg/g Manganese, 0.02 ± 0.00 mg/g Niobium, 87.35 ± 0.03 mg/g Potassium, 2.51 ± 0.01 mg/g Rubidium, 22.51 ± 0.04 mg/g Sodium, 0.02 ± 0.00 mg/g Strontium, 0.03 ± 0.01 mg/g Zirconium (Dahiru et al., 2018). These nutrients play a vital role in supplying support for microalgae growth. Studies have shown that agricultural residues and food waste, either alone or integrated with wastewater, could be used for microalgal cultivation (Ammar et al., 2020).

References

Ahmad S, Iqbal K, Kothari R, Singh HM, Sari A, Tyagi VV (2022) A critical overview of upstream cultivation and downstream processing of algae-based biofuels: Opportunity, technological barriers and future perspective, Journal of Biotechnology 351:74–98.

Ahmed M Aboul-Enein, Zeinab A Salama, Alaa A Gaafar, Hanan F Aly, Faten A bou-Elella, Habiba A Ahmed (2016) Identification of phenolic compounds from banana peel (*Musa paradaisica* L.) as antioxidant and antimicrobial agents, Journal of Chemical and Pharmaceutical Research. 8(4):46-55.

Ammar EM, Arora N, Philippidis GP (2020) The Prospects of Agricultural and Food Residue Hydrolysates for Sustainable Production of Algal Products, Energies 13:6427.

Arapoglou D, Varzakas Th, Vlyssides A, Israilides C (2010) Ethanol production from potato peel waste (PPW), Waste Management 30:1898–1902.

Aro E-M (2016) From first generation biofuels to advanced solar biofuels, Ambio 45:24-31.

Barros HRM, Ferreira TAPC, Genovese MI (2012) Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil, Food Chemistry 134:1892–1898.

Behera S, Singh R, Arora R, Sharma NK, Shukla M, Kumar S (2015) Scope of algae as third generation biofuels, Front Bioeng Biotechnol 2:90.

Clauser NM, González G, Mendieta CM, Kruyeniski J, Area MC, Vallejos ME (2021) Biomass Waste as Sustainable Raw Material for Energy and Fuels, Sustainability 13:794.

Chowdhury H, Loganathan B (2019) Third-generation biofuels from microalgae: a review, Current Opinion in Green and Sustainable Chemistry 20:39–44.

Christaki E, Bonos E, Giannenas I, Florou-Paneri P (2013) Functional properties of carotenoids originating from algae, J Sci Food Agric 93:5–11.

Dahiru M, Zango ZU, Haruna MA (2018) Cationic Dyes Removal Using Low-Cost Banana Peel Biosorbent, American Journal of Materials Science 8(2):32-38.

Daneshvar E, Ok YS, Tavakoli S, Sarkar B, Shaheen SM, Hong H, Luo Y, Rinklebe J, Song H, Bhatnagar A (2021) Insights into upstream processing of microalgae: A review. Bioresource Technology 329:124870.

Demirbas A (2007) Progress and recent trends in biofuels, Prog Energy Combust Sci 33:1–18.

Demirbas A (2010) Use of algae as biofuel sources, Energy Convers Manag 51:2738–2749.

Dismukes GC, Carrieri D, Bennette N, Ananyev GM, Posewitz MC (2008) Aquatic phototrophs: efficient alternatives to land-based crops for biofuels, Curr Opin Biotechnol 19:235–240.

Dragone G, Fernandes B, Vicente AA, Teixeira JA (2010) "Third generation biofuels from microalgae," in Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, ed. A. Mendez-Vilas (Madrid: Formatex), 1315–1366.

Fabregas J, Herrero C (1990) Vitamin content of four marine microalgae. Potential use as source of vitamins in nutrition, J Ind Microbiol 5:259–263.

Goldemberg J, Guardabassi P (2009). Are biofuels a feasible option? Energy Policy 37:10–14.

Ho SH, Chen CY, Lee DJ, Chang JS (2011). Perspectives on microalgal CO_2-emission mitigation systems-a review, Biotechnol Adv 29:189–198.

Ho DP, Ngo HH, Guo W (2014) A mini review on renewable sources for biofuel, Bioresource Technology 169:742–749.

Hossain SMZ, Sultana N, Razzak SA, Hossain MM (2022) Modeling and multi-objective optimization of microalgae biomass production and CO_2 biofixation using hybrid intelligence approaches, Renewable and Sustainable Energy Reviews 157:112016.

IEA, International Energy Agency 2010. Key World Energy Statistics, Paris, France.

IEA, International Energy Agency 2010. Sustainable production of second-generation biofuels: potential and perspectives in major economies and developing countries.

IEA, International Energy Agency 2013. World Energy Outlook 2013, IEA/OECD, Paris.

Jambo SA, Abdulla R, Azhar SHM, Marbawi H, Gansau JA, Ravindra P (2016) A review on third generation bioethanol feedstock, Renewable and Sustainable Energy, Reviews 65:756–769.

Jekayinfa SO, Linke B, Pecenka R (2015) Biogas production from selected crop residues in Nigeria and estimation of its electricity value, Int J Renewable Energy Technology 6(2):101-118.

Joshi G, Pandey JK, Rana S, Rawat DS (2017) Challenges and opportunities for the application of biofuel, Renewable and Sustainable Energy Reviews 79:850–866.

Joshi S, Kumari R, Upasani VN (2018) Applications of algae in cosmetics: an overview, Int J Innov Res Sci Eng Technol 7:2.

Kabenge I, Omulo G, Banadda N, Seay J, Ahamada Zziwa, Nicholas Kiggundu (2018) Characterization of Banana Peels Wastes as Potential Slow Pyrolysis Feedstock, Journal of Sustainable Development 11(2):14.

Kagan J (2010) Third and Fourth Generation Biofuels: technologies, markets and economics through 2015, GTM Research.

Kaliyan B, Ameer K, Agastian P (2016) Simultaneous Production of Xylitol and Ethanol from Different Hemicellulose Waste Substrates by *Candida tropicalis* Strain Ly15, J Bioprocess Biotech 6:8.

Kandasamy S, Zhang B, He Z, Bhuvanendran N, EL-Seesy AI, Wang Q, Narayanan M, Thangavel P, Dar MA (2022) Microalgae as a multipotential role in commercial applications: Current scenario and future perspectives, Fuel 308:122053.

Khan MI, Shin JH, Kim JD (2018) The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products, Microb Cell Fact 17:36.

Lee K, Eisterhold ML, Rindi F, Palanisami S, Nam PK (2014). Isolation and screening of microalgae from natural habitats in the Midwestern United States of America for biomass and biodiesel sources, J Nat Sci Biol Med 5:333–339.

Lenihan P, Orozco A, O'Neill E, Ahmad MNM, Rooney DW, Walker GM (2010) Dilute acid hydrolysis of lignocellulosic biomass, Chem Eng J 156:395–403.

Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N (2008) Biofuels from microalgae, Biotechnol Progr 24:815–820.

Malakar B, Mohanty K (2021) The budding potential of algae in cosmetics. Algae. Springer, Singapore, 181–199. doi.org/10.1007/978-981-15-7518-1_8.

Mallick N, Bagchi SK, Koley S, Singh AK (2016) Progress and Challenges in Microalgal Biodiesel Production, Fronteirs in microbiology 7:1019.

Martinez-Fernandez JS, Gu X, Chen S (2021) Techno-economic assessment of bioactive compound recovery from potato peels with sequential hydrothermal extraction, Journal of Cleaner Production 282:124356.

Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: A review, Renewable and Sustainable Energy Reviews 14:217–232.

Medipally SR, Yusoff FM, Banerjee S, Sharif M (2015) Microalgae as Sustainable Renewable Energy Feedstock for Biofuel Production, BioMed Research International. doi.org/10.1155/2015/519513.

Milano J, Ong HC, Masjuki HH, Chong WT, Lam MK, Loh PK, Vellayan V (2016) Microalgae biofuels as an alternative to fossil fuel for power generation, Renewable and Sustainable Energy Reviews 58:180–197.

Mohammadjavad R, Moheimani, NR, Parlevliet D (2019) Luminescent solar concentrator panels for increasing the efficiency of mass microalgal production, Renewable and Sustainable Energy Reviews 101:47-59.

Nobre BP, Villalobos F, Barragán BE, Oliveira AC, Batista AP, Marques PASS, Mendes RL, Sovová H, Palavra AF, Gouveia L (2013) A biorefinery from *Nannochloropsis* sp. microalga – Extraction of oils and pigments. Production of biohydrogen from the leftover biomass, Bioresource Technology 135:128–136.

Panwar NL, Kaushik SC, Kothari S (2011) Role of renewable energy sources in environmental protection: A review, Renewable and Sustainable Energy Reviews 15:1513–1524.

Peng L, Fu D, Chu H, Wang Z, Qi H (2020) Biofuel production from microalgae: a review, Environmental Chemistry Letters 18:285–297.

Pimentel FB, Alves RC, Rodrigues F, Beatriz MPP (2018) Oliveira macroalgae-derived ingredients for cosmetic industry—an update, Cosmetics 5:2.

Pittman JK, Dean AP, Osundeko O (2011) The potential of sustainable algal biofuel production using wastewater resources, Bioresource Technology 102(1):17–25.

Raheem A, Prinsen P, Vuppaladadiyam AK, Zhao M, Luque R (2018) A review on sustainable microalgae based biofuel and bioenergy production: Recent developments, Journal of Cleaner Production 181:42-59.

Rajkumar R, Yaakob Z, Takriff MS (2014) Potential of the micro and macro algae for biofuel production: a brief review, Bioresour 9(1):1606–1633.

Rossano R, Ungaro N, D'AmbrosioA LGM, Riccio P (2003) Extracting and purifying R-phycoerythrin from Mediterranean red algae Corallina elongate Ellis & Solander, J Biotechnol 101:289–293.

Rupprecht J (2009) From systems biology to fuel—*Chlamydomonas reinhardtii* as a model for a systems biology approach to improve biohydrogen production, Journal of Biotechnology 142(1):10–20.

Russo C, Maugeri A, Lombardo GE, Musumeci L, Barreca D, Rapisarda A, Cirmi S, Navarra M (2021) The Second Life of Citrus Fruit Waste: A Valuable Source of Bioactive Compounds. Molecules 26:5991.

Saqib A, Tabbssum MR, Rashid U, Ibrahim M, Gill SS, Mehmood MA (2013) Marine macroalgae Ulva: a potential feed-stock for bioethanol and biogas production, Asian J Agri Biol 1:155–163.

Schieber A, Saldana MDA (2009) Potato peels: A source of nutritionally and pharmacologically interesting compounds – A review, Food 3:23-29.

Shimoda H, Tanaka J, Shan SJ, Maoka T (2010) Anti-pigmentary activity of fucoxanthin and its influence on skin mRNA expression of melanogenic molecules, J Pharm Pharmacol 62:1137–1145.

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006). Commercial applications of microalgae. J. Biosci. Bioeng 101:87–96.

Suri S, Singh A, Nema PK (2022) Current applications of citrus fruit processing waste: A scientific outlook Applied Food Research 2:100050.

Tan JS, Lee SY, Chew KW, Lam MK, Lim JW, Ho S-H, Show PL (2020) A review on microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids, Bioengineered 11(1):116-129.

UNDP. World energy assessment 2000 – energy and the challenge of sustain-ability. New York: UNDP; 2000 (ISBN 9211261260).

Zaini HM, Roslan J, Saallah S, Munsu E, Sulaiman NS, Pindi W (2022) Banana peels as a bioactive ingredient and its potential application in the food industry, Journal of Functional Foods 92:105054.

https://vikaspedia.in/energy/energy-production/bio-energy/biofuels (assessed on 17.4.2022).

https://www.dreamstime.com/illustration/renewable-energy.html (assessed on 12.7.22).

https://letstalkscience.ca/educational-resources/stem-in-context/biofuels-alternative-energy source (assessed on 12.7.22).

https://extension.okstate.edu/fact-sheets/downstream-processing-of-algal-cultures.html (assessed on 13.7.22).

https://pib.gov.in/PressReleaseIframePage.aspx?PRID=1831289, India has achieved the target of 10 percent ethanol blending, 5 months ahead of schedule (assessed on 15.08.2022).

CHAPTER 2

LITERATURE REVIEW

2.1. Biomass composition and structure

Lignocellulosic biomass is made up of three polymers associated with each other in a complex heterogenous matrix viz. cellulose, hemicellulose and lignin. Cellulose and hemicellulose are potential origin of fermentable sugars. Bioconversion of biomass to biofuel and biomaterials is a multistep procedure that includes the feedstocks' disintegration followed by hydrolysis and extraction of different elements. Bioconversion of lignocellulosic feedstock to biofuel is primarily composed of four essential process steps: pretreatment, enzyme hydrolysis, fermentation, and product recovery. Pretreatment has a substantial impact on bioconversion efficacy.

2.1.1. Cellulose

The main structural constituent in biomass cell walls is cellulose. Also, it is the most abundant biopolymer on the globe. The long-chain of cellulose, a D-glucose polymer, are linked together by covalent bonds, hydrogen bonds, and Vander Waals bonds (Agbor et al., 2011). Cellulose is highly crystalline, making it water-insoluble and resistant to depolymerization (Mosier et al., 2005).

2.1.2. Hemicellulose

The second ample polymer in nature is hemicellulose and they are branched, heterogenous polymers of pentoses such as xylose, arabinose; hexoses such as mannose, glucose, galactose and acetylated sugar acids (Agbor et al., 2011). Hemicellulose is interlinked to lignin and is interlaced with the cellulosic liters of biomass. It is essential to detach the hemicellulose fragments of the biomass from the other two polymers during pretreatment so that subsequent hydrolysis to recover glucose from cellulose becomes more efficient (Alonso et al., 2010).

2.1.3. Lignin

The third ample polymer in nature is lignin. It is a complex and huge amorphous heteropolymer network of phenyl propane fragments such as coumaryl, coniferyl and sinapyl alcohol, bound jointly by various bonding. The hemicellulose and cellulose fragments of biomass is surrounded by lignin, and a primary purpose of biomass pre-treatment is to depolymerize the lignin seal so that sugar can be liberated from the carbohydrate (Huber et al., 2006; Mosier et al., 2005). Lignin is insoluble in water. Different lignocellulosic constitutes

different quantity of lignin which should be separated through pre-treatment to increase biomass digestibility. Delignification i.e. extraction of lignin via pre-treatment, induces swelling of lignocellulosic raw materials, distortion of lignin form, enhances inner surface area, and enhances cellulolytic enzymes availability to cellulose fibrils (Agbor et al., 2011).

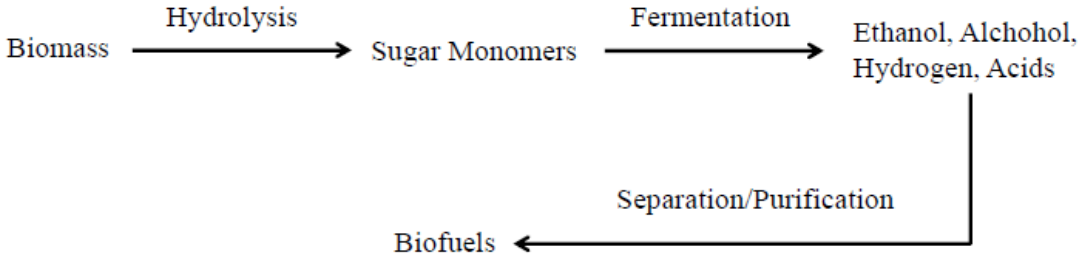


Figure 2.1. Schematic of the conversion of lignocellulosic biomass to biofuel (Source: Kumar et al., 2009).

2.2. Biomass types and categories

Basically, biomass can be categorized into four main types. By far, wood residues are the present largest source of biomass for producing bioenergy. It is derived from the wood industry comprising paper mills, sawmills and furniture processing. The second largest, is the Municipal solid waste, followed by agro residues and dedicated energy crops. Dedicated energy crops such as grasses and aquatic plants seem to have the most potential as future biomass resource as a single planting can produce numerous harvests, thereby significantly reducing average yearly value for handling energy crops compared to traditional crops. These resources contain varied quantities of cellulose, hemicellulose, lignin, and extractives (Agbor et al., 2011).

Fermentation from any biomass which constitutes sugar could procure ethanol. The raw substrates utilized in ethanol processing through fermentation are categorized into three major feedstock categories: sugars, starches, and cellulosic substrates. Sugars from sugarcane bagasse molasses and fruits can be processed into ethanol. Starches from agricultural residues viz. oats, rice, wheat, corn, cassava, potato, and root crops are first hydrolyzed to fermentable sugars. Cellulose from wood residues, and wastes from pulp and paper mills should be formed to sugars similarly. Once these simple sugars are produced, microbes can ferment them to alcohol.

However, molasses is the most extensively utilized substrate for ethanol fermentation (Lin et al., 2006).

2.3. Properties of biomass: Parameters responsible for effective pre-treatment of lignocellulosic biomass

The overall biomass digestibility is determined by parameters such as content of lignin, cellulose accessibility to cellulase and crystallinity of cellulose. Biomass resistance to pre-treatment or hydrolysis is straight associated to the inherent parameters of the biomass resource. The parameters which impart to recalcitrance of biomass includes cellulose crystallinity, degree of polymerization of both cellulose and hemicellulose, Available surface area or porosity, cellulose shielding by lignin, cellulose sheathing by hemicellulose, strength of fibres and acetylation degree of hemicellulose. The accessible surface area for enzymatic action are linked to crystallinity of cellulose and lignin and hemicellulose content (Lin et al., 2006).

Evaluation of pre-treatment methods depends on a criterion known as the "severity factor", which is described as the amalgamated impact of temperature, acidity, and time span of the pre-treatment process.

Various key constituents that affect the degradation of lignocellulosic biomass are discussed below.

Crystallinity of cellulose

The crystallinity of cellulose has been regarded as one of the most significant properties in governing the hydrolysis rate. The cellulosic fibres have both crystalline and amorphous areas. However, the maximal fraction of cellulose (around 2/3 of the total cellulose) is in the crystalline form. The enzyme cellulose easily hydrolyses the more accessible amorphous fraction of cellulose unlike the less accessible crystalline segment. Hence it is anticipated that highly crystalline cellulose will be further repellent to enzyme attack and it is a recognized fact that reducing the crystallinity will enhance the lignocellulosic digestibility (Maurya et al., 2015).

Surface area accessibility

Reports have specified a proper association between the pore volumes (accessible surface area for cellulase and hemicellulose enzyme) and the enzyme digestibility of the

biomass. The major benefit of this association is the enhancement of enzymatic hydrolysis through lignin removal. Two kinds of surface area are present in lignocellulosic biomass: external and internal. The external surface area is linked to the shape and size of the molecules and the internal surface area is depended on the capillary composition of microfibrils present in the cellulose (Maurya et al., 2015).

Effect of lignin content

The existence of Lignin protects the cellulose and hemicellulose of lignocellulosic material. Lignin is liable for the biomass's integrity, structural firmness and swelling prevention. It prevents the availability of enzymes to cellulose and hemicellulose, thereby limiting the efficacy of the hydrolysis process. It is the most significant accepted tool for the recalcitrance of biomass. Hence, effective delignification techniques can enhance the rate and extent of hydrolysis by enzymes (Maurya et al., 2015).

Impact of hemicellulose content

Hemicellulose is a physical hindrance that shields the cellulosic microfibrils from the enzymatic attack during hydrolysis. Studies have shown that the removal of hemicellulose enhances the material's mean pore size, thereby increasing its accessibility and cellulose hydrolysis probability. The other significant feature of the acetylation degree in the hemicellulose is that lignin and acetyl groups are interlinked to the hemicellulosic complex and can obstruct the disintegration of polysaccharides (Chang and Holtzapple, 2000).

2.4. Goals of pre-treatment

The major objective of pre-treatment process is to break down lignin and hemicelluloses fractions, diminish cellulose crystallinity, and enhance the porosity of the lignocellulosic biomass. Also, pre-treatment should boost the production of sugars or the potential to create them during the successive enzyme hydrolysis, limit the waste of carbohydrate, avoid the production of inhibitors that could inhibit the growth of fermentative microbes for subsequent hydrolysis and fermentation procedures, reduces energy demands and decrease cost, thereby making the process economical (Sanchez and Cardona, 2008).

An efficient pre-treatment is depicted by various criteria. Hence it should focus on: (i) enhancement of the accessible surface area (ii) cellulose and hemicellulose depolymerization (iii) solubilization of hemicelluloses and lignin, (iv) modification of the structure of lignin (v)

increase the enzyme digestibility of the pre-treated biomass (vi) decrease the degradation of sugars (vii) cellulose decrystallization (viii) minimize operating costs (ix) avoid formation of inhibitory substances (Maurya et al., 2015).

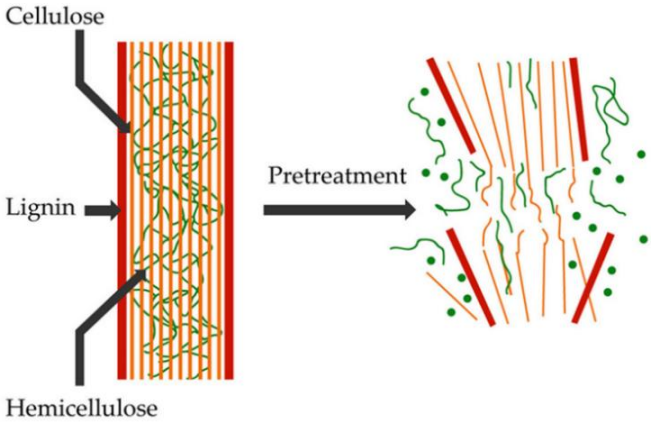


Figure 2.2. Effect of pre-treatment on the lignocellulosic biomass (Source: https://www.cleantechloops.com/pretreatment-lignocellulosic-biomass).

2.5. Biomass pre-treatment

Lignocellulosic biomass pre-treatment is a necessary step and is needed to modify the biomass composition and uncover the lignocellulosic fragments for effortless access to enzymes through enzyme hydrolysis thereby increasing the rate and yield of reducing sugars. Primarily, pre-treatment is categorized into two significant processes viz., non-biological and biological, based on the variety of the treatment technique associated. Non-biological pre-treatment techniques do not include any action caused by microorganisms and are separated into various classifications: physical (mechanical), chemical, and physico-chemical techniques (Kumar and Sharma, 2017). Newly investigated combinatorial/ mixed pre-treatment approaches are normally more efficient in maximizing the digestibility of biomass. Summary of some widely used pre-treatment methods are summarized in Table 2.1.

Mechanical pre-treatment enhances the surface area of the biomass by shrinking its size. Since reactions in the physico-chemical process happen at elevated temperatures and pressure, a considerable impact on operating conditions is necessary (Taherzsadeh and Karimi, 2007). Chemical methods break down hemicellulose and lignin, thereby detaching the structure of lignin holocellulose matrix. Biological pre-treatment techniques are widely utilized for the

delignification of lignocellulosic substrates. An overview of different pre-treatment processes are mentioned in Figure 2.3.

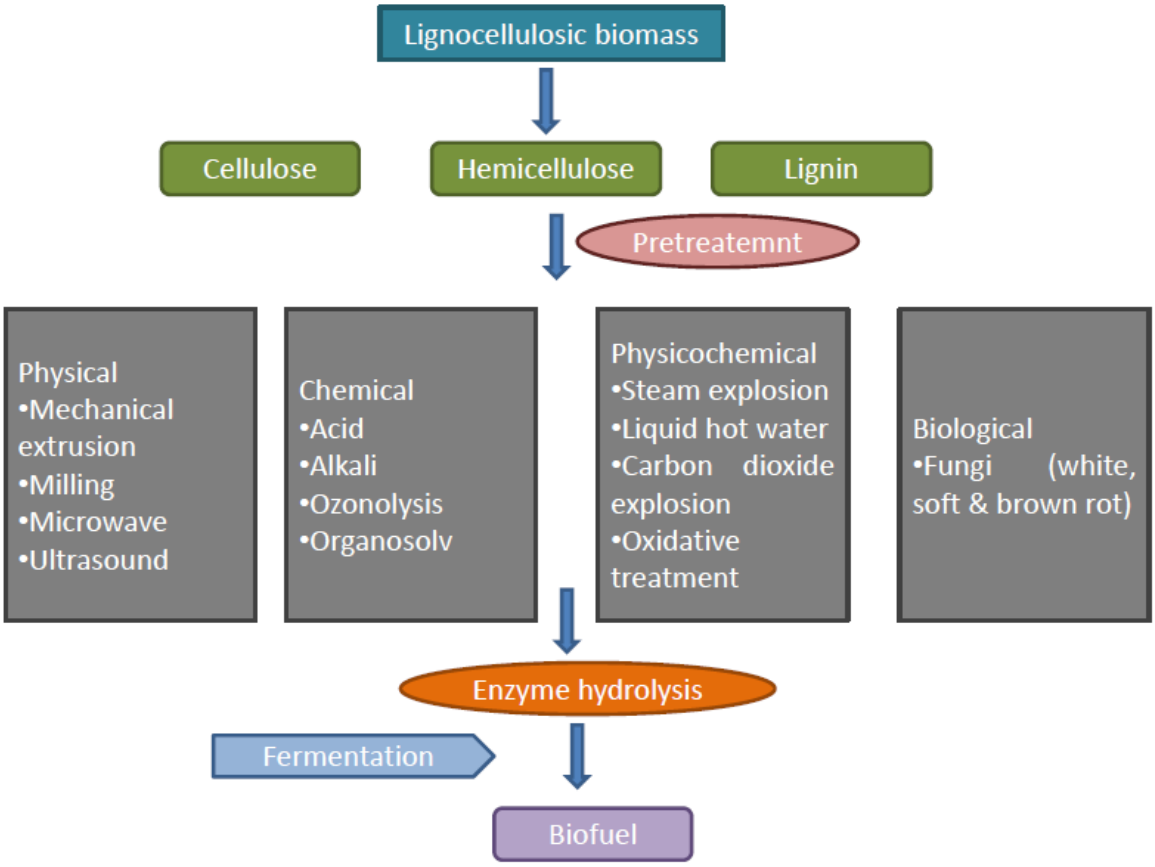


Figure 2.3. Overview of different pre-treatment processes (Source: Kumar and Sharma, 2017).

2.5.1. Physical (mechanical) methods

Mechanical Extrusion

It is the most traditional technique of biomass pre-treatment, where the raw materials are treated with the heating process (>300 °C) under shear mixing. It basically produces gases and biochar from the pre-treated lignocellulosic residues (Shafizadeh and Bradbury, 1979). Extrusion had a vital impact on degradation of cellulose and hemicellulose fractions and exhibited maximal sugar recovery from a broad spectrum of lignocellulosic materials, as reported by Karunanithy and Muthukumarappan, 2011. When merged with additional pre-treatment techniques, this method performs better by enhancing the overall yield of the

reducing sugars. However, this technique needs very high energy, making it a costly process (Zhu and Pan, 2010).

Milling/Mechanical Comminution

Comminution of lignocellulosic biomass via amalgamation of chipping, grinding and milling can decrease cellulose's crystallinity. The aim of mechanical comminution is to decrease the size of the particles and biomass crystallinity to enhance the specific surface area and to decrease the degree of polymerization of cellulose. The biomass size is generally 10–30 mm after chipping and 0.2–2 mm after milling or grinding. This technique is cost intensive due to high power consumption for acquiring desired particle size of the materials (Kumar et al., 2009; Maurya et al., 2015).

Ultrasound

Sonication is a new pre-treatment process that uses ultrasound waves that produce both physical and chemical reactions, thereby altering the biomass morphology. Little cavitation bubbles that disrupts the cellulose and hemicellulose fraction are formed during ultrasound which increases the accessibility to cellulose degrading enzymes for effectual disintegration into simple monomeric sugars. Time span and frequency of sonication has maximal consequence on biomass pre-treatment. A frequency of 10–100 kHz had been sufficient for cell breakdown and polymer degradation as per reports. Prolonged sonication beyond a particular limit had no extra outcome in terms of delignification and liberation of reducing sugars (Rehman et al., 2013)

Microwave pre-treatment

Microwave irradiation can alter the cellulose structure by breaking down lignin and hemicellulose, thereby enhancing the enzymatic susceptibility of biomass (Lu et al., 2011). It is a technique which has been widely accepted due to several rationales such as (1) trouble-free performance (2) less power requisite (3) maximal heating potential in short span of time (4) minimal formation of inhibitory compounds (5) time-efficient i.e. the residence period in microwave treatment varies from 5 to 20 min.

2.5.2. Physicochemical methods

Steam explosion

Steam explosion is broadly classified into an Uncatalyzed steam explosion and catalyzed steam explosion. Uncatalyzed steam explosion refers to a pre-treatment method in which biomass is swiftly heated by high-pressure steam without the inclusion of any catalyst. Water can behave as an acid (chemical catalyst) at elevated temperatures. The vital chemical and physical alterations to biomass by steam treatment are often attributed to the removal of hemicelluloses (Mosier et al., 2005). This technique is also known as 'autohydrolysis' due to the changes that occur during this treatment. In the course of this treatment, the reaction of hemicellulose into glucose and xylose is executed by the acetic acid formed from the acetyl groups of hemicellulose; thus, this technique is referred to as autohydrolysis too. Steam pre-treatment is basically an amalgamation of mechanical forces (pressure drop) and chemical effects (hydrolysis of the acetyl group of hemicellulose). In this method, raw materials are treated with elevated pressure saturated steam at high temperature (160 to 260 °C) for a few seconds to minutes which triggers hydrolysis and liberation of hemicellulose. The steam invades the biomass, swells the walls of cells leading to partial hydrolysis, enhancing the enzymatic accessibility for cellulose. Then the pressure is decreased to atmospheric condition (Kumar and Sharma, 2017). Steam treatment (steam-explosion) can be efficiently increased by including sulphuric acid, Carbon dioxide or Sulphur dioxide as a catalyst. The utilization of acid catalyst enhances the recovery of hemicellulose sugars and boost the enzymatic hydrolysis on the solid remnant. Acid catalyst has been seen to be majorly effective in hemicellulose sugar recovery, reduced formation of inhibitory substances and enhanced enzyme hydrolysis among all the catalysts. Low temperature and longer residence time (190 °C for 10 min) are more desirable compared to high temperature and lower residence time (270 °C for 1 min) because of less fermentation inhibitors production in the earlier process. The elements that influences steam pre-treatment are temperature, residence time, particle size of biomass and moisture content and the combined action of temperature and time. It is an appealing technique as it makes restricted usage of chemicals, it needs less energy input with no recycling or environmental cost, low energy requirement, environment friendly, significant enhancement in enzyme hydrolysis, lesser environmental effect, minimal capital investment, more prospective for energy efficacy, less hazardous process chemicals and conditions and substantial sugar recovery. The limitations are incomplete breakdown of lignin-carbohydrate matrix resulting in

making the biomass less digestible and possible generation of fermentation inhibitors at higher temperatures, the probability of production of fermentation inhibitory compounds at high temperature, incomplete digestion of lignin-carbohydrate matrix (Agbor et al., 2011), the formation of inhibitory/degrading compounds, partial destruction of hemicelluloses and the production of toxic elements which could impact on the enzymatic hydrolysis and fermentation method.

Liquid hot water

Liquid hot water has exhibited the ability to liberate hemicellulosic sugar fraction and decrease the undesired disintegration of products (Mosier et al., 2005). Water at high temperature (170–230 °C) is utilized in this process, leading to hydrolysis of hemicellulose, lignin removal making cellulose more approachable, avoiding the production of fermentation inhibitors at elevated temperatures. Liquid hot water has been referred with multiple terms such as solvolysis, hydro thermolysis, aqueous fractionation and aquasolv. Low temperature necessity, minimal production of inhibitory components and less price of solvent are few of the benefits related to it. However, it needs substantial power in downstream processing because of the large amount of water associated in the technique (Agbor et al., 2011).

Carbon dioxide explosion

Carbon dioxide explosion incorporates the usage of supercritical carbon dioxide under pressure to increase the digestibility of lignocellulosic feedstock (Agbor et al., 2011). This method executes the pre-treatment of feedstock by passing supercritical carbon dioxide. The gas carbon dioxide acts like a solvent in this case. An elevated pressure reactor vessel holding feedstock passes the supercritical carbon dioxide. The reactor is heated to the requisite temperature and retained for several minutes at elevated temperature. Carbon dioxide invades the biomass at elevated pressure forming carbonic acid that enhances the hydrolytic rate thereby hydrolyzing the hemicellulose fraction. When liberated, the pressurized gas distorts the raw materials layout, enhancing the accessible surface area. Low moisture content biomass is not suitable to be processed in this technique. Therefore, more the moisture content, more the hydrolytic yield of the biomass. Less cost of carbon dioxide, less temperature necessity and no toxin production make it an appealing technique. Although, substantial cost of reactor vessel that can endure elevated pressure is a significant hindrance in its implementation (Kumar and Sharma, 2017).

Oxidative pre-treatment

Treatment of feedstocks by oxidizing mediums such as hydrogen peroxide, ozone, oxygen or air causes delignification, which converts lignin to acids. The main disadvantage of oxidative pre-treatment is that it destructs a noteworthy quantity of hemicellulose making it inaccessible for fermentation. Hydrogen peroxide is often used as the oxidizing medium. Removal of lignin and hemicellulose from biomass increases cellulose accessibility leading to enhanced enzymatic hydrolysis (Kumar and Sharma, 2017).

2.5.3. Chemical methods

Acid pre-treatment/hydrolysis

The goal of the acid treatment is chemical hydrolysis that can generate solubilization of hemicelluloses and lignin, and to make cellulose available to enzymes. This method can be carried out with concentrated or diluted acid; however, concentrated acids are environmentally unsafe and dangerous, and toxic, corrosive and corrosion-resistant apparatus are needed in the process. Diluted acid hydrolysis is a feasible technique for biomass pre-treatment. Two kinds of dilute acid pre-treatment methods are present: high temperature (beyond 180 °C) short time (1–5 min) and lower temperature (120 °C), longer time (30–90 min) (Maurya et al., 2015). Hydrochloric acid, Nitric acid, Phosphoric acid, Sulfuric acid, and Dicarboxylic acids: oxalic and maleic acid have been used for biomass pre-treatment, however, dilute sulfuric acid is frequently employed as the acid of choice. Dilute acid is incorporated with feedstock to solubilize hemicellulose thereby enhancing cellulose. The acid liberates oligomers and monomeric sugars via influencing the reactivity of the carbohydrate polymers present in the biomass (Agbor et al., 2011). Acid pre-treatment can enhance the enzymatic hydrolysis of lignocellulosic feedstock to liberate fermentable sugars (Kumar et al., 2009). Acid pre-treatment could be inexpensive as acids are relatively cheap, but, since the technique is executed at elevated temperature, high energy input is needed, making the process costly. Acid hydrolysis is the most frequently utilized traditional treatment technique of lignocellulosic feedstock, but, is less appealing because of the production of high quantity of inhibitors such as furfurals, 5-hydroxymethylfurfural, phenolic acids, and aldehydes mostly in case of concentrated acid hydrolysis, which are needed to be removed before further processing (Kumar and Sharma, 2017).

Alkali pre-treatment

Alkali such as sodium, potassium, calcium and ammonium are treated with biomass at normal temperature and pressure in this process. The major benefit of the technique is effective lignin removal from the feedstock thereby enhancing the reactivity of the other polysaccharides. Alkali reagents distort the side chains of esters and glycosides of the biomass leading to structural alterations of lignin, swelling and decrystallization of cellulose and solvation of hemicellulose. Alkali treatment can be carried out at moderate temperature, pressure and time-varying from hours to days. Lime is another extensively operated alkali as it eliminates acetyl groups and lignin-carbohydrate ester and increases the digestibility of cellulose (Maurya et al., 2015). The advantage of lime treatment is the minimal cost of lime as compared to other alkaline mediums. Being a cost-effective process, the only limitation of alkali pre-treatment is its high cost of downstream processing as it utilizes a large amount of water (Kumar and Sharma, 2017).

Ozonolysis

Ozone (O_3) is a strong oxidizing medium. Its degradation is majorly restricted to lignin. However, hemicellulose is moderately affected in the process but cellulose remains unaffected. Ozonolysis is basically executed at room temperature and pressure. The significant parameter that impacts the pre-treatment of ozone is the moisture content of the feedstock i.e. higher the moisture content, the lower the lignin oxidization. The main limitation of ozonolysis is the necessity of substantial ozone, thereby making it a costly method (Sun and Cheng, 2002). However, ozonolysis is an efficient pre-treatment technique as it does not produce toxins which may hinder the subsequent hydrolysis and fermentation.

Organosolv

Introduction of aqueous organic solvents such as ethanol, methanol, ethylene glycol, and acetone to the feedstock under particular temperature and pressure specifications. Basically, this technique is carried out in the existence of an acid, base or salt catalyst such as hydrochloric acid, sulphuric acid, oxalic, acetylsalicylic and salicylic acid (Bajpai, 2016) for hemicellulose solubilization and lignin extraction (Sun and Cheng, 2002). The temperature in organosolv treatment is determined by the biomass type and catalyst involved and may be extended to 200 °C. Lignin removal from the feedstock exhibits the cellulose fibres for enzymatic hydrolysis leading to greater feedstock transformation. Major disadvantage of this

method is the high cost of the solvents and the catalysts. Formasolv is a kind of organosolv incorporating formic acid, water, and hydrochloric acid. Lignin is soluble in formic acid and the method can be executed at low temperature and pressure. Ethanosolv (incorporating ethanol) is executed at elevated temperature and pressure and cellulose, hemicellulose, and pure lignin can be recovered in the process. Low operation cost is a considerable benefit of this process (Kumar and Sharma, 2017).

2.5.4. Biological method

Biological pre-treatment is regarded as an effective, non-hazardous and moderate energy technique. Mother earth has plenty cellulolytic and hemicellulolytic microorganisms that are particularly selected for efficient biomass pre-treatment (Vats et al., 2013). This pre-treatment is performed through microbes that can specifically deteriorate lignin and hemicellulose structure and less quantity of cellulose, such as brown, white, and soft-rot fungi (Sanchez, 2009). Lignin-degrading enzymes peroxidases and laccases can occur the disintegration of lignin by utilizing white rot and soft rot fungi (Kumar et al., 2009). *Phanerochaete chrysosporium, Ceriporia lacerata, Cyathus stercolerus, Ceriporiopsis subvermispora, Pycnoporus cinnarbarinus* and *Pleurotus ostreaus* are few of the examples of white rot fungi used commonly for biological pre-treatment. Biological pre-treatments are substantially related to the activity of fungi proficiently creating enzymes which deteriorates the feedstock's lignin, hemicellulose, and polyphenols. Brown-rot fungi basically degrade cellulose, while white and soft-rot degrades both lignin and cellulose, by producing enzymes such as lignin peroxidases, polyphenol oxidases, manganese-dependent peroxidases, and laccases that deteriorate the lignin. Depolymerization of lignin through white rot fungi such as *Phanerochaete chrysosporium, Phlebia radiata, Dichmitus squalens, Rigidosporus lignosus,* and *Jungua separabilima* takes weeks but yields significantly efficient and selective result (Hatakka, 1994; Hatakka et al., 1993). White-rot fungi are the most efficient at biological treatment (Sun and Cheng, 2002; Lee, 1997). Other basidiomycetes species, reported to exhibit high delignification property, such as *Bjerkandera adusta, Fomes fomentarius, Ganoderma resinaceum, Irpex lacteus, Phanerochaete chrysosporium, Trametes versicolor, Lepista nuda, Ceriporia lacerata, Cyathus stercolerus, Ceriporiopsis subvermispora, Pycnoporus cinnarbarinus* and *Pleurotus ostreaus* were also investigated for degradation of various biomass. Biological treatment has some major benefits over other techniques: minimal energy requirement, moderate environmental conditions, and no chemical requirement. However, the

major limitation in this process is that the hydrolysis rate is very low in this process. The reality that some of the carbohydrate fraction is devoured by the microbe itself and the necessity of careful growth conditions also makes this method less attractive (Sanchez and Cardona, 2008).

Table 2.1. Summary of some widely used Pre-treatment methods.

S. N.	Pre-treatment	Advantages	Limitations
1	Mechanical comminuition/ Milling	Particle size reduction to increase specific surface area and pore size	High power and energy consumption
2	Extrusion	High yield of reducing sugars	High energy is required
3	Liquid water treatment	Simple method, minimum formation of inhibitory compounds	High energy requirement
4	Steam explosion	Hemicelluloses degradation and lignin transformation, Economic process	Incomplete disruption of lignin
5	Microwave treatment	Simple operation, low energy requirement	Formation of inhibitory compounds
6	Oxidative treatment	High yield of sugars	Damages significant amount of hemicellulose
7	Ultrasonic treatment	New and feasible method	Higher sonication power adversely affects the pre-treatment process
8	Carbon dioxide Explosion	Increases accessible surface area, Economic process	No modification of hemicelluloses and lignin

9	Ozonolysis	Reduces lignin content	Expensive as high amount of ozone is required
10	Acid hydrolysis	Hemicellulose is hydrolyzed to sugars; lignin structure is altered	Formation of toxic substances, high cost
11	Alkaline hydrolysis	Increases accessible area, hemicelluloses and lignin is removed	Long residue time is required
12	Organosolv	Hydrolyses hemicelluloses and lignin	High cost
13	Biological (involving microbes)	Lignin and hemicellulose degradation, low energy requirements, no chemicals required	Hydrolysis rate is low

2.6. Biomass: Feedstock for biorefinery

A biorefinery system is a facility that merges biomass transformation techniques and equipments to form biofuels, biochemicals, and energy from biomass. The main idea behind a biorefinery is to produce biofuel and biochemicals from waste residues. A biorefinery aims to convert biomass into proficient materials by incorporating technological processes. The utility of green techniques to convert cheap value feedstock residue to green products such as waxes and ethanol, etc., has been studied by Clark et al., 2006.

2.6.1. Types of biorefinery

Whole-crop biorefinery

In a whole-crop biorefinery, the whole crop, such as oil seed, is processed as the raw material and consumed to obtain valuable biomaterials. For syngas production, the biomass synthesized in the refinery operations acts as the starting feed to the biorefinery. Syngas can be utilized as the primary substance for the production of methanol.

Green biorefinery

Green biorefinery is a multiproduct paradigm. It utilizes natural wet biomass procured from non-treated feedstock, such as grass, green plants, and green crops, as feed for the production of biochemicals, syngas, and synthetic fuels.

Forest and lignocellulosic based biorefinery

As discussed earlier, biomass constitutes two kinds of polysaccharides, cellulose and hemicellulose, held together via a third element, lignin. It contains three main chemical fragments (a) polymer of glucose, cellulose (b) sugar molecule of basically pentoses, hemicellulose (c) polymer of phenols, and lignin (Tyson et al., 2005). The polymers cellulose and hemicellulose are transformed to their monomeric sugars via hydrolysis. The hydrolysis of these polymers to glucose is done by enzyme or chemical technologies that creates various important materials such as ethanol, acetic acid, acetone, butanol, and other fermentation substances. Agro oil crops such as soybean, rapeseed, and palm oil are extensively utilized for biodiesel production.

Aquatic or algae-based biorefinery

Algae and cyanobacteria are photosynthetic microbes that transform sunlight, water and carbon dioxide to lipid. These lipids consist of neutral lipids, polar lipids, waxes, hydrocarbons, phenyl derivatives such as tocopherols, carotenoids, terpenes, and phenylated pyrole derivatives such as chlorophylls. For biooil extraction and biodiesel production, algal and cyanobacteria biomass are widely explored. For example, *Bortyococcus braunii* has been reported to create vast quantities of (upto 80% Dry Cell Weight) extended hydrocarbon chain, homologous to those in petroleum and hence exploited as biomass for biofuel and biomaterials.

Integrated biorefinery

Biorefinery is a capital-intensive task. Hence, the price of output products generated from such biorefineries is enhanced if it is based on just one conversion method. Thus, various conversion techniques such as thermochemical, biochemical, etc. are integrated to minimize the overall price and increase product creation flexibility. An integrated biorefinery can be employed to produce bioelectricity.

2.7. Applications of biomass pre-treatment: Conversion of biomass to biofuel

Several value-added materials can be attained via pre-treatment of lignocellulosic resources. Microbial and enzymatic action on biological residues can lead to the creation of predominantly ethanol and, less frequently, propanol and butanol. Biobutanol, also known as bio gasoline, is usually proven to be a direct substitution for gasoline, since it can be directly

utilized in a gasoline engine, just as biodiesel can be utilized in diesel engines. Distillation or amalgamation of distillation and adsorption is applied to recover the biofuels from the fermentation broth. Residual lignin, unreacted cellulose and hemicellulose, and enzymes gather at the distillation column's lower part (Kumar et al., 2009).

Lignocellulosic biomass represents a rising possibility as a resource for bioethanol production taking into consideration their output/input ratio, abundance, cost effective nature and ethanol yield. Other than bioethanol, various biofuels are attained via feedstock hydrolysis such as bio-oil, biohydrogen, biomethanol, biobutanol, biogas, syngas etc. Lignocellulosic is a renewable reservoir that can be processed in various ways for creation of several materials such as synthesis gas and electricity (Chum and Overend, 2001).

Bio-oil, coupled with tar, gases and biochar, is processed via pyrolysis. Bio-oil is manufactured by fast depolymerizing lignocellulosic elements such as sugars, carboxylic acids, and phenols (Kumar and Sharma, 2017). Bio-oil, also known as pyrolysis oil is processed by fast pyrolysis. Organic components such as cellulose, hemicellulose, and lignin are thermally degraded in this process at temperature (400–600 C) in lack of oxygen to manufacture liquid components such as bio-oil (60–70%), char (13–25%), and gas such as hydrogen, hydrocarbons (13–25%) and these wide range of ‘‘Green Chemicals’’ are separated from bio-oil through solvent extraction.

Ethanol fabricated biochemically from lignocellulosic resources such as agricultural feedstock (rice straw, barley straw, wheat straw, corn) grasses (switch grass, miscanthus) is called as biomass ethanol or bioethanol. Three categories of microbes are employed for bioethanol production viz. most commonly yeast (*Saccharomyces* sp.), moderately bacteria (*Zymomonas* sp.), and less frequently mold (mycelium). These microorganisms are able to create alcohol from starch and sugar via fermentation. Fermentation is described as a metabolic method in which organic compounds undergoes chemical alterations because of the activities of enzymes released by microbes. Aerobic and Anaerobic are two types of fermentation, based on whether or not oxygen is required in the process.

Biohydrogen is created via thermochemical (gasification and fast/ flash pyrolysis) and biological ways i.e. photo fermentation (dependent on light) and dark fermentation (independent of light) (Putro et al., 2016; Ni et al., 2006).

Biomethanol production in liquid fuel form has various benefits. Firstly, less sulphur and ash fuels can be produced for commercial utility. Secondly, it is easier to maintain, reserve and transfer than gaseous components.

Biobutanol, valued as a fuel additive, is formed from biomass by fermentation of sugars liberated after pre-treatment by the microbe *Clostridium acetobutylicium* (Wackett, 2008).

Biogas and syngas have similar compounds viz. Carbon dioxide, Methane, Hydrogen, and Nitrogen, but are manufactured via two different procedures. Biogas is created by anaerobic digestion incorporating four steps in total i.e. hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Syngas is manufactured via gasification of biomass executed at low temperature and it can be transformed into huge number of organic elements. Biomass gasification has the potential to transform syngas into green fuels such as hydrogen and green motor diesel, also known as Fisher-Tropsch oil. Syngas constitutes deteriorated materials of biomass such as carbohydrates (cellulose, hemicellulose) and lignin (Kumar and Sharma, 2017).

2.8. Microalgae and its cultivation

2.8.1. Cultivation of microalgae

For the development of a sustainable industry for biomass production and synthesis of value-added products, large scale microalgal cultivation which can cater to a large amount of biomass is required.

Microalgae are cultivated under different conditions using different methods. During their life, they pass through different phases: lag, log/exponential, stationary and death. One of the significant parameters of microalgae cultivation is the selection of the type of bioreactor. The photobioreactor should be designed per the culture's purpose and the type of algal species. For extensive scale cultivation, they are grown in open ponds. This cultivation system is economical but can easily become contaminated. For small-scale cultivation, batch and continuous bioreactors are available, but the biomass obtained is lower.

Wastewater is an excellent source of nutrients for microalgal cultivation. Therefore, the organic effluents from agricultural and food industries could also flourish the microalgae (Grobbelaar, 2004). Microalgae cultivation is dependent on the important parameters such as light, temperature, pH, cultivation medium, stirring and mixing, bioreactor type etc. (Mata et

al., 2010). Some of the significant factors having huge impact and influence on the biomass and bioproduct yield from microalgae are discussed below:

Light

Light intensity and duration is one of the significant parameters in microalgae cultivation as it can directly affect the photosynthesis process and influences the microalgal biochemical composition (Krzemińska et al., 2014). Both very high and low light intensities hinder the microalgal growth and causes growth inhibition. A specific time span of light and dark period is needed for photosynthesis process of algae. Various studies show that 16:08 light: dark hour is the most suitable for algal growth. Daliry et al. (2017) reported that *Chlorella vulgaris* exhibited maximum growth and lipid productivity at light intensities in the 5000-7000 lux range.

Temperature

Temperature is a significant factor that could directly influence photosynthesis's biochemical process. The optimum temperature range for majority of algal species is n the range of 20-30 °C (Singh and Singh, 2015). A decrease or increase in the optimal temperature could retard or stop the algal growth (Bechet et al., 2017). Temperature could also be used as a stress treatment to induce valuable metabolites production in microalgae (Moller et al., 2000). Kitaya et al. (2005) reported that temperature between 27 to 31 °C was found to be optimum for several species of microalgae.

pH

Most microalgal species grows well in the pH range of 6-8. Some algal species are pH sensitive while some could endure a board pH range. *Chlorella vulgaris* is one such example which can grow under a broad pH range (Lam and Lee, 2012).

Mixing

The uniform distribution of nutrients and atmospheric air and CO_2 and, penetration of light inside the microalgae culture could be achieved by mixing. Mixing also prevents the settling of microalgae in the bottom of the bioreactor and provides efficient exchange of gases (Show et al., 2017; Zeng et al., 2011).

2.8.2. Agricultural residues and food waste for sustainable cultivation of microalgae

The global algal product market is expected to reach $44.7 billion by 2023 as it grows at a compound annual growth rate (CAGR) of 5.4% (Ammar et al., 2020). Microalgal biomass's excessive popularity and demand for biofuels and bioproducts generation has initiated a hefty attention towards the evolution of cost-effective economical cultivation mediums and methods. Food and agricultural residues are renewable sources of organic carbon and significant vitamins and minerals. They are abundantly available and contain inexpensive carbon sources that can be hydrolyzed for microalgal cultivation. Pre-treatment or hydrolysis of such waste residues could liberate various sugars, nutrients and trace elements. Different microalgal species could utilize these nutrients and carbon for sustainable production of biomass and bioproducts. Glucose is the preferred carbon source for various microalgal species (Ammar et al., 2020).

Fruit and vegetable peels are often disposed of. But these peels contain nutrients in huge quantity, which can be used as a growth medium for growing microalgae. Conventional medium like BG11, BBM are widely used but is not cost effective as they are high in cost. The high cost effects the large-scale cultivation of microalgae for biofuel and value-added product generation. Hwang et al. reported microalgal cultivation of *Anabaena cylindrica* using fruit peels in different concentrations, such as papaya and pineapple peels, and reported better biomass yield in fruit peels when compared to BG11 medium (Hwang et al., 2021).

Park et al. (2014) showed that microalgae *C. vulgaris* OW-01 grown in OPE (orange peel extract) medium produced 3.4-times more biomass and 4.5-times more fatty acid methyl esters (FAMEs) than cells cultured in glucose-supplemented BG 11 medium (BG-G). Yan et al. (2011) explored waste molasses as alternative to a glucose-based medium of microalgae in their study. The maximum algal cell density and oil content were respectively 70.9 gL^{-1} and 57.6%. Pleissner et al. (2013) used food waste as culture medium and nutrient source for the heterotrophic microalgae. They grew well on the complex food waste hydrolysate by utilizing the recovered nutrients. 10–20 g biomass rich in carbohydrates, lipids, proteins, and saturated and polyunsaturated fatty acid were produced. Agwa et al. (2014) cultured microalgae using Cassava wastes as a growth media. The Cassava peels gave a higher lipid productivity of 40.7mg L^{-1} d^{-1} than the effluent with a lipid productivity of 26.1 mg L^{-1} d^{-1}. Liang et al. (2010) examined Stalk juice from sweet sorghum grown for lipid production through microalgal

cultivation. Juice concentrations at 100%, 75%, 50%, and 25% led to biomass, lipid, and docosahexaenoic acid (DHA) production by *Schizochytrium limacinum*. Cultivation of the green microalga *Scenedesmus acutus* by using agricultural residues namely, pineapple peels and sugarcane bagasse was reported by Rattanapoltee and Kaewkannetra (2014). They reported highest lipid content of 28.05% and 40.89% in case of pineapple peels and sugarcane bagasse respectively. Also, a maximum biomass concentration of 3.85 gL^{-1} and biomass productivity of 160.42 mgL^{-1} d^{-1} was reported. This study proves that agricultural residues as nutrient source for microalgae could increase lipid content and reduce biofuel production price. The culture media needed for microalgal cultivation is a cost intensive medium. The cost of nutrients can be reduced if organic wastes are used as an alternative. This could also help in handing the waste agricultural and food residues (Ryu et al., 2013). Pleissner et al. (2013) reported microalgal cultivation of *Chlorella pyrenoidosa* and *Schizochytrium mangrovei* on food waste hydrolysate. This indicates that food waste residues are a promising nutrient source for microalgal cultivation (Pleissner and Lin, 2013).

2.8.3. Prospective of Lipid extracted microalgal biomass residue (LEMBR) reusability

The lipid extracted microalgal biomass (LEMBR) contains residues of carbohydrates, proteins and nutrients in significant amount which can be used as a potential raw material for biofuel production. The carbohydrates (starch, cellulose, hemicellulose) present in the leftover algal biomass, after lipid extraction, can be hydrolyzed to obtain biofuels of diverse nature. Guldhe et al. (2017) reported that LEMBR contains considerable amount of carbon and nitrogen, which makes it a suitable feedstock for production of biofuels such as biohydrogen, biomethane and bioethanol. Hemaiswarya et al. (2012) found that LEMBR residue can be reused for biomethane production. LEMBR can be utilized to supplement microalgae cultivation as a growth promoter and biomass and lipid yield enhancer. Arora et al. (2016) reported that the presence of sugars such as glucose, xylose and arabinose in LEMBR results in 54.12% of lipid accumulation in *Chlorella minutissima*. Abomohra et al. (2018) reported that de-oiled algal hydrolysate and waste glycerol were found to valorize the rate of growth and lipid accumulation of the microalgae *Scenedesmus obliquus*. Kim et al. (2015) and Bui et al. (2015) found that de-oiled biomass residues of *Chlorella sorokiniana*, *Dunaliella tertiolecta* and *Chlamydomonas* sp. have ample potentiality towards bio-oil production through pyrolysis.

Proper exploitation of spent lipid extracted biomass residue could improve the overall economics of microalgal biofuels. The spent microalgal biomass accounts for approximately

70% of the total algal biomass on dry basis and hence could be used for various applications (Park et al., 2012). Microalgal biomass comprises mainly three components: lipids, proteins and carbohydrates. These components vary depending on the microalgal species, cultivation parameters, harvesting techniques and lipid extraction methods. Yang et al. reported that the spent biomass from *Scenedesmus* consists of 6.5% lipid, 24.7 % carbohydrate, 32.4% protein and 10% ash (Yang et al., 2011).

It has been reported that *Chlorella vulgaris* consists of 14-22% lipid, 51-58% protein and 12-17% carbohydrate; *Chlamydomonas rheinhardii* contains 21% lipid, 17% carbohydrate and 18% protein; *Spirulina platensis* have 4-9% lipid, 46-63% protein and 8-14% carbohydrate; *Dunaliella bioculata* contains 8% lipid, 4% carbohydrate and 49% protein; *Anabaena cylindrica* have 4-7% lipid, 43-56% protein and 25-30% carbohydrate (Becker, 2004; Brown et al., 1997). Lipid extracted algae residues consists of high content of proteins and carbohydrates, enabling its usage in biogas and bioethanol production (Torri et al., 2011). The nutritional content in the spent algal biomass makes it an excellent source for animal, fish and microbe feed. It can also be used as a bio sorbent to treat wastewaters by removing heavy metals and dyes (Gokhale et al., 2008).

Microalgal residue has distinct dominance over cellulosic ad crop-based substrates for bioethanol production. Mirsiaghi et al. reported production of bioethanol using lipid extracted residue of the microalgae *Nannochloropsis salina* (Mona et al., 2011). Also, the addition of small content of algae to animal and fish diet enhances their growth (Singh et al., 2011). However, producing microalgal biomass solely this purpose is not feasible economically. Hence, spent microalgal biomass could be a promising alternative in this case. Lipid extracted biomass can be used for several applications such as additional biofuel production, electricity production, animal and fish feed and waste water treatment (Rashid et al., 2013).

2.9. Microalgal components

2.9.1. Lipids

Microalgae is capable of accumulating a high lipid content, which is as high as 30-50%. High C/N ratio and stress conditions can result in high lipid content in the microalgal cells. To increase the lipid productivity, stress conditions like pH shift, nitrogen starvation, high temperature etc. are taken into consideration. Compared to the other lipid-based energy crops, microalgae lipids are more appealing for biofuel production and nutraceutical supplements as it has better productivity. There are many methods for extracting lipids from the microalgal

cells, but the most commonly followed is the Bligh and Dryer method of lipid extraction. Solvent extraction, microwave assisted extraction, ultrasonic extraction, electroporation etc. are the frequently used lipid extraction technique (Chew et al., 2017). Lipid content of few common microalgal species are listed in Table 2.2.

2.9.2. Proteins

Most algal species are known to produce vital proteins, amino acids and enzymes. One of the main constituents of the microalgae, proteins form 50-70% of the microalgal composition. It is a very important products that can be obtained for microalgal biorefinery, which can be used for human, animal and fish nutrition (Chew et al., 2017).

Microalgae can produce proteins in the range of 2.5-7.5 ton ha^{-1} y^{-1} (Stephen and Hayes, 2017). *Chlorella*, the green algae, is a rich source of widely marketed microalgal proteins. *Arthrospira* is another protein rich microalga. Reports have suggested that microalgal proteins can reduced cholesterol levels. Cyanovirin, is a protein produced from *Nostoc* which has reportedly shown antiviral properties against HIV virus (Zappe et al., 2008). *Porphyridium* and *Anabaena* produces the enzyme superoxide dismutase (SOD) which is known to protect against harmful oxidative damages (Khan et al., 2018).

2.9.3. Carbohydrates

Algal carbohydrates primarily comprise glucose, cellulose, glycogen, starch and different polysaccharides. Traditionally, the glucose and starch from algae is used for bioethanol and biohydrogen production. Reports suggests that the algal polysaccharides are able to regulate the immune system and inflammatory reactions which makes them a promising agent of biologically active molecules. These structural molecules have high applications as cosmetic additives, nutraceuticals and food ingredient additives. In order to convert the carbohydrates to fermentable sugars for biofuel production, pre-treatment needs to be carried out (Chew et al., 2017).

Table 2.2. Lipid content of few common microalgal species (Source: Mata et al., 2010).

Microalgae species	Lipid content (% dry weight biomass)
Botryococcus braunii	25-75
Chlorella protothecoides	14.6-57.8
Chlorella sorokiniana	19-22
Chlorella vulgaris	5-58
Chlorella sp.	10-48
Dunaliella sp.	17.5-67
Nannochloropsis oculata.	22.7-29.7
Nannochloropsis sp.	20-56
Neochloris oleoabundans	29-65
Scenedesmus obliquus	11-55
Scenedesmus sp.	19.6-21.1
Spirulina platensis	4-16.6
Tetraselmis sp.	12.6-14.7

2.9.4. Pigments

Microalgae consists three basic natural pigments: chlorophyll, carotenoids and phycobiliproteins. These pigments have major applications in food and feed industry as precursors of vitamins, food coloring additives in food and cosmetic industries and pharmaceutical industries (Chew et al., 2017).

Algae synthesizes different types of pigments that possesses biological activities and hence are of huge commercial interest. Carotenoids have a crucial role in food, feed, biopharma and cosmetic industries. Some of these important pigments are phycobiliproteins, phycoerythrin, beta-carotene, astaxanthin and phycocyanin. Phycobiliproteins are used as fluorescent agent in microscopy (Perez-Garcia et al., 2011). Phycocyanin have anti-inflammatory and anti-oxidant properties and thus are extensively used in cosmetic and food products. *Dunaliela salina* is a major producer of the carotenoid pigment beta-carotene (Sathasivam and Juntawong et al., 2013). *Haematococcus pluvialis* is a major producer of astaxanthin, another important carotenoid pigment (Sathasivam et al., 2017). As the carotenoids have powerful antioxidant activity, they are therapeutic in oxidative stress related diseases like diabetes, stroke and cancer (Khan et al., 2018). Chlorophyll has anti-inflammatory activities and can be used as a wound healing additive in pharmaceuticals (Christaki et al., 2011). These valuable products microalgae an attractive source (Vanthoor-Koopmans et al., 2013).

2.9.5. Other applications

Microalgae has major applications in different important sectors as biofuel, cosmetic and food additive, nutraceuticals and pharmaceuticals. Apart from this, recently microalgae have been used in various other applications. It has been used to fix CO_2 emitted from industries and to treat wastewater. Various algal species are being used as an organic fertilizer. The alga *Chlorella vulgaris* is being used to develop a filler material in various polymers such as Poly vinyl chloride (PVC), polystyrene, polyethylene and polypropylene (Kirrolia et al., 2013).

Medicinally important microalgal pigments are crucial in therapies for cardiovascular, optical and neuronal disorders. Microalgae produces essential amino acids which increases their potential as protein rich food. The high carbohydrate content in microalgal cells makes them a significant food source. Algal cells store lipid in the form of fatty acids and glycerol. These fatty acids have therapeutic and nutritional effects. Polyunsaturated fatty acids (PUFA) of microalgae have commercial value in the field of cardiac diseases, arthritis and asthma

(Adame-Vega et al., 2011). Eicosatetraenoic acid and docosahexaenoic acid (DHA) are two significant microbial products with high market value (Khan et al., 2018).

Microalgal products with high nutritional and therapeutic value are being marketed as tablets, extracts, capsules and as additives to food products such as pasta, gums, candies and beverages, mainly as food coloring agents or nutrients (Liang et al., 2004). *Chlorella*, *Anthrospira* and *Aphanizomenon* are few microalgal strains which are being commercialized for their high health promoting molecules and protein content (Soletto et al., 2005).

Microalgal biorefinery deals with conversion of biomass into fuels, chemicals, food, cosmetics and other value-added products. Microalgae such as *Tetraselmis*, *Isochrysis*, *Nannochloropsis* can produce valuable heath food supplements in the form of long chain fatty acids such as DHA (Docosahexaenoic acid) and EPA (Eicosapentaenoic acid). Recent market conditions and development in the production technologies have made microalgal biofuels feasible (Chew et al., 2017).

Microalgae also are capable of generating bioelectricity through photosynthetic microbial fuel cell. This can lead to an oxygen rich environment as well as removal of CO_2 (Chew et al., 2017).

Microalgae contains polyphenols, essential vitamins and trace elements in large quantity. B2, B12 vitamin. Microalgae are an abundant source of various vitamins. *Haslea ostrearia* produces vitamin E while *P. cruentum* is a rich source of vitamin A, C and E (Mus et al., 2013). *D. salina* produces high quantity of vitamin A, E, B6, B7, B2, B3, B1 (Hosseini et al., 2009).

Spirulina and *Chlorella* are predominantly exploited in food applications due to the presence of bioactive metabolites such as flavonoids, polyphenols and PUFA. *Chlorella* is a rich source of essential amino acids, macronutrients, vitamins such as B12, C, E, K, essential minerals such as iron, sodium, calcium and dietary fibres, hence it has been used as a dietary supplement and functional food (Koushalya et al., 2022).

2.10. Bioethanol generation from microalgae

Renewable biomass rich in sugar and starch material has been extensively used for first generation bioethanol production, however this creates a huge competition in the food markets, thereby making it non-feasible. Hence, there has been extensive investigations going on for making bioethanol generation possible from microalga by utilizing the carbohydrates present

in its cell wall (Shuba and Kifle et al., 2018). *Chlorella* sp. is reported to be a good source for bioethanol production due to high carbohydrate content (approximately 37% dry weight) from which approximately 65% ethanol conversion efficiency is possible (Hirano et al., 1997). Harun et al., (2010) reported 3.83 gL^{-1} of bioethanol from *Chlorococcum* sp. after fermenting with the yeast *Saccharomyces bayanus*.

Microalgal strains accumulate carbohydrates in excess of 40% of the dry biomass, mainly as starch and cellulose. For making the microalgal cells more accessible to the fermenting microbes for fermentation, the biomass can be pretreated by physical, chemical (acid/alkali) or enzymatic hydrolysis. Acid hydrolysis is considered to be the most common as it is easier, cheaper and faster among the other types of pre-treatment available (Rizza et al., 2017).

Although earlier the focus was being set on the microalgal lipids for biodiesel production for decades, more recent research has been conducted on the conversion of the microalgal carbohydrates into bioethanol (Raheem et al., 2018).

The microalgal carbohydrates are mainly represented by starch, glycogen and cellulose (Wang et al. 2014) and these are important substrates for bioethanol production. The microalgal cell wall disintegration and carbohydrates hydrolysis is achieved by pre-treatment. The hydrolyzed microalgae contain monosaccharides such as glucose, xylose, mannose, galactose etc. for further fermentation (Hernández et al. 2015). The most abundant sugar is glucose which is derived from the hydrolysis of the starch, cellulose or glycogen. Frequently used microbes for the bioethanol fermentation are the yeast *S. cerevisiae* and bacteria *Z. mobilis*. *Saccharomyces* sp. has been considered as generally recognized safe microorganism (GRAS). The most common microbe used since ancient times for alcohol production is *S. cerevisiae*, as it has a remarkable efficiency to convert sugars into ethanol (Lakatos et al., 2019).

Bioethanol is one of the clean and major biofuels used for transportation purpose. It has several advantages over fossil fuels. They are: (a) lesser greenhouse gases emission due to the presence of oxygen content (b) only biofuel that be used directly without any modifications in the current automotive industry (c) it can be blended with other oil/fuel. USA and Brazil are world leaders in the production of bioethanol by contributing approximately 80% of the world total bioethanol production. Global biofuel production has enhanced from 4.8 to 16 billion gallons per litre from the year 2000 to 2007 (Khan et al., 2018).

Although bioethanol was mainly produced from sugarcane and corn earlier, now the recent technology shifting towards algal biomass has been observed. Global bioethanol production has increased aggressively from 1 to 39 billion per litre within the last few years and would soon reach 100 billion in the coming years (Licht, 2015).

Fermentative bioethanol mostly depends on the potential of the fermenting microbe. For economical commercial production of bioethanol, biomass and carbohydrate productivity of algal cells needs to be enhanced. *Chlorella vulgaris* and *Chlamydomonas reinhardtii* are carbohydrate rich microalgae and hence widely considered for bioethanol production (Usher et al., 2014). Companies like Algenol, Seambiotic and Sapphire Energy are involved in the commercial production of bioethanol with an output of 1 billion gallon per year costing 85 cents per year (Duvall and Fraker, 2009).

The quality and yield of the bioethanol are dependent on the process of fermentation as it is strongly affected by several parameters such as, pH, temperature, microalgal biomass and the fermenter microbe.

Scenedesmus dimorphus accumulated 53.7 w/w carbohydrates which can yield 80% fermentable sugars for bioethanol production when hydrolyzed with sulfuric acid (Chng et al., 2017). A study reported 11.7 gL^{-1} of bioethanol yield from the microalga *Chlorella vulgaris* (Ho et al., 2013). For the bioethanol production to be viable and economically feasible, all the algal sugars present must be converted to ethanol.

2.11. Microalgal Biorefinery approach

Microalgae based biorefinery aims to develop a sustainable technology for the development of bioenergy and bioproducts such as biofuels, chemicals, nutraceuticals and pharmaceuticals. Apart from that, they are also a rich source of lipids, proteins, carbohydrates, vitamins and minerals (Raheem et al., 2018).

Microalgal proteins, bio-based chemicals, lipids and carbohydrates could be the substrate to a variety of products. Mostly they are grown in open ponds and closed photobioreactors which involves mixing process. However, these processes are energy intensive and the researchers are focusing on the maximum utilization of microalgal biomass while using minimal energy. Long chain fatty acids found in microalgae can be the precursor for health food supplements, proteins and pigments has important function in the pharmaceutical industries to treat various diseases.

Microalgae does not create competition for land and food crops. Microalgal biomass also has photosynthetic efficiency which when coupled with bioenergy production technology, has the potential to generation sustainable renewable energy. Besides, microalgae also have the capability to bio sequester CO_2 from the flue gases generated and this can contribute to the reduction of greenhouse gas emissions.

The biorefinery approach is a process to acquire energy, biofuels and value added bioproducts. It is a promising concept to mitigate greenhouse gas emission. However, the main hindrance in this concept is the separating procedure of different fractions without causing damage to the remaining fractions. This hurdle can be conquered by using cost effective, low energy consuming scalable separation technologies. Microalgae are considered to be suitable candidate for biorefinery because they have the potential for multiple product synthesis. Also, they have rapid growth and are renewable in nature. The microalgae's carbohydrate, protein, minerals and oil fraction can be used to generate biofuels, biogas, chemicals, feed and high value products. Glycerin, a biodiesel processing residue, could also be converted into value added product.

Microalgal biorefinery has two main stages: Upstream processing (USP) and Downstream processing (DSP). The major factors responsible for USP's efficiency are Microalgal strain selection, illumination source and light intensity, cultivation parameters and predominantly the source of nutrients. DSP involves the extraction and purification processes to attain valuable compounds from microalgae. The harvesting and drying processes are energy and cost-intensive process. Also, mild separation techniques are important so as not to damage the desired fractions from the microalgae during extraction (Chew et al., 2017).

2.12. Research Gap and Challenges

- Very limited studies on peels as a cultivation substrate for microalgae. Most studies are on domestic, industrial, sewage and artificial wastewater when a conventional microalgal growth medium is not used.
- The effect of the rotation frequency of the incubator (rotations per minute, rpm) has not been explored much while carrying out response surface methodology (RSM) during process optimization.

- No significant study focused on the development of a sustainable biorefinery model to integrate peel based microalgal cultivation along with lipid production, synthesis of value-added co-products and lipid extracted biomass residue conversion to biofuel.
- Most microalgal biorefinery approach utilizes the conventional BG11 medium for algal cultivation.

2.13. Objectives

Throughout the research, the following objectives were established and accomplished.

1. Selection, characterization and process optimization of the waste peels towards pre-treatment.
2. Isolation and identification of novel indigenous microalgal species from wild freshwater resources.
3. Utilization and evaluation of waste peel hydrolysate as a low-cost substrate for the cultivation of isolated microalgae.
4. Integrated biorefinery approach for simultaneous generation of multiple products from the isolated microalgae.

2.14. Thesis outline and Organization of the Thesis

Thesis outline

The thesis is presented in the following seven chapters.

Chapter I: Introduction

Chapter II: Literature Review and Objectives

Chapter III: Selection, characterization and process optimization of the waste peels towards pre-treatment

Chapter IV: Isolation and identification of novel indigenous microalgal species from wild freshwater resources

Chapter V: Utilization and evaluation of waste peel hydrolysate as a low-cost substrate for the cultivation of isolated microalgae

Chapter VI: Integrated biorefinery approach for simultaneous generation of multiple products from the isolated microalgae

Chapter VII: Conclusion and future scope

Organization of the Thesis

A brief description of the content of each chapter is furnished below:

Chapter I: Introduction

This chapter describes the world's current energy crisis along with the sustainability of renewable resources over non-renewable energy resources. Microalgae as emerging renewable resource for biofuels is briefly discussed. In addition, an introduction to biofuels and microalgae is given. The problems associated with microalgal biofuel technology is also discussed. Lastly, a general introduction is given to the peels used in this study.

Chapter II: Literature Review and objectives

This chapter reports an in-depth literature study on different pre-treatment types and techniques. Major discussion on biomass and biofuels are written elaborately. Subsequent discussion on microalgal cultivation on various agricultural and food substrates are reported in detail. Bioethanol generation process from microalgae is discussed. Lastly, the importance and application of microalgal biorefinery approach and lipid-extracted microalgal biomass residue are reported. Based on the literature survey, the major objectives of the present research project were decided and composed accordingly.

Chapter III: Selection, characterization and process optimization of the waste peels towards pre-treatment

In this chapter, potato, banana and sweet lime peels, otherwise considered waste, were used as substrates for producing glucose. Different pre-treatment processes were carried out for all the three peels and the best conditions yielding a higher amount of glucose concentration were further hydrolyzed by enzyme. The pre-treated extract at 80 min in case of dilute acid hydrolysis with autoclaving conditions gave higher glucose concentration amongst all the different types of pre-treatment processes for both potato and sweet lime peels and hence considered for further analysis. In the case of banana peels, at 40 min in case of autoclaving treatment followed by enzymatic hydrolysis, the pre-treated hydrolysate gave the higher glucose concentration amongst the various treatments executed.

Rationale for the next step of the experiment was designed based on a central composite statistical design (CCD). To obtain a high glucose yield, response surface methodology (RSM) was used to optimize the hydrolysis conditions. The three parameters chosen for the study were; time (h), temperature (^oC) and the rotation frequency of the incubator (revolutions per minute i.e. RPM). Under optimum conditions, 46.17 ±0.77 g L^{-1}, 29.84 ± 0.57 g L^{-1} and 35.90 ±0.43 g L^{-1} of glucose were obtained for potato, banana and sweet lime peels respectively. The experimental results further confirmed the optimum factors acquired from the statistical model.

The waste peels were further characterized through proximate and ultimate analysis, compositional analysis, FESEM, EDX, FTIR and pH study.

Chapter IV: Isolation and identification of novel indigenous microalgal species from wild freshwater resources

This chapter demonstrates the process of isolation and identification of novel microalgal species. Two indigenous strains were isolated from two different lakes present in IIT Guwahati, Assam. The strains were initially identified through Microscopy and FESEM for understanding its cell structure and morphology. To attain a clear distinction between them, Genetic identification was carried out. The strains *Chlorella sorokiniana* KMBM_K and *Chlorella sorokiniana* KMBM_I were maintained at laboratory conditions for further experiments.

Chapter V: Utilization and evaluation of waste peel hydrolysate as a low-cost substrate for the cultivation of isolated microalgae

In this chapter, potato, banana, and sweet lime peels were exploited instead of conventional growth mediums to cultivate microalgal cells. These wastes are zero-value material, which contains vital nutrients and elements for cultivating oleaginous microorganisms like microalgae. The growth of the isolated strains *Chlorella sorokiniana* KMBM_K and KMBM_I in the pre-treated peel hydrolysates were investigated. Growth kinetic parameters of the strains were analyzed in varying culture conditions. While performing the optimization studies, pH 7 was found to the suitable pH for algal cultivation. Biomass yield of 2.1 g L^{-1} and 1.94 g L^{-1} with a lipid content of 25.87 % and 25.12 % are obtained in *Chlorella sorokiniana* KMBM_K and *Chlorella sorokiniana* KMBM_I respectively when cultivated in potato peel extract. *Chlorella sorokiniana* KMBM_K and *Chlorella sorokiniana* KMBM_I displayed a biomass yield of respectively 1.72 g L^{-1} and 1.53 g L^{-1} with a lipid content of 22.83 % and 22.17 % respectively in case of banana peels. 1.97 g L^{-1} and 1.84 g L^{-1} of biomass yield,

and 24.14 % and 23.42 % of lipid content was obtained in *Chlorella sorokiniana* KMBM_K and *Chlorella sorokiniana* KMBM_I in case of sweet lime peels.

Among all the experiments, the highest biomass yield of 2.56±0.09 g L^{-1} and lipid content of 26.34±0.24 % was observed when *Chlorella sorokiniana* KMBM_K was cultivated in the mixed peel extract of potato, banana and sweet lime.

Chapter VI: Integrated biorefinery approach: Simultaneous generation of multiple products from the isolated microalgae

This chapter highlights the potential of a bio-based refinery of two novel microalgal isolates in producing lipid, bioethanol, pigments, etc. The isolates demonstrated significant amount of lipid, protein and carbohydrate. The strains also exhibited significant amount of pigments like chlorophyll-a, chlorophyll-b and carotenoids. The spent biomass after extraction of lipids were reused and recycled for sustainable biofuel synthesis.

The present study describes a thorough protocol for microalgal biorefinery – with lipid as the main product, and pigments, proteins and carbohydrates as the side products, and bioethanol as the secondary product. Bioethanol is produced from the microalgal residue after the lipid extraction. The lipid-extracted microalgal biomass residue (LEMBR) of *Chlorella sorokiniana* KMBM_K is subjected to acid hydrolysis and subsequent fermentation employing yeast to produce bioethanol of 7.16±0.43 g L^{-1}. The lipid extracted microalgal biomass residue (LEMBR) also has various applications in the field of biogas, biochar and bioethanol production, pigments, animal and fish feed, and fertilizer. Further the simultaneous production of microalgal biomass along with lipid and carbohydrate content, which are used to produce biofuel, is considered to be an integrated biorefinery approach.

Chapter VII: Conclusion and future scope

This chapter summarizes the contributions and inferences of all the chapters demonstrated in the thesis. Few suggestions and recommendations are also presented for carrying forward the research work in the future. This present work aimed on obtaining novel indigenous microalgae from the enormous biodiversity rich region of IIT Guwahati campus. This study also evaluated the effect of waste peels as a nutrient source for the cultivation of the microalgae and study of its growth kinetics. This study emphasized to investigate the integrated microalgal cultivation procedure combining the pre-treatment of waste peels and microalgal

growth on the extracted hydrolysate for biomass production and lipid accumulation. Along with that, synthesis of different value-added products was carried out in the process thereby balancing the microalgal biorefinery economically. However, further scale-up of these processes to pilot scale are required in both indoor and outdoor conditions for performing a techno-evaluation evaluation.

References

Abomohra AEF, Eladel H, El-Esawi M, Wang S, Wang Q, He Z, Feng Y, Shang H, Hanelt D (2018) Effect of lipid-free microalgal biomass and waste glycerol on growth and lipid production of *Scenedesmus obliquus*: innovative waste recycling for extraordinary lipid production. Bioresour Technol 249:992–999.

Adame-Vega C, Lim DK, Timmins M, Vernen F, Li Y, Schenk PM (2011) Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production, Microbiol Cell Fact 11:1–11.

Agbor VB, Cicek N, Sparling R, Berlin A, Levin DB (2011) Biomass pretreatment: fundamentals toward application, Biotechnol Adv 29:675–685.

Agwa OK, Neboh HA, Ossai-Chidi LN, Okoli MC (2014) Cultivation of microalgae using Cassava wastes as a growth media, J Algal Biomass Utln 5(2):8–19.

Alonso DM, Bond JQ, Dumesic JA (2010) Catalytic conversion of biomass to biofuels, Green Chem 12:1493–1513.

Ammar EM, Arora N, Philippidis GP (2020) The Prospects of Agricultural and Food Residue Hydrolysates for Sustainable Production of Algal Products, Energies 13:6427.

Arora N, Patel A, Pruthi PA, Pruthi V (2016) Recycled de-oiled algal biomass extract as a feedstock for boosting biodiesel production from *Chlorella minutissima*, Appl Biochem Biotechnol 180:1534–1541.

Bajpai P (2016) In Pretreatment of lignocellulosic biomass for biofuel production, Springer Briefs in Molecular Science 17-70.

Bechet Q, Laviale M, Arsapin N, Bonnefond H, Bernard O (2017) Modeling the impact of high temperatures on microalgal viability and photosynthetic activity, Biotechnol Biofuels 10:136.

Becker EW (2004) Microalgae in human and animal nutrition, in: A. Richmond (Ed.), Handbook of Microalgal Culture, Blackwell Publishing, Oxford.

Brown MR, Jeffrey SW, Volkman JK, Dunstan GA (1997) Nutritional properties of microalgae for mariculture, Aquaculture 151:315–331.

Bui HH, Tran KQ, Chen WH (2015) Pyrolysis of microalgae residues – a Kinetic study, Bioresour Technol 199:362–366.

Chang VS, Holtzapple MT (2000) Fundamental factors affecting biomass enzymatic reactivity, Appl Biochem Biotechnol 84:5–37.

Chew KW, Yap JY, Show PL, Suan NH, Juan JC, Ling TC, Lee D-J, Chang J-S (2017) Microalgae biorefinery: High value products perspectives, Bioresource technology, 229:53–62.

Chng LM, Lee KT, Chan DJ (2017) Evaluation on microalgae biomass for bioethanol production, Mater Sci Eng A 206:12–8.

Christaki E, Florou-Paneri P, Bonos E (2011) Microalgae: a novel ingredient in nutrition, Int J Food Sci Nutr 62(8):794–799.

Chum HL, Overend RP (2001) Biomass and renewable fuels, Fuel Processing Technol 71:187–195.

Clark JH, Buldarni V, Deswarte FIE (2006) Green chemistry and the biorefinery: a partnership for a sustainable future, Green Chem 8:853–860.

Daliry S, Hallajisani A, Mohammadi Roshandeh J, Nouri H, Golzary A (2017) Investigation of optimal condition for *Chlorella vulgaris* microalgae growth, Global J Environ Sci Manage 3(2):217–30.

Duvall MN, Fraker RN (2009) Algae-based biofuels attract incentives and investments. Washington, D.C.: Beveridge & Diamond, P.C.

Gokhale SV, Jyoti KK, Lele SS (2008) Kinetic and equilibrium modeling of chromium (VI) biosorption on fresh and spent *Spirulina platensis*/*Chlorella vulgaris* biomass, Bioresour Technol 99:3600–3608.

Grobbelaar JU (2004) Algal nutrition. In: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Oxford: Blackwell, 97–115.

Guldhe A, Kumari S, Ramanna L, Ramsundar P, Singh P, Rawat I, Bux F (2017) Prospects, recent advancements and challenges of different wastewater streams for microalgal cultivation, J Environ Manage 203:299–315.

Harun R, Danquah MK, Forde GM (2010) Microalgal biomass as a fermentation feedstock for bioethanol production, J Chem Technol Biotechnol 85:199–203.

Hemaiswarya S, Raja R., Carvalho IS, Ravikumar R, Zambare V, Barh D (2012) An Indian scenario on renewable and sustainable energy sources with emphasis on algae, Appl Microbiol Biotechnol 96:1125–1135.

Hatakka AI, Varesa T, Lunn TK (1993) Production of multiple lignin peroxidases by the white rot fungus *Phlebia ochraceofulva*, Enzyme Microb Technol 15:664–669.

Hatakka A (1994) Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation, FEMS Microbiol Rev 13:125–135.

Hernández D, Riaño B, Coca M, García-González M (2015) Saccharification of carbohydrates in microalgal biomass by physical, chemical and enzymatic pre-treatments as a previous step for bioethanol production, Chem Eng J 262:939–945.

Hirano A, Ueda R, Hirayama S, Ogushi Y (1997) CO_2 fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation, Energy 22(2):137–42.

Ho SH, Huang SW, Chen CY, Hasunuma T, Kondo A. Chang JS (2013) Bioethanol production using carbohydrate-rich microalgae biomass as feedstock, Bioresour Technol 135:191.

Huber GW, Iborra S, Corma A (2006) Synthesis of Transportation Fuels from Biomass: Chemistry, Catalysts, and Engineering, Chem Rev 106:4044–4098.

Hwang TY, Qi HA, Kin CM, Shing WL, Djearamane S, Thirunavukkarasu C (2021) Potential of Fruit Peel Waste in Growing Cyanobacteria *Anabaena cylindrica*, International Journal of Technology 12(4):711-719.

Karunanithy V, Muthukumarappan K (2011) Optimizing extrusion pretreatment and big bluestem parameters for enzymatic hydrolysis to produce biofuel using response surface methodology, Int J Agric Biol Eng 4:61–74.

Kim SS, Ly HV, Kim J, Lee EY, Woo HC (2015) Pyrolysis of microalgae residual biomass derived from *Dunaliella tertiolecta* after lipid extraction and carbohydrate saccharification, Chem Eng J 263:194–199.

Kirrolia A, Bishnoi NR, Singh R (2013) Microalgae as a boon for sustainable energy production and its future research & development aspects, Renewable and Sustainable Energy Reviews 20:642–656.

Kitaya Y, Azuma H, Kiyota M (2005) Effects of temperature, CO_2/O_2 concentrations and light intensity on cellular multiplication of microalgae, *Euglena gracilis*, Adv Space Res 35(9):1584–8.

Khan MI, Shin JH, Kim JD (2018) The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products, Microb Cell Fact 17:36.

Koushalya S, Hariprasad P, Malik A (2022) *Chlorella minutissima* as a functional food: evaluation on nutritional profle and antioxidant potential of the metabolites, Biomass Conversion and Biorefnery, doi.org/10.1007/s13399-022-02401-8.

Krzemińska I, Pawlik-Skowrońska B, Trzcińska M, Tys J (2014) Influence of photoperiods on the growth rate and biomass productivity of green microalgae, Bioprocess Biosyst Eng 37(4):735–41.

Kumar P, Barrett DM, Delwiche MJ, Stroeve P (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production, Ind Eng Chem Res 48:3713–3729.

Kumar AK, Sharma S (2017) Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review, Bioresour Bioprocess 4:7.

Lakatos GE, Ranglová K, Manoel JC, Grivalský T, Kopecký J, Masojídek J (2019) Bioethanol production from microalgae polysaccharides, Folia Microbiologica 64:627–644.

Lam MK, Lee KT (2012) Potential of using organic fertilizer to cultivate *Chlorella vulgaris* for biodiesel production, Appl Energy 94:303–8.

Lee J (1997) Biological conversion of lignocellulosic biomass to ethanol, J Biotechnol 56:1-24.

Liang S, Xueming L, Chen F, Chen Z (2004) Current microalgal health food R&D activities in China, Hydrobiologia 512:45–8.

Liang Y, Sarkany N, Cui Y, Yesuf J, Trushenski J, Blackburn JW (2010) Use of sweet sorghum juice for lipid production by *Schizochytrium limacinum* SR21. Bioresource Technology 101: 3623–3627.

Licht FO (2006) World ethanol markets: the outlook to 2015. Agra Europe Special Report: Tunbridge Wells.

Lin Y, Tanaka S (2006) Ethanol fermentation from biomass resources: current state and prospects, Appl Microbiol Biotechnol 69: 627–642.

Lu X, Xi B, Zhang Y, Angelidaki I (2011) Microwave pretreatment of rape straw for bio-ethanol production: focus on energy efficiency, Bioresour Technol 102:7937–7940.

Maurya DP, Singla A, Negi S (2015) An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol, 3 Biotech 5:597–609.

Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: A review, Renewable and Sustainable Energy Reviews 14:217–232.

Moller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai PF (2000) Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence, or detoxification ability? Avian Poult Biol Rev 11:137–59.

Mona S, Kaushik A, Kaushik CP (2011) Biosorption of reactive dye by waste biomass of *Nostoc linckia*, Ecol Eng 37:1589–1594.

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–686.

Mus F, Toussaint JP, Cooksey KE, Fields MW, Gerlach R, Peyton BM, Carlson RP (2013) Physiological and molecular analysis of carbon source supplementation and pH stress- induced lipid accumulation in the marine diatom *Phaeodactylum tricornutum*, Appl Microbiol Biotechnol 97:3625–42.

Ni M, Leung DYC, Leung MKH, Sumathy K (2006) An overview of hydrogen production from biomass, Fuel Process Technol 87:461–472.

Park JH, Jeong JY, Hee D, Dong JL (2012) Anaerobic digestibility of algal bioethanol residue, Bioresour Technol 113:78–82.

Park W-K, Myounghoon M, Kwak M-S, Jeon S, Choi G-G, Yang J-W, Lee B (2014) Use of orange peel extract for mixotrophic cultivation of *Chlorella vulgaris*: Increased production of biomass and FAMEs, Bioresource Technology 171:343–349.

Perez-Garcia O, Escalante FME, de-Bashan LE, Bashan Y (2011) Heterotrophic cultures of microalgae: metabolism and potential products, Water Res 45(1):11–36.

Pleissner D, Lin CSK (2013) Valorisation of food waste in biotechnological processes, Sustainable Chemical Processes 1:21.

Pleissner D, Lam WC, Sun Z, Lin CSK (2013) Food waste as nutrient source in heterotrophic microalgae cultivation, Biores Technol 137:139–146.

Putro JN, Soetaredjo FE, Lin SY, Ju YH, Ismadi S (2016) Pretreatment and conversion of lignocellulose biomass into valuable chemicals, RSC Adv 6:46834–46852.

Raheem A, Prinsen P, Vuppaladadiyam AK, Zhao M, Luque R (2018) A review on sustainable microalgae based biofuel and bioenergy production: Recent developments, Journal of Cleaner Production 181:42-59.

Rashid N, Rehman MSU, Han J-I (2013) Recycling and reuse of spent microalgal biomass for sustainable biofuels, Biochemical Engineering Journal 75:101–107.

Rehman MSU, Kim I, Chisti Y, Han JI (2013) Use of ultrasound in the production of bioethanol from lignocellulosic biomass, Energy Sci Res Technol 30(2):1391–1410.

Rizza LS, Smachetti MES, Nascimento MD, Salerno GL, Curatti L (2017) Bioprospecting for native microalgae as an alternative source of sugars for the production of bioethanol, Algal Research 22:140–147.

Sanchez OJ, Cardona CA (2008) Trends in biotechnological production of fuel ethanol from different feedstocks, Bioresour Technol 99:5270–5295.

Sánchez C (2009) Lignocellulosic residues: biodegradation and bioconversion by fungi, Biotechnol Adv 27:185–194.

Sathasivam R, Juntawong N (2013) Modifed medium for enhanced growth of *Dunaliella* strains, Int J Curr Sci 5:67–73.

Sathasivam R, Radhakrishnan R, Hashem A, Abd_Allah EF (2017) Microalgae metabolites: a rich source for food and medicine, Saudi J Biol Sci, doi.org/10.1016/j.sjbs.2017.11.003.

Shafizadeh F, Bradbury AGW (1979) Thermal degradation of cellulose in air and nitrogen at low temperatures, J Appl Poly Sci 23:1431–1442.

Shuba ES, Kifle D (2018) Microalgae to biofuels: 'Promising' alternative and renewable energy, review, Renewable and Sustainable Energy Reviews 81:743–755.

Show PL, Tang MSY, Nagarajan D, Ling TC, Ooi C-W, Chang J-S (2017) A holistic approach to managing microalgae for biofuel applications, Int J Mol Sci 18(1):215.

Singh A, Poonam SN, Jerry DM (2011) Mechanism and challenges in commercialisation of algal biofuels, Bioresour Technol 102:26–34.

Singh SP, Singh P (2015) Effect of temperature and light on the growth of algae species: a review, Renew Sust Energy Rev 50:431–44.

Soletto D, Binaghi L, Lodi A, Carvalho JCM, Converti A (2005) Batch and fedbatch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources, Aquaculture. 243:217–24.

Stephen B, Hayes M (2017) Algal Proteins:Extraction, application, and challenges concerning production, Foods 6:33.

Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review, Bioresour Technol 83:1-11.

Rattanapoltee P, Kaewkannetra P (2014) Utilization of Agricultural Residues of Pineapple Peels and Sugarcane Bagasse as Cost-Saving Raw Materials in *Scenedesmus acutus* for Lipid Accumulation and Biodiesel Production, Appl Biochem Biotechnol 173(6):1495–1510.

Ryu B-G, Kim K, Kim J, Han J-I, Yang J-W (2013) Use of organic waste from the brewery industry for high-density cultivation of the docosahexaenoic acid-rich microalga, *Aurantiochytrium* sp. KRS101, Biores Technol 129:351–359.

Tafreshi AH, Shariati M (2009) *Dunaliella* biotechnology: methods and applications, J Appl Microbiol 107(1):14–35.

Taherzsadeh MJ, Karimi K (2007) Enzymatic-based hydrolysis processes for ethanol from lignocellulosic materials: a review, Bioresources 2:707–738.

Torri C, Samori C, Alessio A, Daniele F, Cecilia F, Giuseppe T (2011) Preliminary investigation on the production of fuels and bio-char from *Chlamydomonas reinhardtii* biomass residue after bio-hydrogen production, Bioresour Technol 102:8707–8713.

Tyson KS, Bozell J, Wallace R, Petersen E, Moens L (2005) Biomass oil analysis: research needs and recommendations. NREL Technical Report, http://www.eere.energy.gov/biomass/pdfs/34796.pdf.

Usher PK, Ross AB, Camargo-Valero MA, Tomlin AS, Gale WF (2014) An overview of the potential environmental impacts of large scale microalgae cultivation. Biofuels 5:331–49.

Vanthoor-Koopmans M, Wijffels RH, Barbosa MJ, Eppink MHM (2013) Biorefinery of microalgae for food and fuel, Bioresource Technology 135:142–149.

Vats S, Maurya DP, Shaimoon M, Agarwal A, Negi S (2013) Development of a microbial consortium for the production of blend enzymes for the hydrolysis of agricultural waste into sugars, J Sci Ind Res 72:585–590.

Wackett LP (2008) Biomass to fuels via microbial transformations, Current Opinion in Chem Biol 12(2):187–193.

Wang H, Ji C, Bi S, Zhou P, Chen L, Liu T (2014) Joint production of biodiesel and bioethanol from filamentous oleaginous microalgae *Tribonema* sp., Bioresour Technol 172:169–17.

Yang Z, Rongbo G, Xiaohui X, Xiaolei F, Shengjun L (2011) Hydrogen and methane production from lipid-extracted microalgal biomass residues, Int J Hydrogen Energy 36:3465–3470.

Zappe H, Snell ME, Bossard MJ (2008) PEGylation of cyanovirin-N, an entry inhibitor of HIV, Adv Drug Deliv Rev 60(1):79–87.

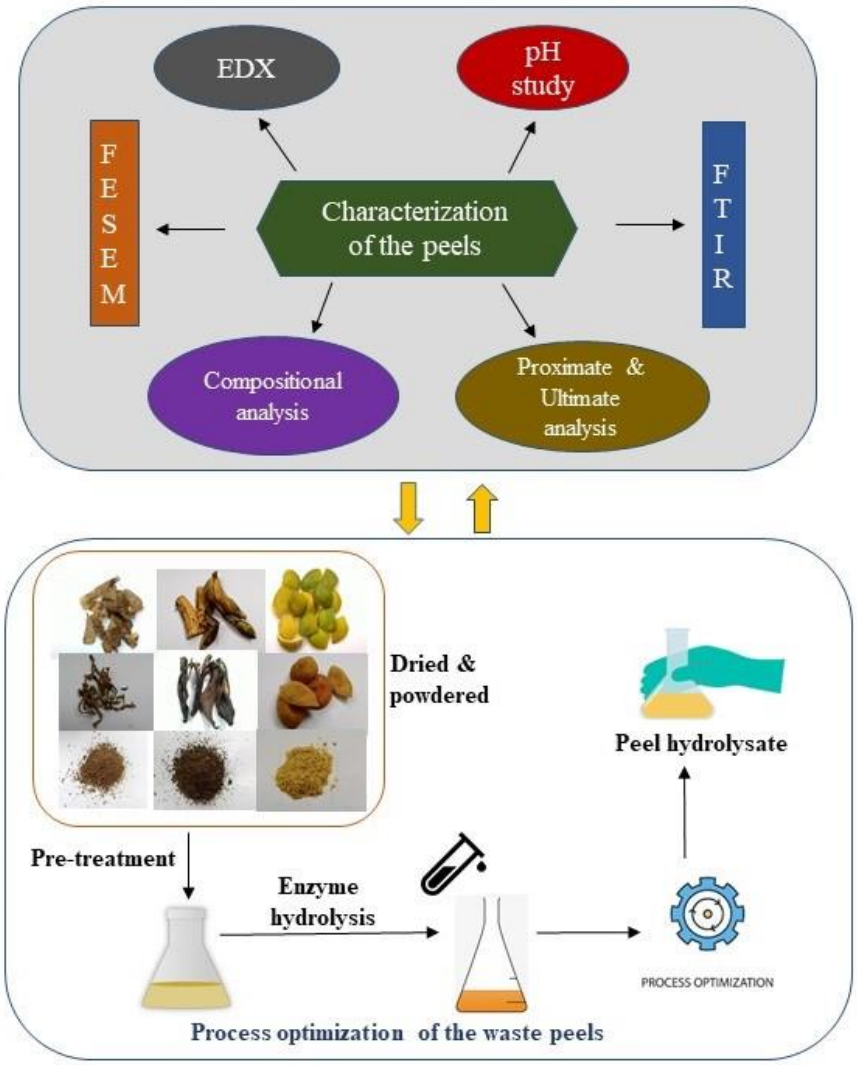
Zeng X, Danquah MK, Chen XD, Lu Y (2011) Microalgae bioengineering: from CO_2 fxation to biofuel production, Renew Sustainable Energy Rev 15:3252–60

Zhu JY, Pan XJ (2010) Woody biomass pretreatment for cellulosic ethanol production technology and energy consumption evaluation, Bioresour Technol 101:4992–5002.

https://www.cleantechloops.com/pretreatment-lignocellulosic-biomass (assessed on 12.7.22).

CHAPTER 3

SELECTION, CHARACTERIZATION AND PROCESS OPTIMIZATION OF THE WASTE PEELS TOWARDS PRE-TREATMENT



3.1. Overview

The most abundant renewable energy resource available in the world is biomass, which is composed of cellulose, hemicelluloses, and lignin (Roberto et al., 2003). Fruit and vegetable peels are generated in huge amounts in industries, hostels, juice shops, and households. They are mostly dumped along with other wastes without segregation making it unfit for further use. This study is an attempt to utilize these waste peels for the recovery of sugars towards producing biofuels and improving the environment.

Biomass cellulose is composed of crystalline and amorphous components and enzymes more easily digest the amorphous component than the crystalline component (Laureano-Perez et al, 2005). Highly specific cellulose enzyme carries out enzymatic hydrolysis and reducing sugars like glucose is the product of such hydrolysis. To enhance the hydrolysis of cellulose, it is required to pre-treat the biomass, which in turn decreases the crystallinity of cellulose. Hence, before enzymatic hydrolysis, it is crucial to pre-treat the biomass (El-Zawawy et al., 2011). Different pre-treatment techniques have been employed on various biomass that include physical methods (microwave assisted or ultrasonic wave assisted), chemical methods (acid and alkali assisted) and biological method (enzyme-assisted hydrolysis). Each method has its own benefits and drawbacks. Although physical and chemical methods are widely used for pre-treatment purpose, they have disadvantages in the form of higher energy consumption. Enzyme assisted hydrolysis has its advantages in the form of less energy consumption, no corrosion problems, however the cost hinders its large-scale application (Tao et al., 2016). Each pre-treatment method has its specific effect on these three main biomass constituents. It is always necessary to pre-treat the biomass before enzymatic hydrolysis to make the cellulose more accessible to enzymes, to give rise to reducing sugars as the end product (El-Zawawy et al., 2011).

The three types of pre-treatment widely followed are Physical, Chemical and Biological. Several physico-chemical, structural and compositional parameters affect the enzymatic digestibility of cellulose present in the biomass. All pre-treatment methods aim to enhance the rate of enzyme hydrolysis and get higher yields of fermentable sugars from cellulose or hemicellulose. Hence, physico-chemical modifications were executed by the pre-treatment methods to obtain the desired results (Mosier et al, 2005). Parameters such as the amount of lignin, cellulose crystallinity and particle size etc. limits the digestibility of the hemicelluloses and cellulose existing in the substrate. Each pre-treatment has its own particular

effect on the three main components of biomass: cellulose, hemicelluloses and lignin (Hendriks and Zeeman, 2009). Hemicellulose is more easily hydrolyzed than cellulose.

Cellulose is a highly stable high molecular weight crystalline polymer which is recalcitrant to enzyme hydrolysis, hence the need for an efficient pre-treatment process. During pre-treatment process, disruption in lignin and crystalline structure of cellulose occurs resulting in enhancement of the enzyme accessibility to the cellulose (Mohapatra et al., 2010). Hot water treatment permits selective solubility of hemicelluloses, enhancing the penetrability of enzymes for hydrolysis of cellulose (Alvira et al., 2010). Autoclaving treatment or Steam explosion is broadly classified into uncatalyzed steam explosion and catalyzed steam explosion. Uncatalyzed steam explosion refers to a pre-treatment method in which biomass is swiftly heated by high-pressure steam without inclusion of any catalyst. Water can behave as a chemical catalyst at elevated temperatures. The vital chemical and physical alterations to biomass by steam treatment are often attributed to the removal of hemicelluloses (Mosier et al., 2005). In steam explosion or autoclaving pre-treatment, the steam enters the feedstock, inflates the cell walls directing to partial hydrolysis, in turn intensifying the enzyme availability for cellulose. Steam treatment can be efficiently increased by inclusion of sulfuric acid, Carbon dioxide or Sulphur dioxide as a catalyst, which is actually catalyzed steam explosion. The utilization of acid catalyst enhances the recovery of hemicellulose sugars and boosts the enzymatic hydrolysis on the solid remnant (Agbor et al., 2011; Kumar et al., 2017). Dilute acid treatment is an effective method for feedstock pre-treatment. Acid hydrolysis can intensify the enzyme treatment of lignocellulosic biomass to release reducing sugars. Dilute acid treatment can enhance the sugars available for enzyme hydrolysis. Acid hydrolysis is an efficient widely used method for biomass structure conversion, and it highly facilitates enzyme hydrolysis (Kumar et al., 2009a). Sulfuric and hydrochloric acids are the most popularly used catalysts for hydrolysis of biomass (Lenihan et al, 2010). Lime pre-treatment removes lignin from biomass without causing any significant depletion of structural carbohydrate materials e.g., glucose, xylose, arabinose etc. Lime treatment effectively separates lignin from the biomass and increases digestibility of cellulose (Maurya et al., 2015).

It is very important to optimize the pre-treatment procedure to maximize the concentration of recovered glucose from the peel. Response surface methodology (RSM) is a useful statistical tool that can illuminate and analyze the acts of the studied variables (Kim et al., 2011).

In the present study, various pre-treatment processes were carried out concerning divergent time profiles (20, 40, 60, 80 and 100 min) to achieve the best conditions yielding higher glucose concentration. The pre-treated extract at 80 min in case of dilute acid hydrolysis with autoclaving conditions followed by enzyme hydrolysis, to achieve maximum recovery of glucose, gave higher glucose concentration amongst all the different types of pre-treatment processes for both potato and sweet lime peels and hence considered for further analysis. At 40 min in case of autoclaving treatment followed by enzymatic hydrolysis, the pre-treated hydrolysate gave the higher concentration of glucose amongst the various treatments executed and hence was chosen for further analysis. This study put emphasis on optimization (utilizing the response surface methodology) of acid hydrolysis to improve the enzymatic digestibility. Three process variables (time, temperature and rotation frequency or agitation speed of the incubator) were considered for the RSM study. To find the optimum conditions, experimental values were fitted to the second order polynomial model. To predict the most efficient combinations, a RSM model, an integrated model and an optimization approach was developed based on the experimental results. RSM is an efficient tool that provides high degree of statistical significance in the results.

3.2. Materials and methods

3.2.1. Procurement of Potato, banana and sweet lime peels

Potato, banana and sweet lime peels were collected from the hostels, juice shops and canteens inside IIT Guwahati campus, Assam, India. The peels were cut into small pieces and cleaned with water followed by drying at 80 ^{o}C in a hot air oven till constant weight was achieved. Size of dried pieces were reduced further by using a grinder to obtain powder like fine particles.

3.2.2. Characterization of the waste peels

3.2.2.1. FESEM

FESEM (Field emission scanning electron microscopy) analysis (Zeiss, sigma 300) was carried out to examine the surface structure and morphology of the three biomasses before and after the pre-treatment, according to the standard method by Sadiq et al. (2011).

3.2.2.2. EDX

Using the energy dispersive x-ray spectroscopy (EDX) coupled with the FESEM, the composition of the components in the biomass was determined.

3.2.2.3. Ultimate and proximate analysis

Ultimate analysis

Using EuroEA elemental analyzer, the elemental analysis of the sample for the determination of CHNS(O) composition was carried out.

The high heating value (HHV, MJ Kg^{-1}) or gross calorific value (GCV) and the low heating value (LHV, MJ Kg^{-1}) or net calorific value (NCV) based on the CHNS(O) elemental analysis of the biomass was determined using the following equations (Pandey et al., 2019),

$$GCV \text{ or } HHV = 0.383 \times C + 1.422 \times (H - O/8) \quad (1)$$

$$NCV \text{ or } LHV = HHV - 2.447 \times (H/100) \times 9.011 \quad (2)$$

Where C, H and O are the weight in percentages (%) of carbon, hydrogen and oxygen in the biomass respectively.

Proximate analysis

To determine the Moisture content, Ash and Volatile matter (Proximate analysis) of the three peel feedstocks, National Renewable Energy Laboratory (NREL, 2008) protocol was used.

The content of Fixed carbon (FC) has been calculated by deducting the values of the moisture content (M), volatile matter (VM) and ash (A_S) content from 100 (Artemio et al., 2018).

$$FC = 100 - (M + VM + A_S) \quad (3)$$

3.2.2.4. Compositional analysis

The cellulose, hemicellulose and the lignin present in the peel feedstocks were determined according to the chemistry of Van Soest method of Fibre analysis.

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3.2.2.5. FTIR analysis

FTIR (PerkinElmer, Spectrum 2) analysis of the biomass was executed to recognize the chemical changes before and after the hydrolysis. FTIR spectrum was noted from 4000 cm^{-1} to 400 cm^{-1} for scanning the chemical properties of the samples.

3.2.3. Pre-treatment of the biomass

Different pre-treatment methods employed in this study are mentioned below;

1. Hot water treatment (H)
2. Autoclaving treatment or steam explosion (A)
3. Dilute Acid treatment (DA)
4. Lime treatment (L)
5. Dilute Acid hydrolysis in hot water (DA+H)
6. Dilute Acid hydrolysis in autoclaving conditions (DA+A)
7. Lime treatment in hot water (L+H)
8. Lime treatment in autoclaving conditions (L+A)

These methods were employed with few modifications to the existing commonly used methods. For all the treatment methods, 10 g peel biomass (10% w/v) was combined with 100 mL distilled water in a 250 mL Erlenmeyer flask. These tests were performed in different time profiles (20, 40, 60, 80, 100 min).

In hot water treatment, the flask was incubated in a hot water bath at 100oC; in autoclaving treatment, the flask was autoclaved at 121^{o}C for 15 psi pressure. 1% H_2SO_4 (Sulphuric acid) and 1% calcium hydroxide (with stirring at regular intervals at room temperature) were used in the case of acid and lime treatment, respectively.

For the remaining pre-treatments, combinations of two techniques with slight modifications to the traditional methods were employed. 1% H_2SO_4 was used in both the cases of acid hydrolysis in hot water and acid hydrolysis in autoclaving conditions. Similarly, 1% calcium hydroxide was utilized in both the cases of lime treatment in hot water and lime treatment in autoclaving conditions. Incubation at 100^{o}C was done in case of acid and lime

treatment in hot water while autoclaving was done at 121°C at 15 psi pressure in case of acid and lime treatment in autoclaving conditions. The characteristic parameters of the mentioned pre-treatment methods are discussed in Table 3.1.

After the treatment was complete in all the cases, the flask was allowed to cool down to room temperature, followed by filtration to obtain the extract. Then, enzyme-assisted hydrolysis was performed for 24 h at 60°C and 120 RPM on each treatment condition to check which treatment yields the higher glucose concentration. Two additional sets of untreated blank (control) experimental flask were also set in which peel powder was immersed in distilled water, (1) kept at room temperature with periodic shaking and stirring at systemic intervals in case of the above pre-treatments except enzyme hydrolysis and (2) in case of enzyme assisted hydrolysis, kept in shaker incubator at 60°C for 24 h without the inclusion of the enzyme.

Table 3.1. Characteristic parameters of the pre-treatment methods.

Pre-treatment method	Characteristic parameters
H- Hot water treatment	100°C
A- Autoclaving treatment	121°C, 15 psi
L- Lime treatment	1% calcium hydroxide
DA- Dilute acid hydrolysis	1% H_2SO_4
DA+H- Dilute acid hydrolysis and Hot water treatment	100°C, 1% H_2SO_4
DA+A- Dilute acid hydrolysis and Autoclaving treatment	121°C, 15 psi, 1% H_2SO_4
L+H- Lime treatment and Hot water treatment	100°C, 1% calcium hydroxide
L+A- Lime treatment and Autoclaving treatment	121°C, 15 psi, 1% calcium hydroxide

3.2.4. Enzymatic hydrolysis through RSM

Since the glucose concentration was found to be highest in acid hydrolysis with autoclaving conditions (after enzyme assisted hydrolysis) compared to the other techniques for both potato and sweet lime peels, it was selected for enzymatic hydrolysis through RSM. Autoclaving treatment followed by enzymatic hydrolysis gave the highest glucose concentration in case of banana peels, compared to the other treatments and hence it was further considered for optimization by RSM. The cellulose enzyme complex (Accelerase 1500), was kindly gifted from Genencor International, B.V., Netherlands. 6 mL L^{-1} of enzyme was added to the pretreated peels in a 250 mL Erlenmeyer flask and incubated in a shaker incubator (Orbitek, Scigenics Biotech, India) for specified duration as per the experimental design and layout of RSM. For stopping the enzymatic reaction, the conical flask was autoclaved for 20 min at 121 °C. Then the conical flask was let to reach room temperature and filtered to attain the hydrolysate eventually.

3.2.5. Experimental design and statistical analysis by RSM

The Minitab v. 16.0 software was employed for regression and graphical analyses of the experimental data obtained. To obtain increased glucose recovery, successive trials were outlined with response surface methodology (RSM). Values of three process factors deployed on single-factor experiment were optimized further and analyzed employing central composite design (CCD). Twenty trials consisting of six central points were fabricated with three variables, and glucose concentration was adopted as the response. The six central points were employed to assess the proposed model's pure error and lack of fit (Selvakumar et al., 2019).

All the experiments were conducted in triplicate and the mean values were reported. The optimum data of the variables were acquired by analyses using the 'RSM-CCD' program and the criterion of desirability.

A quadratic polynomial regression was used to illustrate the respective and interactive outcome of the three factors deployed on the obtained numerical data using RSM. Then, the predicted optimal data were established by the verification trials using the adjusted values of the three factors.

The generalized second-order polynomial model employed in the response surface analysis is given below:

$$Y = a_0 + \sum_{i=1}^{n} a_1 X_i + \sum_{i=1}^{n} a_2 X^2{}_i + \sum \sum_{j=i+1}^{n} a_3 X_i X_j \quad (4)$$

where, *Y* is the experimental response i.e., glucose; *Xi* and *Xj* are independent variables in coded values; α_0 is the constant; α_1 is the linear coefficient; α_2 is the quadratic coefficient and α_3 is the interaction coefficient.

The experimental design in this investigation was inclusive of three parameters: time (h), temperature (°C) and the agitation speed or rotation frequency of the incubator shaker (RPM). The experimental values were fitted to the second-order polynomial model to acquire the regression coefficients. The experimental runs were randomized in order to lessen the results of the unexpected variability in the observed responses. Lack of fit, the coefficient of determination (R^2) and the F-test value attained from the analysis of variance (ANOVA) were used to assess the efficacy of the model developed. To decide the optimum conditions and analyze how the interactions of independent variables affected the overall response, regression analysis and response surface plots were generated. Validation of model rationality was established by assessing and comparing the experimental data with the predicted outcome of the optimized model.

3.2.6. Experimental and Statistical Analyses

Glucose estimation was done through HPLC (Shimadzu, RF-20A) furnished with Repromer H+ column. Minitab software was used to perform the optimization by Response surface methodology (RSM). All the investigations were executed in triplicate and demonstrated as Mean ± Standard deviation (SD). Origin pro 9.0 was employed for the statistical evaluations.

3.3. Results and Discussion

3.3.1. Compositional analysis of the biomass

The phases of the biomass preparation for the hydrolysis process are depicted in Figure 3.1, 3.2 and 3.3. Compositional analysis of Cellulose, Hemicellulose, Lignin of the feedstocks are presented in Table 3.2. The proximate (fresh and powdered peel) and ultimate analysis (powdered peel) of the three feedstocks were carried out and are reported in Table 3.3 and 3.4. The higher percent of ash in the peels indicates that they are an abundant source of various

minerals. Further, the structure and morphology of the peel powder was observed by FESEM. The structural difference of the three waste peels before and after pre-treatment can be observed in Figure 3.4. The cell breakdown due to the application of the pre-treatment techniques in the three peels can be observed in Figure 3.4. EDX analysis was carried out to investigate the elemental composition of the three feedstocks and the various minerals present in the waste peel biomass are reported in Table 3.5. These minerals could aid in the growth of the microalgae, which is the next step of this project. As microalgae needs an optimum pH for their growth and since an integral aim of this project is to evaluate the growth of microalgae in the peel waste extracts, the extracts' pH was measured and presented in Table 3.6.

Figure 3.1. Stages of preparation of potato peel for pre-treatment.

Figure 3.2. Stages of preparation of banana peel for pre-treatment.

Figure 3.3. Stages of preparation of sweet lime peel for pre-treatment.

Table 3.2. Composition of the waste peels.

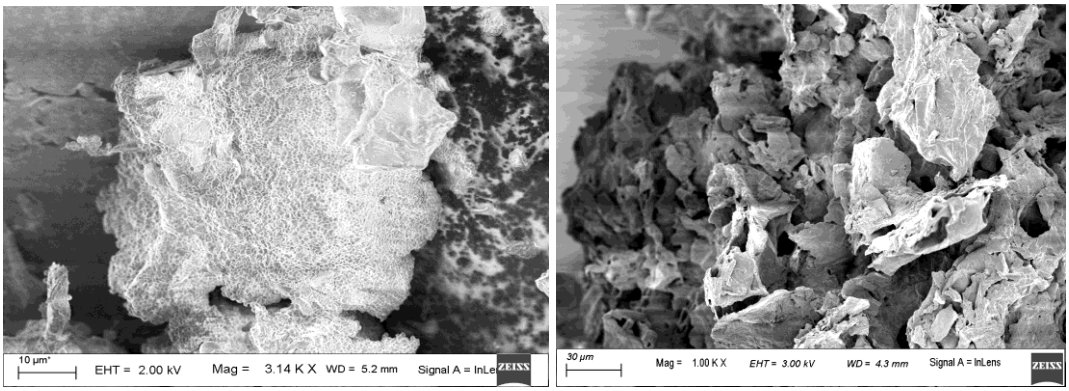
Sample	Cellulose (%)	Hemicelluloses (%)	Lignin (%)
Potato peel	47.29	10.93	13.22
Banana peel	13.98	21.56	9.28
Sweet lime peel	17.07	25.18	7.34

Table 3.3 Proximate analysis of the fresh peels.

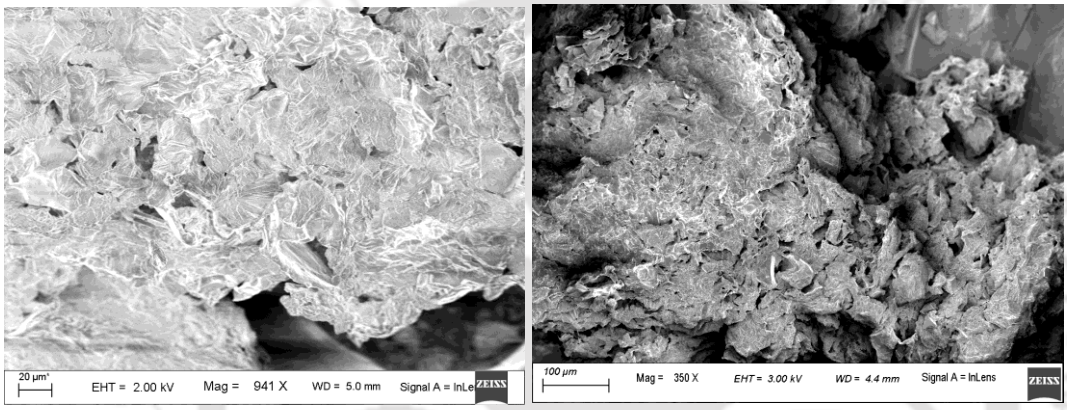
Sample	Moisture content (%)	Ash (%)	Volatile matter (%)	Fixed carbon (%)
Potato peel	76.38±0.36	8.23±0.23	1.46±0.17	13.93±0.24
Banana peel	86.66±0.21	10.09±0.32	1.39±0.29	1.86±0.18
Sweet lime peel	73.44±0.39	4.13±0.19	1.97±0.25	20.46±0.17

Table 3.4. Proximate and ultimate analysis of the powdered peels.

Parameters	Potato peel	Banana peel	Sweet lime peel
	Proximate analysis		
Moisture (%)	1.7±0.06	3.3±0.09	2.6±0.19
Volatile matter (%)	87.5±0.63	83.3±0.52	83.05±0.45
Ash (%)	9.73±0.23	13.31±0.32	14.2±0.39
Fixed carbon (%)	1.07±0.05	0.09±0.01	0.15±0.02
	Ultimate analysis		
C (%)	39.64	42.34	43.11
H (%)	7.05	6.61	7.19
N (%)	2.42	1.52	0.89
O (%)	41.16	36.22	34.61
C/N (%)	16.38	27.86	48.4
O/C (%)	1.04	0.86	0.80
HHV or GCV (MJ Kg^{-1})	17.89	19.17	20.59
LHV or NCV (MJ Kg^{-1})	16.34	17.71	19



(A)



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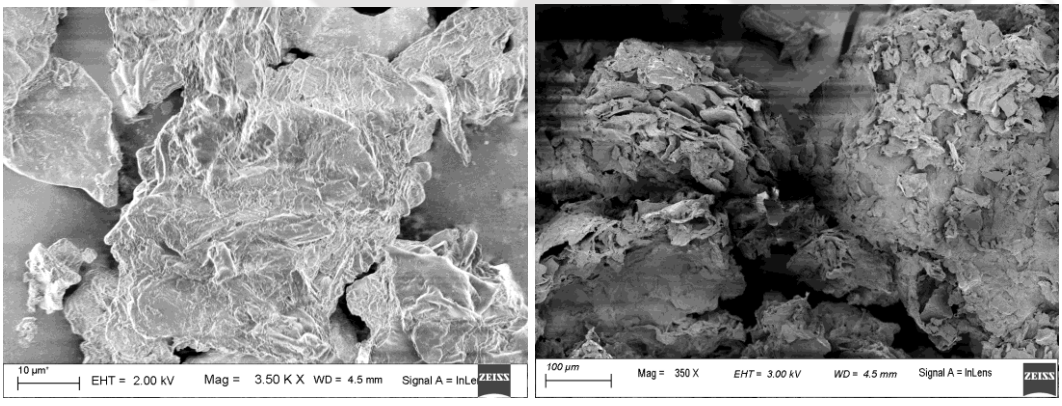


(C)

Figure 3.4. FESEM analysis:

(A) FESEM images of Potato peel powder before and after pre-treatment.

(B) FESEM images of Banana peel powder before and after pre-treatment.

(C) FESEM images of Sweet lime peel powder before and after pre-treatment.

Table 3.5. EDX analysis.

	Elemental composition (%)		
	Potato peel	Banana peel	Sweet lime peel
C	53.4	54.8	52.9
O	38.8	36.1	43.1
K	4.9	6.1	1
Cl	0.9	1.1	-
P	0.1	0.5	0.2
Si	-	0.4	0.1
Ca	0.3	-	0.9
Cd	0.2	0.4	-
Cu	0.2	-	0.5
Co	0.4	-	0.1
Fe	0.5	-	-
Al	0.1	0.2	-
Mg	0.2	0.2	0.2
Zn	-	-	0.6
Na	-	0.2	-
Mn	-	-	0.4

Table 3.6. pH of the extracts after the enzyme hydrolysis process.

Treatment	pH		
	Potato peel	Banana peel	Sweet Lime peel
Control	5.82±0.21	5.37±0.24	4.23±0.37
Hot water	5.58±0.36	5.16±0.39	4.45±0.25
Autoclaving	5.48±0.26	5.10±0.34	4.42±0.26
Lime	8.99±0.21	7.23±0.29	7.12±0.37
Acid	1.43±0.13	1.45±0.17	1.28±0.22
Acid+hot water	1.57±0.18	1.51±0.25	1.36±0.16
Acid+autoclaving	1.16±0.11	1.38±0.19	1.23±0.22
Lime+hot water	8.37±0.27	7.14±0.23	6.58±0.21
Lime+autoclaving	9.02±0.19	6.31±0.19	6.77±0.27

3.3.2. Pre-treatment and enzymatic hydrolysis of the biomass

Different techniques of hydrolysate development used in this study have resulted in release of varied amount of glucose from the treated peels. Based on the glucose concentration with different treatment techniques in banana peels, autoclaving treatment followed by enzyme hydrolysis yielded the best glucose concentration of 13.04 ± 1.56 g L^{-1}. When treated with high temperature and pressure, the treatment method resulted in significant release of sugars probably due to the exposure of the peels to steam under pressure.

Based on the glucose yields with various hydrolysis methods used in the other two peels, 1% acid treatment in autoclaving conditions assisted by enzymatic treatment has yielded the best result in case of both potato and sweet lime peels. 39.33 g L^{-1} and 32.19 g L^{-1} of glucose in case of potato peel and sweet lime peel respectively has been seen thereby showing a significant release of glucose. The pre-treatment process for potato and sweet lime peels included two steps: (1) addition of dilute acid (1% sulphuric acid) to the peel and (2) autoclaving of acidified peel solution with the application of high pressure and high temperature. Exposure to steam under pressure in the autoclaving step could result in a substantial release of sugar.

On comparing the detailed results presented in Figure 3.2, It can be seen that in case of potato peel on the addition of enzyme, the highest glucose concentration from the acid treatment in autoclaving conditions is followed by acid treatment in hot water (19.94 g L^{-1}), acid treatment (18.20 g L^{-1}), autoclaving treatment (13.34 g L^{-1}), hot water treatment (7.67 g L^{-1}), lime treatment in autoclaving conditions (1.47 g L^{-1}), lime treatment (0.71 g L^{-1}) and eventually lime treatment in hot water conditions (0.64 g L^{-1}). However, in case of sweet lime peel on the addition of enzyme, the pattern of results for glucose concentration has been seen different from the potato peel. The highest glucose concentration from the acid treatment in autoclaving conditions is followed by autoclaving treatment (22.27 g L^{-1}), lime treatment in autoclaving conditions (16.93 g L^{-1}), hot water treatment (16.83 g L^{-1}), acid treatment (15.86 g L^{-1}), acid treatment in hot water conditions (12.20 g L^{-1}), lime treatment (10.05 g L^{-1}) and eventually lime treatment in hot water conditions (10.03 g L^{-1}). Finally in case of banana peels, the maximum glucose concentration after the addition of the enzyme was found in autoclaving conditions followed by acid treatment (11.38 g L^{-1}), acid treatment in hot water (10.86 g L^{-1}), acid treatment in autoclaving conditions (10.25 g L^{-1}), hot water treatment (10.21 g L^{-1}), lime treatment (8.79 g L^{-1}), lime treatment in autoclaving conditions (8.02 g L^{-1}) and finally lime treatment in hot water treatment (6.20 g L^{-1}).These results are reported in detail in Table 3.7 and 3.8.

Lower concentration of glucose in some of the cases here specifies deficient or incomplete hydrolysis. The treatment parameters might not be able to degrade the specific type of biomass structure properly, resulting in lower glucose concentration for a particular treatment type. The pre-treatment technique and the inherent properties of the biomass are mainly accountable for its breakdown, hydrolysis and release of sugars. Sometimes, combined pre-treatment is considered to yield higher sugar concentration. It is observed in the study that enzyme hydrolysis of the sample after treatment resulted in higher glucose yield. This can be due to the fact that pre-treatment alters the structure of a particular biomass so that the cellulose or hemicellulose becomes more available to the enzymatic attack during the enzyme hydrolysis so as to liberate more sugars.

As the acid treatment with autoclaving conditions assisted by enzymatic treatment in case of potato and sweet lime peels, and autoclaving conditions assisted by enzyme hydrolysis in case of banana peels yielded the maximum amount of glucose, hence, enzymatic hydrolysis (through RSM) was carried out to further increase the release of sugar from the pre-treated biomass.

In case of different pre-treatment methods performed without the addition of enzyme, the control was kept at room temperature with shaking at regular intervals and studied at different time profiles (20, 40, 60, 80 and 100 min) and the best glucose yield was obtained at 80 min for all the three samples as Potato 1.19 g L^{-1}, Banana gL^{-1} 6.19 g L^{-1} and Sweet lime 6.62 g L^{-1}. In case of enzyme assisted hydrolysis, the control (untreated) was kept at 60ºC for 24 h at 120 RPM without the addition of enzyme, and glucose yield was obtained as Potato1.24 g L^{-1}, Banana 6.41 g L^{-1}, Sweet lime 11.95 g L^{-1}.

Michelin and Teixeira (2016) reported a glucose concentration of 0.40 g L-1, 0.45 g L-1, 0.15 g L-1 and 0.10 g L-1 from corn husk, corn cob, wheat straw and wheat straw and luffa sponge respectively after hot water treatment. 28.2 g L^{-1} of glucose concentration was reported in case of autoclaving treatment along with enzymatic hydrolysis from banana peels under optimized conditions (Oberoi et al., 2012). Lemons e Silva et al., (2015) reported 13.9 g L^{-1} glucose from Arundo biomass (1.1% w/w) when pretreated with sulphuric acid. Samsal et al., (2013) reported 475 mg of glucose/biomass (g) from Moj in case of lime pre-treatment.

Table 3.7. Glucose yield in g L^{-1} before enzyme hydrolysis.

Treatment types	Glucose yield (g L^{-1})			
	Time (min)	Potato peel (*Solanum tuberosum*)	Banana peel (*Musa* sp.)	Sweet lime peel (*Citrus limetta*)
Without pre-treatment (control) at room temperature shaked at regular intervals	20	0.93±0.29	1.16±0.33	4.95±0.47
	40	0.88±0.24	1.22±0.26	4.52±0.41
	60	1.15±0.22	1.19±0.21	4.46±0.51
	80	1.19±0.31	6.19±0.76	6.62±0.74
	100	0.81±0.35	5.21±0.68	5.19±0.58
Hot water treatment at 100°C	20	0.31±0.15	6.47±0.81	6.28±0.67
	40	0.35±0.13	5.45±0.66	4.69±0.62
	60	0.27±0.11	6.45±0.78	4.43±0.59
	80	0.31±0.12	4.89±0.65	5.03±0.55
	100	0.19±0.09	5.26±0.71	4.79±0.49
Autoclaving condition at 121°C	20	0.47±0.21	5.05±0.23	4.94±0.51
	40	2.13±0.35	6.54±0.53	6.41±0.46
	60	2.36±0.26	6.40±0.27	4.42±0.26
	80	1.24±0.19	2.49±0.24	5.44±0.54
	100	0.88±0.16	1.53±0.32	3.91±0.49
Lime treatment (1% calcium hydroxide)	20	0.08±0.03	2.36±0.27	7.52±0.69
	40	0.09±0.03	1.95±0.33	9.05±0.95
	60	0.12±0.05	2.39±0.31	7.86±0.77
	80	0.103±0.04	1.99±0.30	6.88±0.72
	100	0.08±0.03	3.44±0.33	3.78±0.66
Dilute acid hydrolysis in plain water (1% v/v H_2SO_4)	20	1.02±0.53	1.46±0.23	4.92±0.51
	40	1.03±0.51	9.08±0.75	4.77±0.55
	60	3.10±0.63	8.89±0.94	5.57±0.61

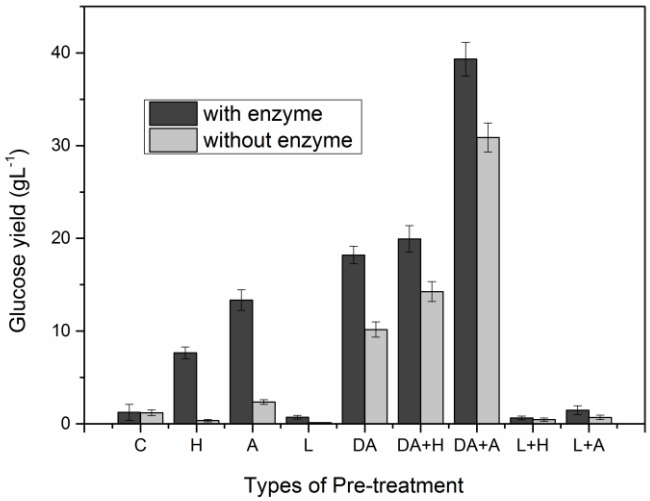
	80	10.17±0.93	10.33±1.21	5.62±0.44
	100	0.55±0.58	3.06±0.55	5.42±0.61
Dilute acid hydrolysis in hot water	20	1.67±0.36	0.79±0.59	3.56±0.29
	40	4.31±0.45	1.71±0.31	4.28±0.48
	60	5.71±0.41	1.33±0.27	4.11±0.62
	80	14.26±1.08	1.81±0.37	5.15±0.56
	100	10.24±0.98	1.65±0.47	4.81±0.28
Dilute acid hydrolysis in autoclaving condition	20	17.4±1.11	1.75±0.66	5.32±0.68
	40	28.01±1.15	1.29±0.58	6.35±0.69
	60	21.18±0.95	2.13±0.52	6.77±0.52
	80	30.87±1.57	1.78±0.49	7.49±0.61
	100	30.82±1.42	3.23±0.58	6.48±0.39
Lime treatment in hot water	20	0.26±0.18	1.53±0.31	3.66±0.36
	40	0.42±0.17	1.15±0.34	5.58±0.66
	60	0.43±0.20	0.56±0.22	5.51±0.58
	80	0.45±0.18	0.55±0.26	5.86±0.61
	100	0.44±0.16	0.36±0.28	6.48±0.76
Lime treatment in autoclaving condition	20	0.18±0.24	0.79±0.58	2.45±0.55
	40	0.32±0.34	0.96±0.59	4.54±0.64
	60	0.41±0.47	1.09±0.48	5.59±0.48
	80	0.48±0.47	1.95±0.55	5.45±0.61
	100	0.70±0.25	1.20±0.38	5.68±0.82

Table 3.8. Glucose yield in g L^{-1} after enzyme hydrolysis w.r.t treatment time.

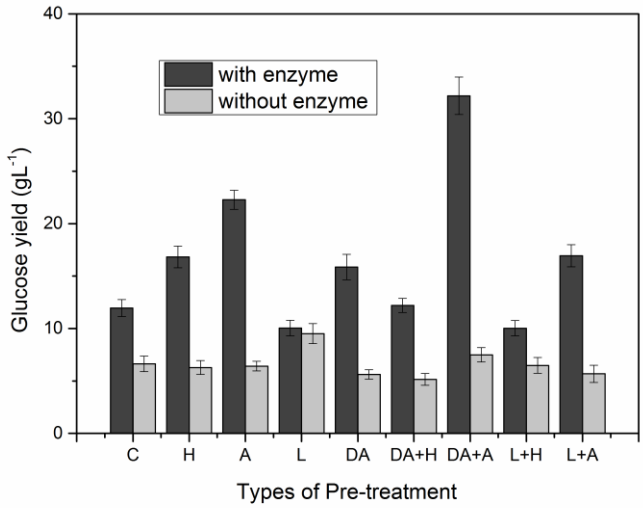
Treatment	Potato peel		Banana peel		Sweet Lime peel	
	Glucose yield (g L^{-1})	Treatment time	Glucose yield (g L^{-1})	Treatment time	Glucose yield (g L^{-1})	Treatment time
Control (Untreated, Kept in shaker incubator at 60°C for 24 h without the addition of the enzyme)	1.24±0.87	24 h	6.41±0.97	24 h	11.95±0.81	24 h
Hot water	7.67±0.63	40 min prior to enzyme hydrolysis	10.21±1.07	20 min prior to enzyme hydrolysis	16.83±1.03	20 min prior to enzyme hydrolysis
Autoclaving	13.34±1.11	60 min prior to enzyme hydrolysis	13.04±1.56	40 min prior to enzyme hydrolysis	22.27±0.91	40 min prior to enzyme hydrolysis
Lime	0.71±0.21	60 min prior to enzyme hydrolysis	8.79±0.55	100 min prior to enzyme hydrolysis	9.51±0.74	40 min prior to enzyme hydrolysis
Acid	18.20±0.95	80 min prior to enzyme hydrolysis	11.38±0.86	40 min prior to enzyme hydrolysis	15.86±1.21	80 min prior to enzyme hydrolysis
Acid+hot water	19.94±1.44	80 min prior to enzyme hydrolysis	10.86±0.79	80 min prior to enzyme hydrolysis	12.20±0.68	80 min prior to enzyme hydrolysis
Acid+autoclaving	39.33±1.81	80 min prior to enzyme hydrolysis	10.25±0.89	100 min prior to enzyme hydrolysis	34.19±1.78	80 min prior to enzyme hydrolysis
Lime+hot water	0.64±0.20	80 min prior to enzyme hydrolysis	6.20±0.53	20 min prior to enzyme hydrolysis	10.03±0.73	100 min prior to enzyme hydrolysis

Lime+autoclaving	1.47±0.48	100 min prior to enzyme hydrolysis	8.02±0.66	80 min prior to enzyme hydrolysis	16.93±1.05	100 min prior to enzyme hydrolysis

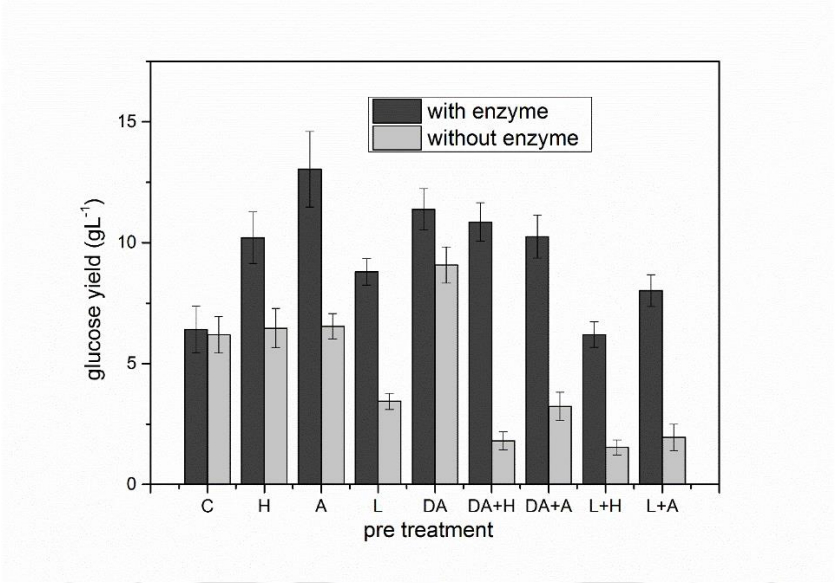
All the samples were incubated for 24 h at 60°C in a shaker incubator after the addition of enzyme.



(A) Potato peel



(B) Sweet lime peel



(C) Banana peel

Figure 3.5. Comparative analysis of glucose (g L^{-1}) released before and after enzyme hydrolysis.

#C=Control (untreated); H=Hot water treatment; A=Autoclaving treatment; L=Lime treatment; DA=Dilute acid hydrolysis; DA+H= Dilute acid hydrolysis and Hot water treatment; DA+A= Dilute acid hydrolysis and Autoclaving treatment; L+H= Lime treatment and Hot water treatment; L+A= Lime treatment and Autoclaving treatment.

3.3.3. Statistical modelling by response surface methodology

The experimental test for attaining a quadratic model consists of twenty trials constituting six replicates at the central point and were investigated by multiple regression analysis and ANOVA. Central composite design did the optimization of the parameters (time, temperature and rotation frequency). The range and levels of the independent variables and coded values were depicted in Table 3.9. The experimental layout and glucose concentration were taken as the dependent variables or responses and are given in Table 3.10.

RSM analysis was performed for examining the combined effects of the three independent factors time (X_1), temperature (X_2) and rotation frequency or agitation speed of the incubator shaker (X_3) on glucose yield by enzymatic hydrolysis.

Based on obtained numerical data, the reduced quadratic polynomial regression model for glucose concentration of potato peel, banana peel and sweet lime peel are expressed as follows:

$Y= 35.3132 -0.2403X_1+0.04953X_2+0.05688X_3+0.0034X_1^2- 7.5945x10^{-4}X_2^2 - 1.4373x10^{-4}X_3^2 + 0.0031X_1X_2 – 8.3333x10^{-5}X_1X_3 – 1.5652x10^{-4}X_2X_3$(For potato peel) (5)

$Y=32.1851-0.2281X_1+0.0412X_2-0.0552X_3+0.0035X_1^2-3.7289x10^{-4}X_2^2+0.0002X_3^2+ 0.0015X_1X_2 + 0.0003X_1X_3 + 4.3478x10^{-6}X_2X_3$ (For sweet lime peel) (6)

$Y= 23.3593 -0.4221X_1 -0.1883X_2 + 0.0610 X_3 + 0.0036X_1^2 + 0.0003X_2^2 – 3.2827x10^{-4}X_3^2 + 0.0056 X_1X_2 + 0.0004X_1X_3 + 0.0004X_2X_3$(For banana peel) (7)

Table 3.9. Experimental range and levels of independent process variables and coded values in CCD for the peels.

Independent variable	Range and levels		
	-1	0	+1
Time (h)	24	36	48
Temperatue (°C)	37	48.5	60
Rotation frequency (RPM)	100	125	150

Table 3.10. Experimental design and results obtained.

Run	Time	Temperature	RPM	Glucose concentration of potato peel (g L^{-1})	Glucose concentration of banana peel (g L^{-1})	Glucose concentration of sweet lime peel (g L^{-1})
1	48	37	100	41.10	21.55	31.19
2	36	48.5	125	40.29	21.13	30.23
3	24	37	150	39.88	20.12	29.77
4	24	60	100	39.95	20.07	29.98
5	36	48.5	125	40.87	24.04	29.84
6	48	37	150	41.34	22.17	31.45
7	24	37	100	38.76	19.44	28.67
8	36	48.5	125	39.64	20.98	30.89
9	24	60	150	40.11	20.65	30.81
10	48	60	100	43.23	24.76	33.33
11	48	60	150	44.07	26.47	34.03
12	36	48.5	125	41.37	20.67	30.54
13	36	48.5	125	40.97	21.05	30.57
14	36	68	125	41.43	23.78	31.34
15	16	48.5	125	38.12	18.87	28.21
16	36	48.5	125	40.57	20.91	30.77
17	56	48.5	125	45.38	26.91	35.68

18	36	48.5	83	39.78	20.44	29.84
19	36	48.5	167	40.48	21.13	30.97
20	36	29	125	38.77	19.23	28.78

Regression analysis was performed to find the best-fitting response function with the experimental data. The *F*-test (ANOVA) was executed to evaluate the statistical significance of the model equation for glucose yield, and it revealed that the regression of the response was statistically significant at 92.12%, 90% and 92.82 % of confidence level in case of potato, banana and sweet lime peels respectively. Also, the models did not exhibit lack-of-fit and showed a high determination coefficients R^2=0.9212, 0.9 and 0.9282, in case of potato peel, banana peels and sweet lime peel, respectively. Coefficients of determination (R^2) values were employed to evaluate the validity of the models generated and its best fitting response. The ANOVA results for the reduced quadratic model for the response of glucose yield are shown in Table 3.11 for potato and sweet lime peels, and Table 3.12 for banana peels. A high statistically significant multiple regression relationship is seen between the response variable and the independent variables. The lack of fit test in the ANOVA can ascertain the adequacy of the model to fit the experimental values. The *F*-test for the lack of fit were known to be insignificant if $p > 0.05$ indicating that the model well fitted the experimental values for the response variables generated. The multiple linear regressions were used to generate the best-fitting model and to eliminate the insignificant terms ($p > 0.05$) from the suggested quadratic model. Thus, well-fitting models for the optimization of potato, banana and sweet lime peel treatment were successfully generated.

F-test analysis of variance (ANOVA) was employed to examine the statistical significance of the model. Results showed the F-value for the lack of fit of 1.56 and 4.49 ($p > 0.05$) respectively for potato peel and sweet lime peel, thus, implying that the lack of fit was not significant relative to pure error. On the contrary if the p-value for lack of fit for a model is significant ($p < 0.05$), a more complex model would be necessary to fit the data (Kim et al., 2011). In this case, the p-value for lack of fit for potato peel and sweet lime peel were respectively 0.32 and 0.062, i.e., insignificant, hence, well-fitting the quadratic model. In the banana peel, the *F*-value for the lack-of-fit was found as 0.21 ($p > 0.05$), implying that the lack-of-fit was not significant relative to the pure error. The p-value for lack-of-fit was 0.946,

indicating it was insignificant, which in turn meant that it well fitted the quadratic model. R^2 values were 0.9212, 0.90, and 0.9282 in the case of potato, banana and sweet lime peels respectively. The R^2 value closer to 1 determines the strength of the model in better predicting the response. F-value can represent the effects of time, temperature, and rotation frequency (RPM) on enzymatic hydrolysis and the larger the F-value, the higher the effect. Based on the F-values, the order of significance for the three factors through the enzymatic hydrolysis was time > temperature > rotation frequency (RPM) for potato peel, banana peel and sweet lime peel.

The 3-D response surface plots graphically assess the significance of individual and integrated interactions on glucose concentration. Interactions between the three factors time (X_1), temperature (X_2) and the rotation frequency (X_3) in case of potato peel, sweet lime peel and banana peel are presented in Figure 3.6, Figure 3.7 and Figure 3.8 respectively. With the increase of the other two factors, glucose concentration would increase initially and then decrease gradually when a single factor was fixed.

Table 3.11. Results of RSM analysis for potato peel and sweet lime peel.

Source	Sum of squares		Degrees of freedom		Mean square		F value		p value	
	Potato peel	Sweet lime peel	Potato peel	Sweet lime peel	Potato peel	Sweet lime peel	Potato peel	Sweet lime peel	Potato peel	Sweet lime peel
Regression	54.37	52.22	9	9	6.04	5.80	13.00	14.36	0.00	0.00
Residual error	4.64	4.04	10	10	0.46	0.40				
Lack of fit	2.82	3.31	5	5	0.56	0.66	1.56	4.49	0.32	0.06
Pure error	1.82	0.73	5	5	0.36	0.14				
Total	59.02	56.26	19	19						

Value of "p" less than 0.05 indicated the term was significant, less than 0.01 highly significant, more than 0.05 non-significant (insignificant).

Table 3.12. Results of RSM analysis for banana peel.

Source	Sum of squares	Degrees of freedom	Mean square	F value	P value
Regression	88.68	9	9.85	10.09	0.00
Residual error	9.76	10	0.97		
Lack of fit	1.67	5	0.33	0.21	0.946
Pure error	8.09	5	1.61		
Total	98.44	19			

Value of "p" less than 0.05 indicated the term was significant, less than 0.01 highly significant, more than 0.05 non-significant (insignificant).

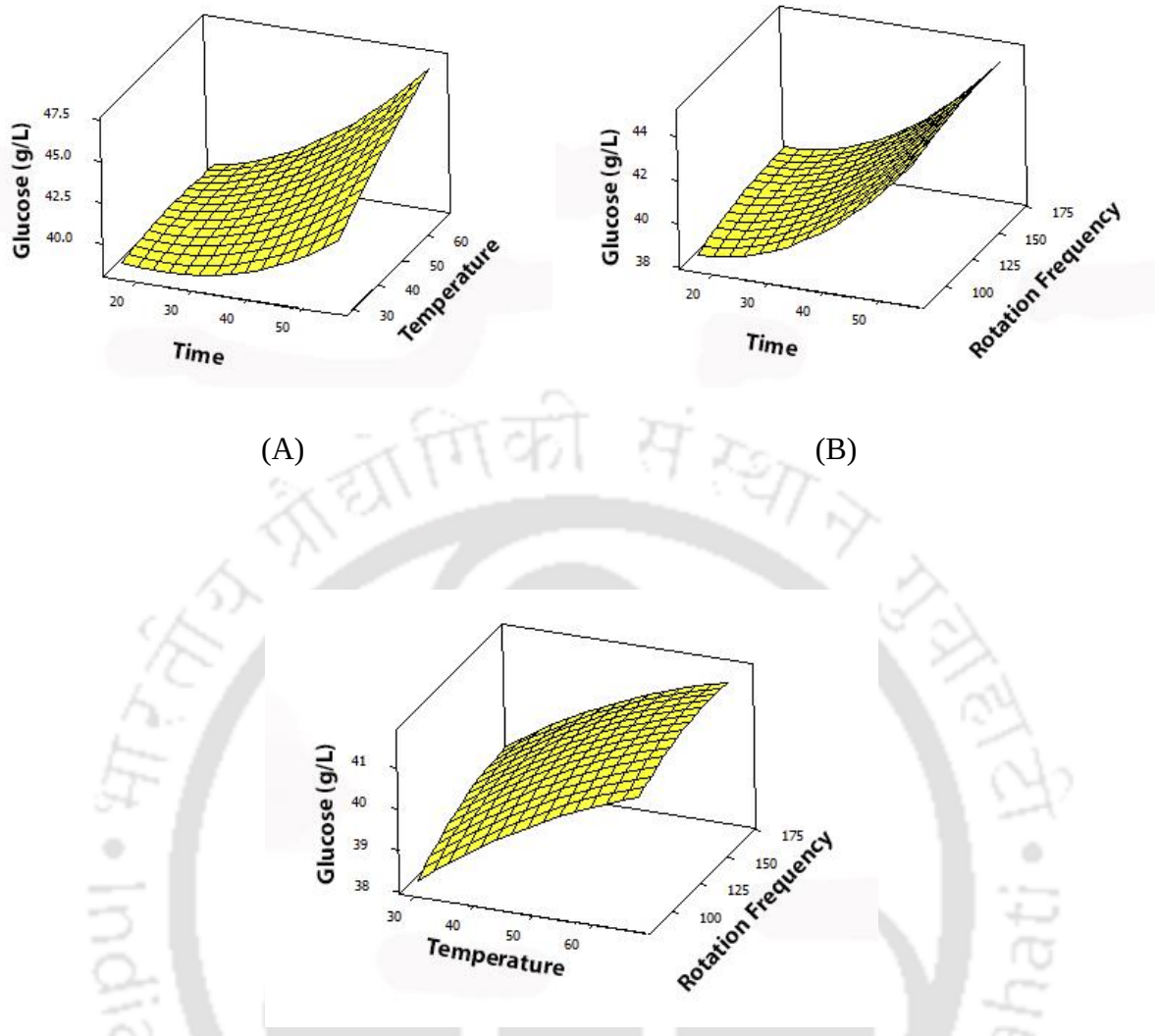


Figure 3.6. 3-D response surface plot by the model which represents glucose concentration of potato peel as a function of (A) Time (h) and Temperature (^{o}C), (B) Time (h) and Rotation frequency (RPM), (C) Temperature (^{o}C) and Rotation frequency (RPM).

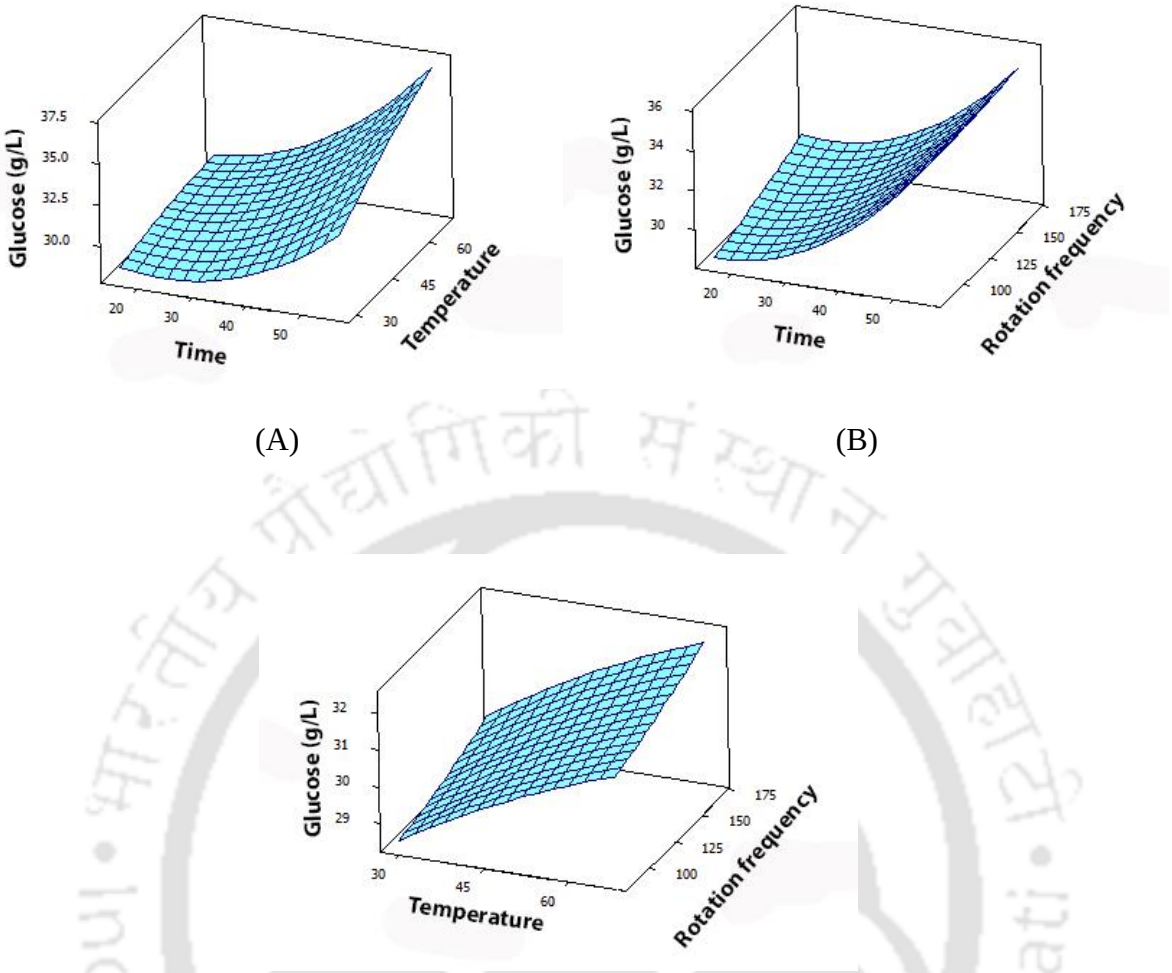
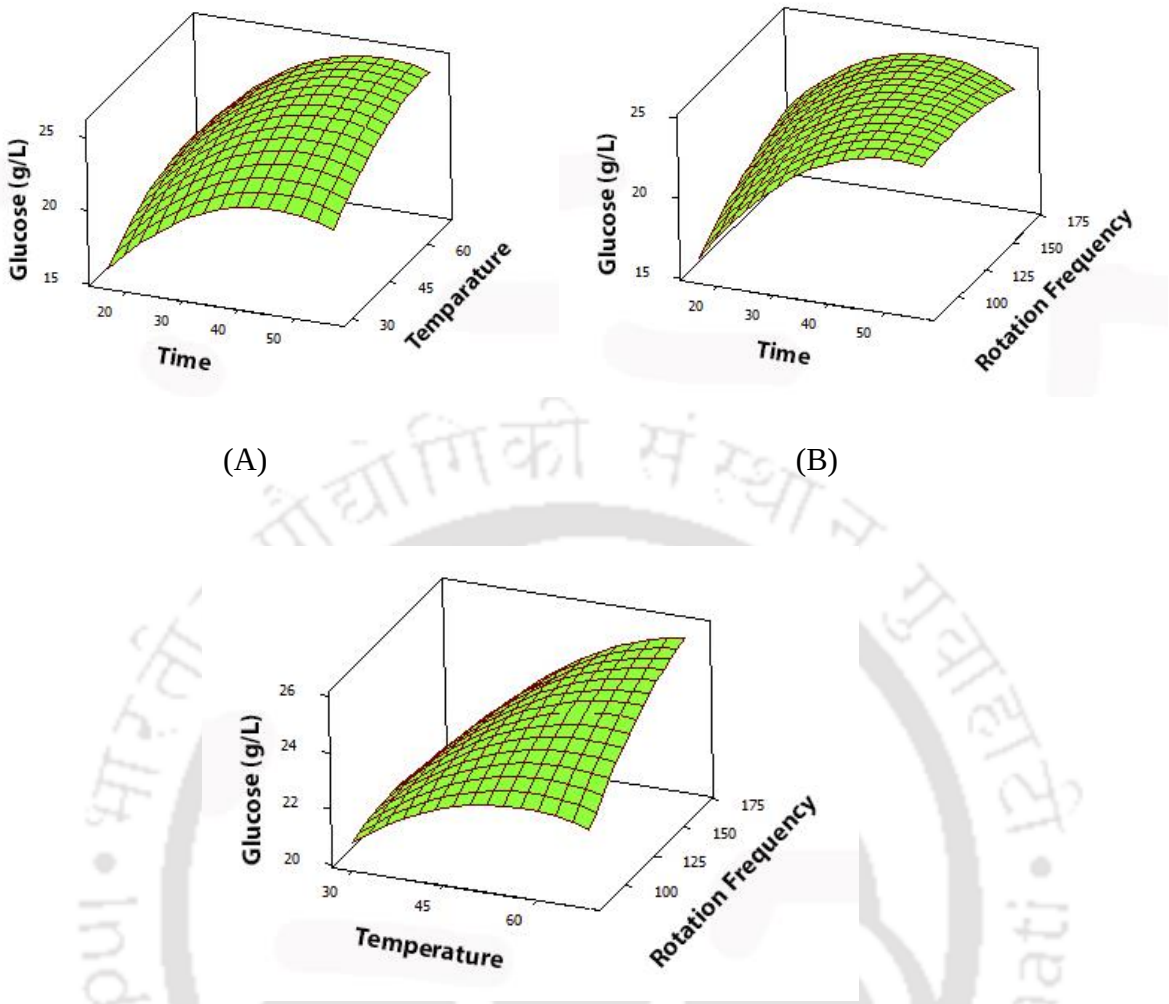


Figure 3.7. 3-D response surface plot by the model which represents glucose concentration of sweet lime peel as a function of (A) Time (h) and Temperature (^{o}C), (B) Time (h) and Rotation frequency (RPM), (C) Temperature (^{o}C) and Rotation frequency (RPM).



(A) (B)

(C)

Figure 3.8. 3-D response surface plot by the model which represents glucose concentration of banana peel as a function of (A) Time (h) and Temperature (°C), (B) Time (h) and Rotation frequency (RPM), (C) Temperature (°C) and Rotation frequency (RPM).

3.3.4. Verification of optimum conditions

Graphical verification of optimum conditions was carried out by using the 'Minitab' program. The optimal working condition to obtain maximum levels of glucose yield was defined using the optimization plot in response surface. The optimum conditions in case of potato peel were found to be 56 h (time), 68^{o}C (temperature) and 144 RPM (rotation frequency of the incubator) while in the case of sweet lime peel, the optimum conditions were 56 h (time), 68^{o}C (temperature) and 167 RPM (agitation speed or rotation frequency of the incubator shaker). 68 °C (temperature), 56 h (time) and 167 RPM (agitation speed or rotation frequency of the incubator shaker) are found to be the optimal working conditions in the case of banana peel. For the confirmation of these results, hydrolysis tests were conducted experimentally under the optimized conditions and glucose yield of 46.17±0.77 g L^{-1}, 29.84±0.57 g L^{-1} and 35.90±0.43 g L^{-1} for potato, banana and sweet lime peel were obtained respectively, which were closer to the predicted values. The obtained results showed that the model fitted well with the experimental data.

The highest glucose yields i.e., 45.38 g L^{-1} in case of potato peel and 35.25 g L^{-1}in case of sweet lime peel were obtained by working with 55.6 h (time), 48.5^{o}C (temperature)and 125 RPM (rotation frequency of the incubator). However, the optimal conditions predicted by Minitab v. 16 software were as follows: 56 h, 68°C and 144 RPM in case of potato peel and 56 h, 68°C and 167 RPM in the case of sweet lime peel. The predicted optimal glucose concentration values were 47.15 g L^{-1} and 37.62 g L^{-1} for potato and sweet lime peel respectively. In case of banana peels, the maximal glucose concentration 26.91 g L^{-1} was attained when tests were performed with 48.5 °C (temperature), 55.6 h (time) and 125 RPM (agitation speed or rotation frequency of the incubator). However, the predicted optimum parameters through Minitab v.16 were 68 °C, 56 h and 167 RPM and the predicted optimum glucose yield was 31.57 g L^{-1}. The validity of the predicted results was verified by the hydrolysis test performed under the optimum parameters. The experiments were performed in triplicate. The mean glucose concentration was 46.17±0.77 g L^{-1}, 29.84±0.57 g L^{-1} and 35.90±0.43 g L^{-1} for potato, banana and sweet lime peels respectively, which were in accordance with the predicted data. A significant correlation between the predicted and experimental data is observed.

3.3.5. Glucose yield from the biomass

The agro and forest residues have been conventionally used as an alternate cheap source of lignocellulosic biomass for biofuels. However, the food waste has not been explored much. Rossgard et al., (2007) reported a glucose yield of 32-39 g L^{-1} in case of acid hydrolysis and steam explosion followed by enzyme hydrolysis of barley straw. Akponah and Akpomie (2011) obtained a glucose yield of 250 mg g^{-1} from acid hydrolysate of cassava root peel and 210.4 mg g^{-1} and 110.4 mg g^{-1} respectively in amylolytic fungi and enzyme hydrolysate of the same. Nweke and Cynthia (2014) studied the effect of variation of enzyme concentration upon cassava peel and found varied amount of glucose yield such as: 3.76 mg mL^{-1}, 4.00 mg mL^{-1} and 4.25 mg mL^{-1}. A comparison of the results of this study with different agricultural residues as reported in literature is given in Table 3.13. The pre-treatment methods, enzymatic hydrolysis and the glucose released after that are compared. It could be seen that the glucose released from potato, banana and sweet lime peels were comparatively higher than most of the other biomass.

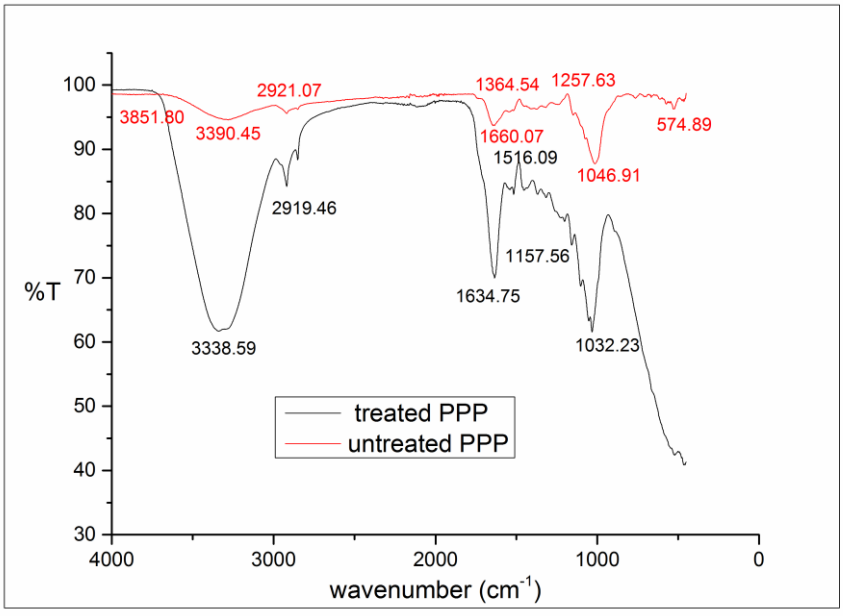
Table 3.13. Comparative assessment of various substrates in terms of glucose released.

Author	Substrate	Pre-treatment	Glucose concentration ($g\ L^{-1}$)
This study	Potato peel	Dilute Acid hydrolysis in autoclaving conditions followed by enzymatic hydrolysis	46.17±0.77
This study	Sweet lime peel	Dilute Acid hydrolysis in autoclaving conditions followed by enzymatic hydrolysis	35.90±0.43
This study	Banana peel	Autoclaving followed by enzymatic hydrolysis	29.84±0.57
Bhatia and Johri (2012)	*Citrus sinensus* peel	NaOH treatment followed by enzyme hydrolysis	40.51±0.42
Bayitse et al (2015)	Cassava peel	Enzyme hydrolysis	14.67
Sininart and Abdullateef (2012)	Banana peel	Alkali pre-treatment followed by acid hydrolysis	4.29
Elechi et al (2016)	Cassava peel	Acid hydrolysis	2.499
Liu et al (2010)	Wheat bran	Dilute acid treatment	21.3
Qureshi et al (2008a)	Corn fibre	a) Dilute acid treatment b) Dilute acid treatment followed by enzyme hydrolysis	4.3 22.4
Qureshi et al (2008b)	Wheat straw	a) Dilute acid treatment b) Dilute acid treatment followed by enzyme hydrolysis	17.8 19.1

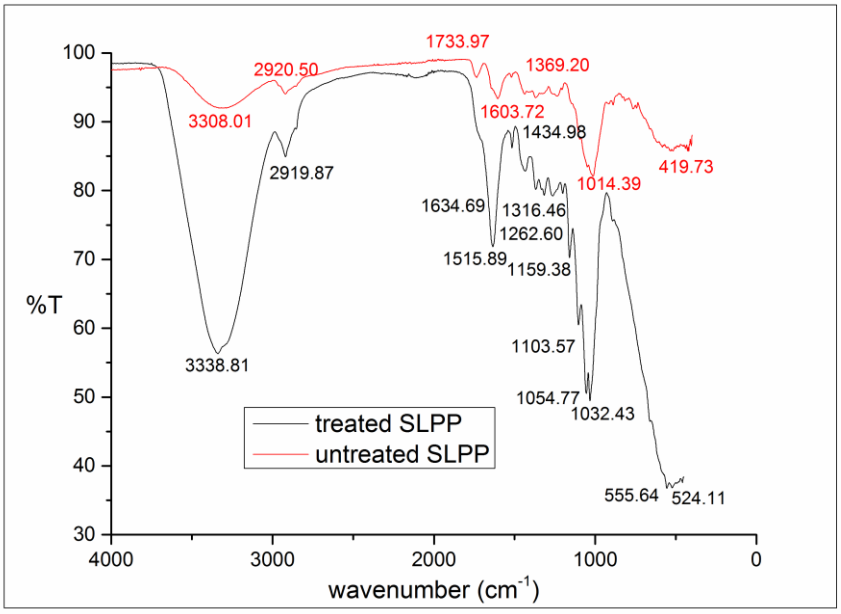
3.3.6. Fourier-transform infrared spectroscopy analysis (FTIR) analysis

FTIR studies were carried out to examine the alterations in chemical properties of the biomass potato peel powder (PPP), banana peel powder (BPP) and sweet lime peel powder (SLPP) before and after pre-treatment. FTIR spectra of treated and untreated potato peel, banana peel and sweet lime peel are presented in Figure 3.9. In the FTIR spectra, notable peaks were noticed depicting the presence of lignin, cellulose and hemicelluloses which are interlinked via hydrogen bonding. The covalent bonds bind lignin to cellulose and hemicelluloses, establishing lignin–carbohydrate networks. FTIR spectra of both untreated and treated samples demonstrated peaks at some similar areas, however the treated sample illustrated broader peaks and a drop in the peak intensity which can be attributed to the efficient removal of the different biomass components. The peak at 3851.80 cm^{-1} in case of untreated PPP corresponded to free and intermolecular OH stretch. OH stretching indicates rupture of cellulose hydrogen bonds. Band positions at 3390.45 cm^{-1} (untreated PPP) 3338.59 cm^{-1} (treated PPP), 3308.01 cm^{-1} (untreated SLPP) and 3338.81 cm^{-1} (treated SLPP) corresponded to hydrogen bonded OH stretching of cellulose. Peaks at 2921.07 cm^{-1} (untreated PPP), 2919.46 cm^{-1} (treated PPP), 2920.50 cm^{-1} (untreated SLPP) and 2919.87 cm^{-1} (treated SLPP) indicated C-H stretching of methyl and methylene groups of cellulose. C–H stretching indicates rupture of methyl and methylene groups of cellulose. Band positions at 1733.97 cm^{-1} (untreated SLPP) were related to the C=O stretch of hemicelluloses. Peaks at 1660.07 cm^{-1} (untreated PPP), 1634.75 cm^{-1} (treated PPP), 1603.72 cm^{-1} (untreated SLPP) and 1634.69 cm^{-1} (treated SLPP) represented C=O stretching vibration due to carbohydrate linked with lignin. Peaks at 1516.09 cm^{-1} (treated PPP) and 1515.89 cm^{-1} (treated SLPP), which appeared only in case of the treated samples were related to C=C stretching of the aromatic benzene rings of lignin. This band corresponds to the reduction in the lignin content of the treated sample. Peak at 1434.98 cm^{-1} (treated SLPP) is assigned to cellulose band. The peak at 1364.54 cm^{-1} (untreated PPP) 1369.20 cm^{-1} (untreated SLPP), 1316.46 cm^{-1} (treated SLPP) is assigned to hemicellulose band. Peaks at 1257.63 cm^{-1} (untreated PPP) and 1262.60 cm^{-1} (treated SLPP) corresponded to C-O stretching in lignin. Band positions at 1157.56 cm^{-1} (treated PPP), 1159.38 cm^{-1} (treated SLPP) and 1103.57 cm^{-1} (treated SLPP) were related to C-O-C ring vibrational stretching of cellulose. Peaks at 1046.91 cm^{-1} (untreated PPP), 1032.23 cm^{-1} (treated PPP), 1014.39 cm^{-1} (untreated SLPP), 1054.77 cm^{-1} (treated SLPP) and 1032.44 cm^{-1} (treated SLPP) represented C-O, C-C and C-OH stretching vibrations of the cellulose, hemicelluloses and lignin, indicating the presence of cellulose, hemicelluloses and lignin in the two feedstocks. Band positions at 574.89

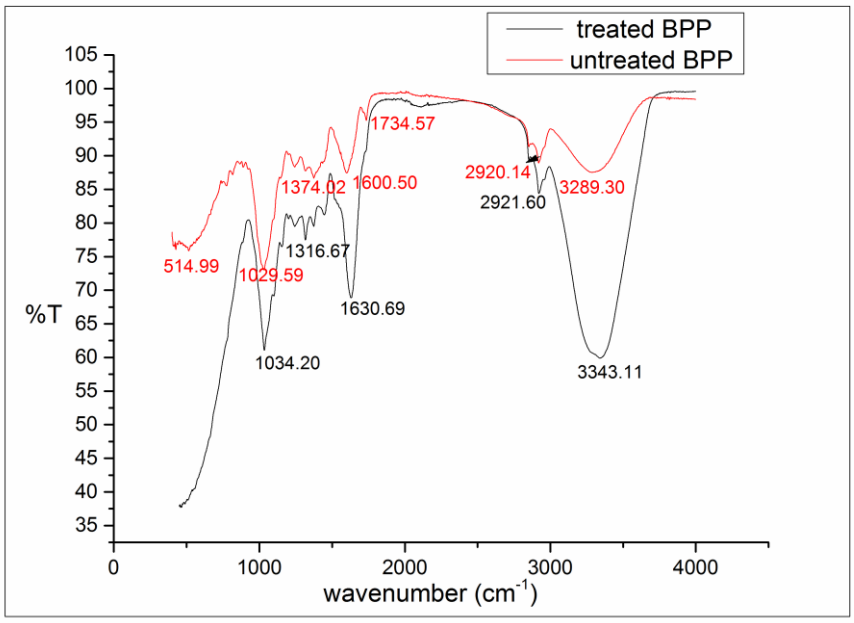
cm^{-1} (untreated PPP), 419.73 cm^{-1} (untreated SLPP), 555.64 cm^{-1} (treated SLPP), 524.11 cm^{-1} (treated SLPP) indicated of C-CO-C- bend in ketones. In case of BPP, Peaks at 3343.11 (treated) and 3289.30 (non-treated) corresponded to hydrogen bonded OH stretching of cellulose. Band positions at 2921.60 (treated) and 2920.14 (non-treated) represented C–H stretching of methylene and methyl groups of cellulose. The peak at 1734.57 (non-treated) indicated C=O stretch of hemicelluloses. Band positions at 1630.69 (treated) and 1600.50 (non-treated) was related to the C=O vibration due to carbohydrate-lignin linkage. Band positions at 1316.67 (treated) and 1374.02 (non-treated) represented hemicellulose band. Band positions at 1034.20 (treated) and 1029.59 (non-treated) indicated C-O, C-OH, C-C stretching vibrations of the hemicelluloses, cellulose and lignin, recommending the hemicellulose, cellulose and lignin presence in the peels. Peak at 514.99 (non-treated) is assigned to C-CO-C- band in ketones. The decreased peak intensity and broader peaks implied delignification and lower cellulose. Change in the peak intensity of FTIR spectra depicts changes in the composition of the samples while the broad peak indicated the presence of weaker intra and intermolecular hydrogen bonding and lesser crystallinity (Kuhad et al., 2010; Kumar et al., 2009b; Peng et al., 2015; Sarma and Mohanty, 2018). The possible range of IR-wavelength (cm^{-1}) with respect to presence of functional group from FTIR spectra of untreated and treated peel powder is depicted in Table 3.14.



(A)



(B)



(C)

Figure 3.9. FTIR spectra of treated and untreated (A) potato peel powder (PPP) (B) sweet lime peel powder (SLPP) (C) banana peel powder (BPP).

Table 3.14. Possible range of IR-wavelength (cm^{-1}) with respect to presence of functional group from FTIR spectra of untreated and treated peel powder.

IR Range	Functional group	Functional Moiety	Untreated PPP	Treated PPP	Untreated BPP	Treated BPP	Untreated SLPP	Treated SLPP
3700-3000	Free and intermolecular OH	OH stretch	3851.80	-	-	-	-	-
3520-3320	NH_2 in aromatic amines	NH stretch	3390.45	3338.59	-	3343.11	-	3338.81
3420-3250	OH in alcohols and phenols	OH stretch	-	-	3289.30	-	3308.01	-
2990-2850	CH_3 and $–CH_3$ in aliphatic compound	CH antisym and asym stretch	2921.07	2919.46	2920.14	2921.60	2920.50	2919.87
2000-1650	Substituted benzene ring	Several bands from overtone and combination	-	-	1734.57	-	1733.97	-
1680-1620	C=O and NH_2 in primary amides	C=O stretch	1660.07	1634.75	1600.50	1630.69	1603.72	1634.69
1515-1485	Benzene ring in aromatic compounds	Ring stretch	-	1516.09	-	-	-	1515.89
1400-1310	COO- group in carboxylic acid	Antisym stretch	1364.07	-	1374.02	1316.67	1369.20	1434.98 1316.46
1260-1150	Hydrated sulphonic acid	SO_3 stretch	1257.63	1157.56	-	-	-	1262.60 1159.38 1103.57

1060-1025	CH2-OH primary alcohols	in	C-O stretch	1046.91	1032.23	1029.59	1034.20	1014.39	1054.77 1032.44
630-535	C-CO-C ketones	in	C-CO-C-bend	574.89	-	514.99	-	419.73	555.64 524.11

3.4. Conclusion

Continuously escalating energy demand and depletion of conventional fossil fuels has challenged and urged our globe to search for alternative resources which are economically viable, renewable and sustainable. Potato, banana and sweet lime peels, otherwise considered waste, were used as substrates for producing glucose. Different pre-treatment processes were carried out and the best conditions yielding higher amount of glucose concentration were further hydrolyzed by enzyme. After studying the various types of pre-treatment procedures, it was found that in case of potato and sweet lime peels, dilute acid hydrolysis with autoclaving conditions at 80 min gave the highest glucose yield amongst all and hence was considered for further studies. The best conditions (40 min, autoclaving) furnishing the higher glucose concentration in case of banana peels was chosen for further investigation. Rationale for the enzyme hydrolysis of the pre-treated peels was created through central composite design (CCD) using response surface methodology (RSM) to attain a higher concentration of glucose to optimize the hydrolysis parameters. Temperature (°C), Time (h) and the rotation frequency or agitation speed (revolutions per minute, RPM) of the incubator were the three chosen parameters. Under the selected conditions of enzymatic hydrolysis, potato peel, banana peel and sweet lime peel promised to be good source of glucose. It was found that the enzyme treatment time, enzyme treatment temperature and rotation frequency of the incubator had a notable effect on the enzymatic hydrolysis of the pre-treated biomass. A statistical model was developed to predict the pre-treatment process for this study. As demonstrated in this study, 46.17±0.77 g L^{-1} and 35.90±0.43 g L^{-1} of glucose could be attained under the optimum conditions for potato peel and sweet lime peel respectively. 29.84±0.57 g L^{-1} glucose was obtained from the banana peels. The experimental results further confirmed the optimum factors acquired from the statistical model.

The study showed that the duration of time plays the major role during hydrolysis of these biomasses followed by temperature and rotation frequency of the incubator. Comparing the analysis of this study with other lignocellulosic biomass, it can be concluded that these three renewable biomasses have great potential for their conversion into biofuel in future by using appropriate microorganisms.

References

Agbor VB, Cicek N, Sparling R, Berlin A, Levin DB (2011) Biomass pretreatment: fundamentals toward application, Biotechnol Adv 29:675–685.

Akponah E, Akpomie OO (2011) Analysis of the suitability of yam, potato and cassava root peel for bioethanol production using *Saccharomyces cerevisae*, Int Res J Microbiol. 2:393-398.

Alvira P, Tomas-Pejo E, Ballesteros M, Negro MJ (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review, Biores Technol 101:4851–4861.

Artemio CP, Maginot NH, Serafin CU, Rahim FP, Guadalupe RQJ, Fermin CM (2018) Physical, mechanical and energy characterization of wood pellets obtained from three common tropical species, PeerJ 6:5504.

Bayitse R, Hou X, Bjerre AB, Saalia FK (2015) Optimisation of enzymatic hydrolysis of cassava peel to produce fermentable sugars, AMB Express 5.

Bhatia L, Johri S (2014) Fourier Transform Infrared Mapping of Peels of *Citrus sinensis* var mosambi after Physiochemical Pretreatment and its SSF for Ethanol Production by Saccharomyces cerevisiae MTCC 3821- An Economic & Ecological Venture, Int J Science & Res 3.

El-Zawawy WK, Ibrahim MM, Abdel-Fattah YR, Soliman NA, Mahmoud MM (2011) Acid and enzyme hydrolysis to convert pretreated lignocellulosic materials into glucose for ethanol production, Carbohydrate Polymers 84(3):865–871.

Elechi OO, Tagbo NJ, Mary OC, Emmanuel AO (2016) Acid Hydrolysis of Cassava peel, Int J Sci Technol Res 5.

Kim JK, Um BH, Kim TH (2011) Bioethanol production from micro-algae, *Schizocytrium* sp., using hydrothermal treatment and biological conversion, Korean J Chem Eng 29:209-214.

Kuhad RC, Gupta R, Khasa YP, Singh A (2010) Bioethanol production from *Lantana camara* (red sage): Pretreatment, saccharification and fermentation, Biores. Technol. 101(21):8348–8354.

Kumar P, Barrett DM, Delwiche MJ, Stroeve P (2009a) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production, Ind Eng Chem Res. 48:3713–3729.

Kumar R, Mago G, Balan V, Wymand CE (2009b) Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies, Biores Technol 100:3948–3962.

Kumar AK, Sharma S (2017) Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review, Biores Bioprocess 4:7.

Laureano-Perez L, Teymouri F, Alizadeh H, Dale BE (2005) Understanding Factors That Limit Enzymatic Hydrolysis of Biomass Characterization of Pretreated Corn Stover, Applied Biochem Biotechnol 121–124:1081-1099.

Lemons e Silva CF, Schirmer MA, Maeda RN, Barcelos CA, Pereira Jr. N (2015) Potential of giant reed (*Arundo Donax* L.) for second-generation ethanol production, Electronic J Biotechnol 18:10-15.

Liu Z, Yu Y, Fuli L, Cuiqing M, Ping X (2010) Butanol production by *Clostridium beijerinckii* ATCC 55025 from wheat bran, J Ind Microbiol Biotechnol 37:495–501.

Maurya DP, Singla A, Negi S (2015) An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol, 3 Biotech. 5:597–609.

Michelin M, Teixeira JA (2016) Liquid hot water pretreatment of multi feedstocks and enzymatic hydrolysis of solids obtained thereof, Biores Technol 216:862–869.

Mohapatra D, Mishra S, Sutar N (2010) Banana and its by-product utilization: an overview, J Sci Ind Res 69:323-329.

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass, Biores Technol 96:673–686.

National renewable energy laboratory (NREL) 2008.

Nweke FN, Abiamere OC (2014) Glucose syrup production from cassava peels and cassava pulp, Int J Current Microbiol Appl Sciences 3:781-787.

Oberoi HS, Sandhu SK, Praveen VV (2012) Statistical optimization of hydrolysis process for banana peels using cellulolytic and pectinolytic enzymes, Food & bioproducts processing 90:257–265.

Pandey A, Srivastava S, Kumar S (2019) Isolation, screening and comprehensive characterization of candidate microalgae for biofuel feedstock production and dairy effluent treatment: A sustainable approach, Bioresource Technology 293:121998.

Peng W, Wang L, Ohkoshi M, Zhang M (2015) Separation of Hemicelluloses from *Eucalyptus* Species: Investigating the residue after Alkaline Treatment, Cellulose Chemistry and Technology 49(9):757-764.

Qureshi N, Ezeji TC, Ebener J, Dien BS, Cotta MA, Blaschek HP (2008a) Butanol production by *Clostridium beijerinckii*. Part I: Use of acid and enzyme hydrolyzed corn fiber, Biores Technol 99:5915–5922.

Qureshi N, Saha BC, Cotta MA (2008b) Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii*. Part I: Fed batch fermentation, Biomass Bioenergy 32:168–175.

Roberto IC, Mussatto SI, Rodrigues RCLB (2003) Dilute-acid hydrolysis for optimization of xylose recovery from rice straw in a semi-pilot reactor, Ind Crop Prod 17:171-176.

Rosgaard L, Pedersen S, Anne S (2007) Comparison of Different Pretreatment Strategies for Enzymatic Hydrolysis of Wheat and Barley Straw, Appl Biochem Biotechnol 143:284–296.

Sadiq IM, Dalai S, Chandrasekaran N, Mukherjee A (2011) Ecotoxicity study of titania (TiO2) NPs on two microalgae species: *Scenedesmus* sp. and *Chlorella* sp., Ecotoxicol Environ Saf 74:1180–1187.

Sarma PJ, Mohanty K (2018) *Epipremnum aureum* and *Dracaena braunii* as indoor plants for enhanced bio-electricity generation in a plant microbial fuel cell with electrochemically modified carbon fiber brush anode, Journal of bioscience and bioengineering 126(3):404-410.

Sasmal S, Goud VV, Mohanty K (2013) Delignification kinetics of lime pre-treatment- an ineluctable tread for augmenting saccharification, J Biobased Mater Bioenergy 7:660-664.

Schieber A, Saldana MDA (2009) Potato peels: A source of nutritionally and pharmacologically interesting compounds – A review, Food 3:23-29.

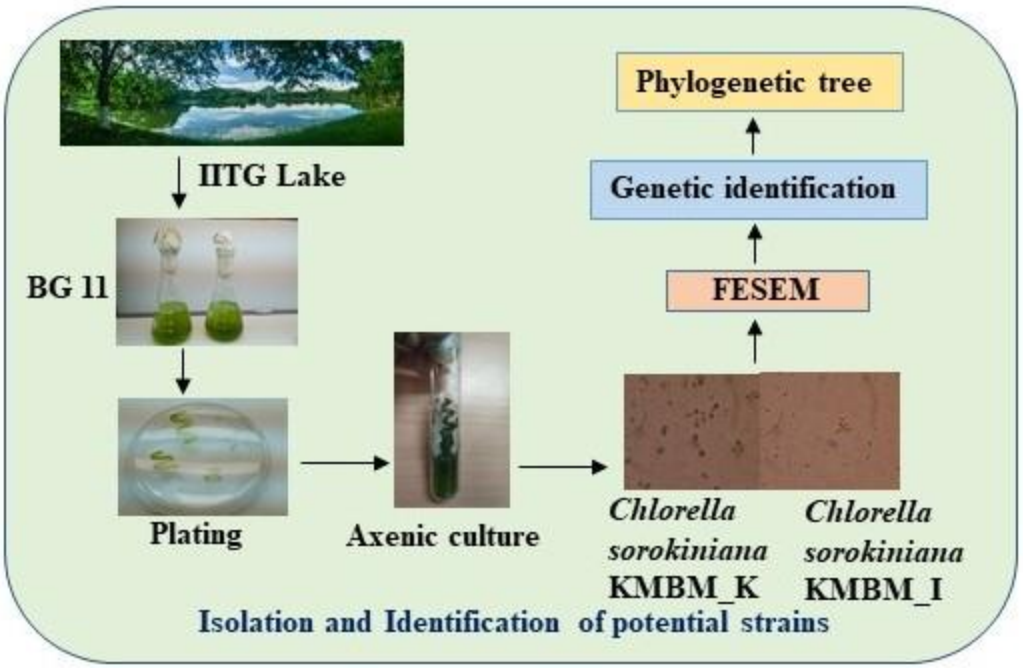
Selvakumar P, Kavitha S, Sivashanmugam P (2019) Optimization of Process Parameters for Efficient Bioconversion of Thermo-chemo Pretreated *Manihot esculenta* Crantz YTP1 Stem to Ethanol, Waste and Biomass Valorization 10:2177–2191.

Sininart C, Abdullateef D (2012) Alkali-pretreatment and acid-hydrolysis of banana peels. The 10th International PSU Engineering Conference.

Tao Y, Xiekun L, Shiyuan X, Ying G, Weizheng Z, Jingliang X, Zhenhong Y (2016) Microalgae pretreatment with liquid hot water to enhance enzymatic hydrolysis efficiency, Biores Technol 220:530–536.

CHAPTER 4

ISOLATION AND IDENTIFICATION OF NOVEL INDIGENOUS MICROALGAL SPECIES



4.1. Overview

This present work aimed to obtain novel indigenous microalgae from the enormous biodiversity rich region of IIT Guwahati campus. The strains were identified through sequencing and submitted to NCBI. The microalgal strains were then tested for its growth kinetics, biomass and lipid productivity.

4.2. Materials and Methods

4.2.1. Sampling, Isolation and maintenance of the microalgal strains

Microalgal samples were collected in 50 mL centrifuge tubes from two different water bodies located in IIT Guwahati, Assam, India. For isolation of microalgae, 5 mL of the collected samples were initially inoculated into 500 mL of BG11 medium in 1000 mL Erlenmeyer flasks. 1.5% agar was added to the BG11 medium for preparation of the solid media required for isolating the axenic cultures through the plating and slant method. Then the single colonies were again sub-cultured in the BG11 medium in 250 mL conical flasks. All the culture medium was sterilized in autoclave at 121 ºC for 20 min. Fresh BG11 medium was used to revive and maintain the microalgae at regular intervals.

The BG11 nutrient medium comprises of 0.075 g L^{-1} magnesium sulfate ($MgSO_4.7H_2O$), 0.036 g L^{-1} calcium chloride ($CaCl_2.2H_2O$), 0.006 gL^{-1} citric acid ($C_6H_8O_7$), 0.006 g L^{-1} ferric ammonium citrate (C_6H_5 + 4yFexNyO_7), 0.006 g L^{-1} EDTA ($C_{10}H_{14}N_2Na_2O_8.2H_2O$), 0.02 g L^{-1} sodium carbonate (Na_2CO_3), 1.5 g L^{-1} sodium nitrate ($NaNO_3$), 0.04 gL^{-1} dipotassium hydrogen phosphate (K_2HPO_4), and 1 mL L^{-1} trace element mix. The trace element mixture contains 2.86 g L^{-1} boric acid (H_3BO_3), 1.81 g L^{-1} manganese chloride ($MnCl_2.4H_2O$), 0.222 g L^{-1} zinc sulfate ($ZnSO_4.7H_2O$), 0.39 gL^{-1} sodium molybdate ($Na_2MoO_4.2H_2O$), 0.079 g L^{-1} copper sulfate ($CuSO_4.5H_2O$) and 0.0494 g L^{-1} cobalt nitrate ($Co(NO_3)_2.6H_2O$).

The isolates were maintained at 25 ± 2 °C by artificial illumination with light intensity of approximately 5000 lux, light: dark period of 16:08 h using white fluorescent tubes, incubated at 150 RPM in a shaker incubator (Table 4.1). To maintain the culture medium's pH, external CO_2 was sparged briefly at regular intervals. The chemicals and CO_2 cylinder (99.99% purity) needed for this study were procured respectively from Hi-Media® and Assam Air Products Pvt. Ltd.

Table 4.1. Microalgal culture conditions.

Parameters	Conditions
Inoculum	10% (v/v)
Temperature	25 ± 2 ^{o}C
Light intensity	5000 lux (approx.)
Photoperiod (light: dark hours)	16:08

4.2.2. Morphological and taxonomic identification

Purity check and preliminary identification of the isolates were done using a light microscope (Olympus CX41, Japan) equipped with digital camera (Olympus E330, Japan), upon which the axenic cultures were sent for rRNA gene sequencing. The data obtained from the sequencing results were submitted to NCBI (National Center for Biotechnology) GenBank database (http://www.ncbi.nim.nih.gov) and the accession numbers for both the isolated strains were obtained.

The genetic identification of the isolates was conducted by Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka using 18S ribosomal RNA (rRNA) gene sequencing technique. ITS1 (5′-TCCGTAGG TGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATT GATATGC-3′) are used as the forward and reverse primers respectively for the partial gene sequencing. To identify the closest relative species of these strains, the generated nucleotide sequences were subjected to BLAST (Basic local alignment search tool) search in NCBI databank. Further, construction of the phylogenetic tree was done by Maximum Likelihood method using MEGA 7 (Molecular Evolutionary Genetics Analysis) software.

The surface morphology and structure were examined using FESEM (Field Emission Scanning Electron Microscope) images (Zeiss, sigma 300). For the FESEM analysis, the microalgal cells were prepared by first washing with phosphate buffer saline (PBS). The cells were then fixed with 4% (w/v) glutaraldehyde for 24 h, followed by rinsing with distilled water

and dehydrating the cells for three minutes gradually with 30%, 50%, 75%, 85%, 95% and 100% ethanol. Finally, the cells were rinsed with acetone and vacuum dried overnight. The dried cells were eventually coated with gold particles and loaded for FESEM analysis (Sadiq et al., 2011).

4.2.3. Evaluation of cell growth

For the determination of the cell density of the culture, spectrophotometric method of growth evaluation was followed by measuring the optical density (OD) at wavelength 680 nm in an UV-vis spectrophotometer (Thermo Fisher Scientific, USA) at regular intervals during the entire batch. Once the stationary phase is reached, the cultures were harvested by centrifugation (Harmle Z300, Hettich, Germany) for 15 min at 6000 RPM. The pellet is then oven dried at 70 ºC till a constant weight is obtained. Standard curves were used for estimation of dry cell weight (DCW) for the two strains. The biomass concentration for both strains was determined by properly calibrating the absorbance values at OD_{680} with respect to the dry cell weight (DCW).

The linear relationship used is:

Biomass concentration = conversion factor x OD_{680} x dilution factor (if any) (1)

4.2.4. Lipid extraction

The modified Bligh and Dyer (1959) method was used for lipid extraction. The cells were harvested through centrifugation, dried and powdered. Extraction was done through ultrasonication using chloroform and methanol at the ratio of 2:1 (v/v) followed by centrifugation. The pellets were subjected to repeated extractions with the chloroform and methanol mixture. The supernatants were collected together and the solvents were evaporated until constant weight is achieved. The total lipid content (%) was determined gravimetrically.

4.2.5. Determination of growth kinetic parameters, biomass and lipid productivity

The specific growth rate is the estimation of number of generations or doublings that happen per unit of time in a culture with exponential growth. Once the specific growth rate is measured, the generation time or doubling time and divisions per day can be calculated. The cell doubling time is the time needed to attain a doubling of the number of cells.

The specific growth rate (μ, d^{-1}), divisions per day (K, d^{-1}) and the doubling time or generation time of cells (T_D, d^{-1}) are obtained from the following equations, where C_2 and C_1 are the biomass concentration (g L^{-1}) at time t_2 and t_1 respectively.

$$\mu = ln\ (C_2 - C_1)/(t_2 - t_1) \quad (2)$$

$$K = \mu/ln\ 2 = \mu/0.6931 \quad (3)$$

$$T_D = ln\ 2/\mu = 0.6931/\mu \quad (4)$$

The biomass productivity (P_B, mg L^{-1} d^{-1}) and lipid productivity (P_L, mg^{-1} L d^{-1}) was calculated using the following equations,

$$P_B = (Y_1 - Y_0)/(t_1 - t_0) \quad (5)$$

$$P_L = P_B\ X\ L_C \quad (6)$$

where Y_1 and Y_0 are the biomass concentration (g L^{-1}) at cultivation time t_1 and t_0 respectively, and L_C is the lipid content (%).

4.3. Results and Discussion

4.3.1 Morphological identification

Indigenous microbes have innate potential to adapt to a particular ecosystem and are always endorsed to select as model strains to obtain notable results. Microalgae were isolated into pure cultures by repeated plating and microscopic observations. The initial culture of the microalgae is presented in Figure 4.1, while the plating of the microalgal colonies are shown in Figure 4.2 and axenic slant cultures are shown in Figure 4.3. The pure cultures were presented in Figure 4.4. The strains were identified based on its preliminary investigation of morphological characteristics like cell shape and size via optical microscope and it indicated that they might belong to the division Chlorophyta. Initial microscopic identification (Figure 4.5) and FESEM analysis (Figure 4.6) suggested the strains were of genus *Chlorella*. The genus *Chlorella* belongs to the Chlorellaceae family, phylum Chlorophyta, class Trebouxiophyceae and order Chlorellales. The strains showed similar morphological characteristics such as green colored spherical shaped cells. Besides morphological study, genetic identification of the

strains was conducted to attain clarity. The isolated strains turned pale green to eventually dark green during the batch cultivation. *Chlorella* sp. is however reported to exhibit diverse cell morphology and biochemical composition under different environmental conditions.

4.3.2. Molecular identification

The partial 18S rRNA gene sequence coding for the ribosomal RNA of the two strains were sequenced and submitted to GenBank. BLAST analysis in the nucleotide database showed that the strains "KMBM_K" & "KMBM_I" are the closest relative to *Chlorella sorokiniana* with maximum sequence similarity (Figure 4.7). The sequences were submitted in NCBI GenBank database and the Accession Nos. MH244847 and MH244502 were acquired for the isolated strains "KMBM_K" and "KMBM_I" respectively.

The strains will be henceforth mentioned in the Thesis as "K" and "I" respectively for "KMBM_K" & "KMBM_I".

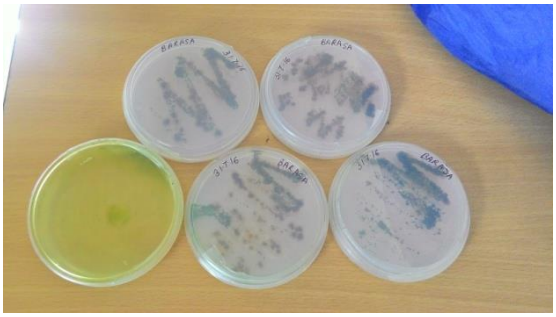


Figure 4.1. Microalgal culture.

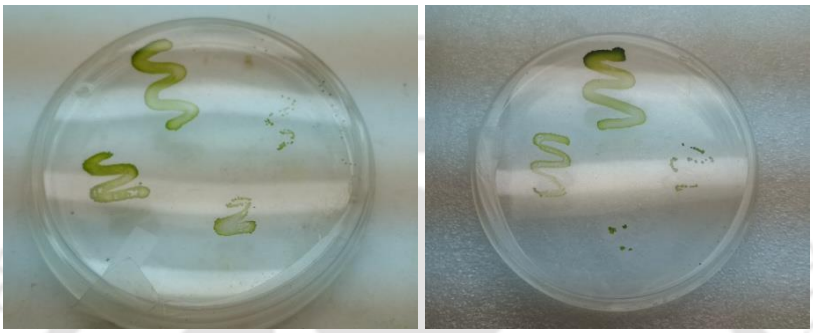
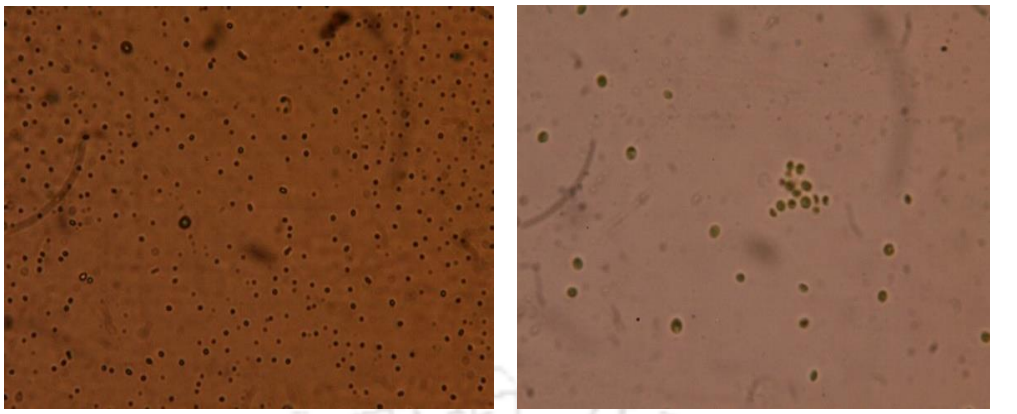


Figure 4.2. Plating of microalgal colonies.

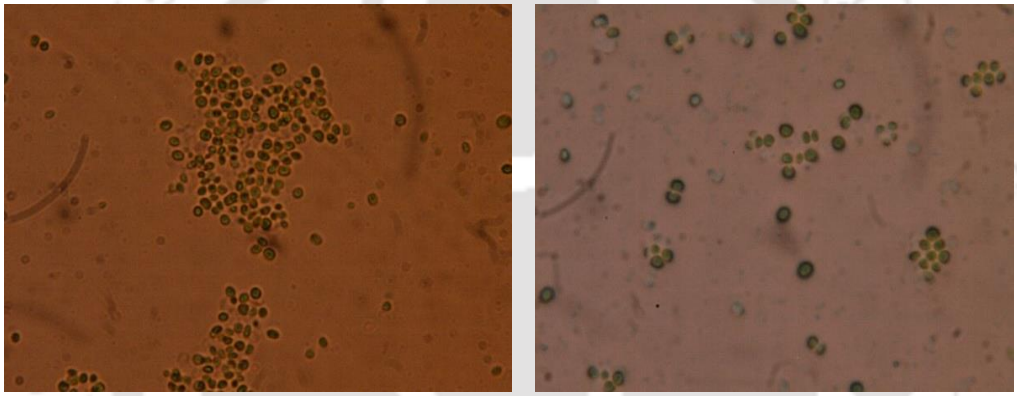
Figure 4.3. Axenic Slant culture of microalgal colonies.

Figure 4.4. Pure cultures of microalga.



40X magnification 100X magnification

(A)



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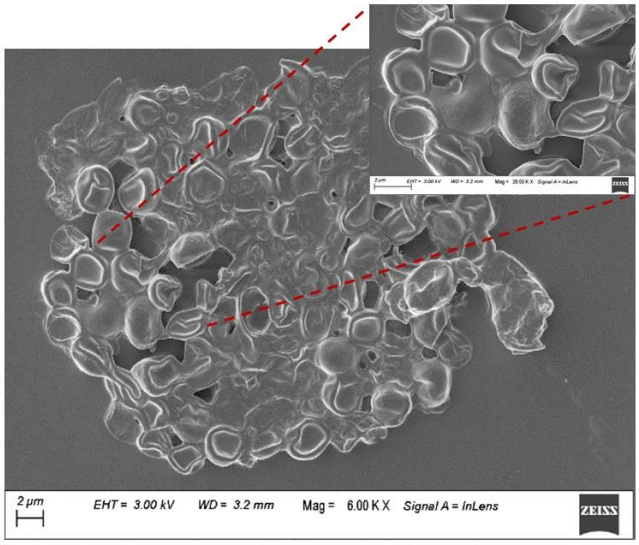
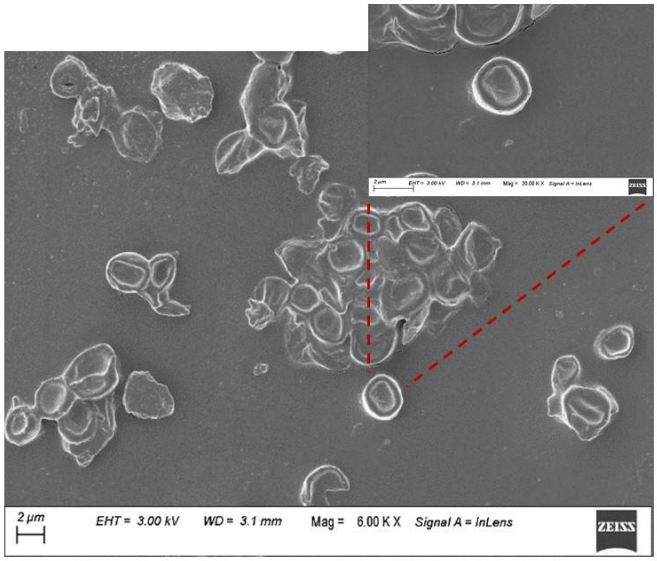
(B)

Figure 4.5. Microscopic observation of the strains "I" (A) and "K" (B).

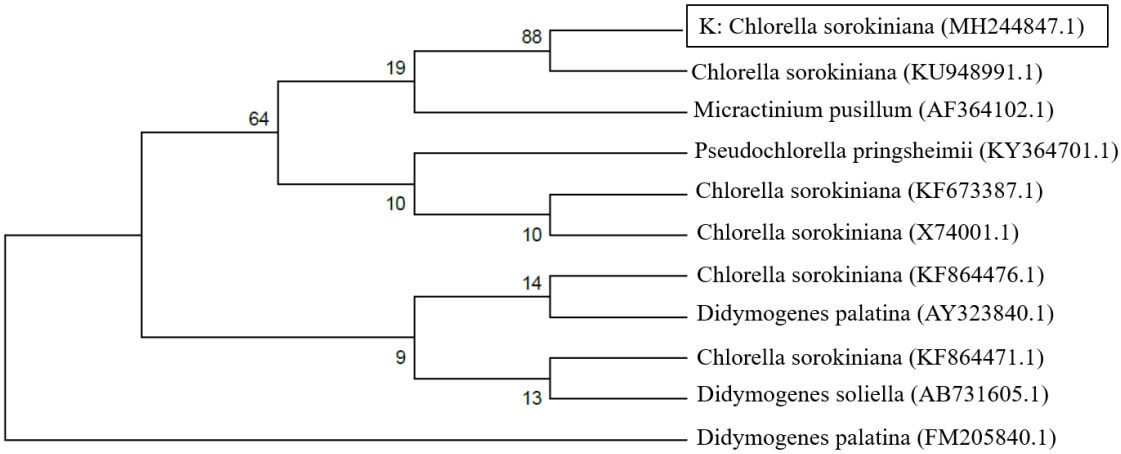


(A)

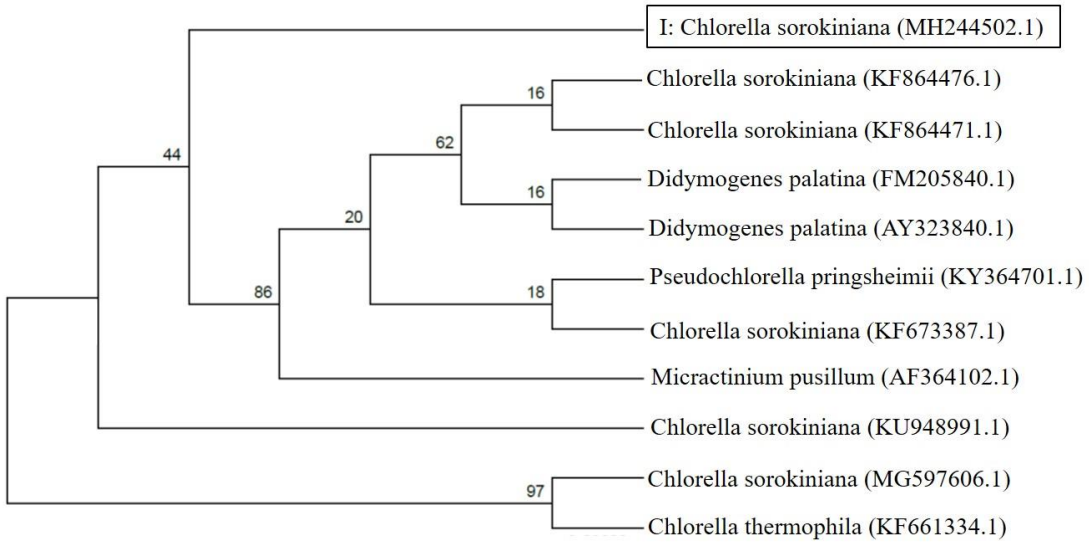


(B)

Figure 4.6. FESEM images of the strains "I" (A) and "K" (B).



(A)



(B)

Figure 4.7. Phylogenetic tree for the isolated strains (A) *Chlorella sorokiniana* KMBM_K (B) *Chlorella sorokiniana* KMBM_I.

4.3.3. Evaluation of the growth kinetics, biomass and lipid productivity of the microalgal strains

The two isolated microalgal strains were cultivated on the BG11 medium to evaluate its growth kinetics, biomass, and lipid productivity. The findings are reported in Table 4.1.

Table 4.2. Growth Kinetics of the isolated strains in BG11 medium.

Strain	Biomass concentration (DCW, g L^{-1})	Lipid content (%)	Biomass productivity (g L^{-1} d^{-1})	Lipid productivity (mg L^{-1} d^{-1})	Specific growth rate (μ, d^{-1})	Divisions per day (K, d^{-1})	Doubling time/rate (T_D, d^{-1})
I	1.59±0.13	21.65±0.51	0.145±.01	31.39±0.56	0.21±0.02	0.30±0.03	3.30±0.27
K	1.65±0.07	21.87±0.38	0.161±0.01	35.21±0.43	0.22±0.01	0.32±0.05	3.15±0.21

4.4. Conclusion

Two indigenous novel microalgal strains were isolated from the water bodies inside the IIT Guwahati, Assam campus. The strains were found to be a good source of biomass and lipid content when grown in BG11 medium. These strains were further cultivated and evaluated on the waste peel hydrolysate medium.

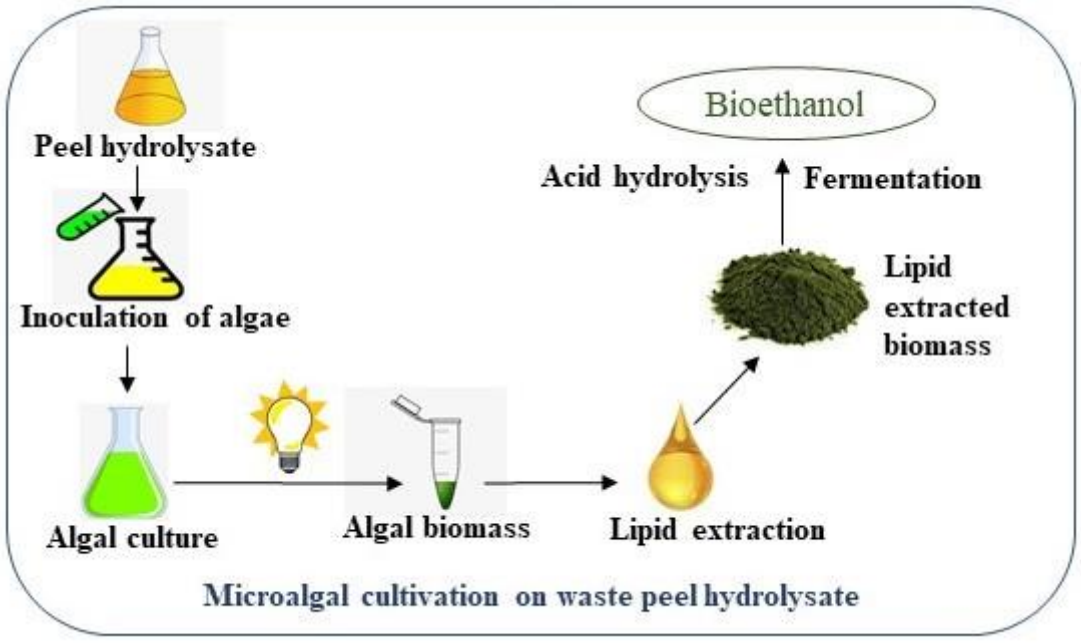
References

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Sadiq IM, Dalai S, Chandrasekaran N, Mukherjee A (2011) Ecotoxicity study of titania (TiO2) NPs on two microalgae species: *Scenedesmus* sp. and *Chlorella* sp. Ecotoxicology and Environmental Safety 74:1180–1187.

CHAPTER 5

UTILIZATION AND EVALUATION OF WASTE PEEL HYDROLYSATE AS A LOW-COST SUBSTRATE FOR MICROALGAL CULTIVATION



5.1. Overview

Microalgal fuels has the budding possibility to aid the sector of renewable energy, although the nutrient and water dependence are seen as crucial issues for mass cultivation. Growing them on a commercial scale seems even more expensive. It has been reported that per year US needs more than 85% of the annual freshwater to create one billion gallons of microalgal biodiesel (Yang et al., 2011a). The continuous usage of fossil fuels has paved its way into one of the most vital world-wide environmental challenges i.e. global warming. The decrease in oil deposits and increase in fuel rates have forced the mankind in search of alternative sustainable energy resources as a substitute for conventional fuels. As the existing fuel reserves are left only for a few more decades, developing alternative fuel resources is the need of the hour.

Biofuels derived from plants and microbial origin, acts as an alternative to fossil derived fuels due to similar properties (Berg and Boland, 2014; Gaurav et al., 2017). However, biofuel sustainability depends on the water supply, land use, demand of nutrients and fertilizer, and competition with food crops. Due to their rapid and sustainable biomass production, faster growth rate, higher photosynthetic efficacy and high oil content, microalgae are considered advantageous than terrestrial plants (Ashraful et al., 2014; Jin et al., 2015; Chisti, 2007). Oleaginous microbes like microalgae, fungi and bacteria has the ability to accumulate lipid (Hashem et al., 2021). However, the commercial production of microalgal biomass has a major limitation because of the high cost involved.

The cost of growth medium is a major issue in the large-scale algal growth and commercial production of microalgal fuels. Substitution of the chemical growth medium with low cost organic biomass feedstock could help in dealing the cost related problems associated with cultivation of microalgae and also in dealing with heaps of agricultural and food waste generated daily. These food and agricultural wastes are found to be high source of nutrients and carbohydrates and hence can be used as the cultivation medium for microalgae to generate various value-added products (Limbu and Sibi, 2017). A review by Slade and Bauen (2013) proposed that a notable reduction in cultivation cost can be attained if nutrients and water can be acquired at economical cost. One such approach for microalgal cultivation is utilization of industrial wastewater or agricultural waste. In their study, Bhatnagar et al. (2010) found that Chlorella minutissima maximizes its growth in municipal sewage water with carbon source addition, compared to its growth with no carbon source addition. Hence the requirement for

cost-intensive carbon sources is of utmost importance. Experiments done by Wong (1985) have shown that food waste such as soy bean waste extract could be utilized for the growth of microalgae. One of the best choices to use as a raw material for microbial biomass and lipid production are food residues, as they cheap and are rich in carbon and nitrogen source. Peel wastes are zero value material, rich in a lot of nutrients that could be utilized as microbial cultivation medium. After some pre-treatment or hydrolysis, these peel wastes act as an effective growth medium for oleaginous microbes (Hashem et al., 2020). Hydrolytic treatment on these wastes alters its physiochemical properties, releasing carbohydrates and mineral nutrients (Banerjee et al., 2017; Hao et al., 2015).

Replacement of a chemical growth medium with cost-effective medium seems to be a way to challenge the high cultivation cost of the microalgae. Organic feedstocks are abundant in carbohydrates, sugars, minerals and nutrients. Waste peels of fruits and vegetables are suitable resources as they have zero industrial value and are discarded into the environment. The properties of these wastes can be altered through pre-treatment or hydrolysis so to release maximum sugars and minerals (Limbu and Sibi, 2017). Recently Park et al. (2014) utilized orange peel waste extract for the mixotrophic cultivation of the microalgae, *Chlorella vulgaris* and found significant higher biomass yield and increase in lipid content. Amit and Ghosh (2019) used kinnow peel for the cultivation of the microalgae *Tetraselmis indica*. *Aurantiochytrium* sp. was found to have significant growth in orange peel hydrolysate (Mathias et al., 2019). Fruits and vegetables peels are an abundant source of organic carbons, amino acids, vitamins and numerous macro and micro nutrients. Globally, India is in second position for producing fruits and vegetables. Peels, seeds and stalks, considered as the waste biomass generated during fruit or vegetable processing, constitute 30%-40% of the total fruit or vegetable weight (Selvakumar et al., 2021).

Selection of a suitable pre-treatment is required to produce an appropriate growth medium for algae cultivation as growth medium could influence the growth rate, biomass and lipid production. These organic waste mediums, abundant in carbohydrates and vital nutrients, can make the cultivation process more economical by reducing the cost.

Sweet lime peels contain 35.25% carbohydrates and 9.7% protein. They contain 121.2 of Ca, 142.1 of K, 37.7 of Na, 19.7 of Mg mg per 100 g. Also, they contain 943.4 of Fe, 72.7 of Cu, 200.3 of Mn, 151.4 of Zn µg per 100 g (Barros et al., 2012). Potato peel contain 68.7% carbohydrates, 1.3% nitrogen (Arapoglou et al., 2010) and 17.1% protein. They contain 24.80

ppm of Cu, 22.80 ppm of Zn, 178 ppm of Fe. Also, they contain 0.041% Na, 0.156% Ca, 3.090% K, 1.80% P (Jekayinfa et al., 2015). The composition of the banana peels is reported as: 68.31 ± 0.83 % carbohydrate content, 7.57 ± 0.30 % protein content (Aboul-Enein et al., 2016), 56.24 ± 0.01 mg/g Calcium, 0.92 ± 0.22 mg/g Iron, 69.05 ± 0.42 mg/g Manganese, 0.02 ± 0.00 mg/g Niobium, 87.35 ± 0.03 mg/g Potassium, 2.51 ± 0.01 mg/g Rubidium, 22.51 ± 0.04 mg/g Sodium, 0.02 ± 0.00 mg/g Strontium, 0.03 ± 0.01 mg/g Zirconium (Dahiru et al., 2018). These nutrients play a vital role in supplying support for the growth of microalgae. Studies have shown that food waste alone or integrated with wastewater could be used for microalgal cultivation (Park et al., 2014).

This study aims to increase biomass production and lipid accumulation in microalgae by utilizing a growth medium made up of waste peel extract, undergoing pre-treatment and enzyme hydrolysis. The effect of potato, banana and sweet lime peels as a nutrient-rich cultivation medium for the cultivation of the two isolated microalgae and its growth kinetics parameters were evaluated in this study. The usage of cheaper and economical cultivation medium is emphasized here. This study highlighted to investigate the integrated microalgal cultivation procedure combining the pre-treatment of waste peels and microalgal growth on the extracted hydrolysate using *Chlorella sorokiniana* (KMBM_I and KMBM_K) strains for biomass production and lipid accumulation, also limiting the cost of cultivation substrate.

5.2. Materials and Methods

5.2.1. Preparation of the waste peel hydrolysate

The waste potato and sweet lime peels were procured from the juice shops and canteens of IIT Guwahati campus. Further, they were separately dried in hot air oven and crushed to obtain the peel powders. The peel powders were then separately pre-treated and eventually enzyme hydrolyzed using RSM (Response surface methodology) as described in chapter 3.

5.2.2. Microalgal growth evaluation under different culture conditions

To determine the effect of varying culture conditions on the microalgal growth, various studies were performed. 16:08 h of light:dark cycle, light intensity of 5000 lux (approx.) and temperature of 25 ± 2 ºC were kept constant throughout the experiments. Algal inoculum of 10% (v/v) was used throughout the studies. The studies were carried out till the stationary phase of the microalgal growth was reached. BG11 media was used as the control.

5.2.2.1. Microalgal cultivation on potato and sweet lime peels undergoing acid with autoclaving pre-treatment

The maximum glucose concentration for potato and sweet lime peels were obtained after treating them under acid with autoclaving conditions, followed by enzymatic hydrolysis. The obtained peel hydrolysates were then diluted into various concentrations (20%, 25%, 30%, 40%, 50%) following hydrolysis. Then the pH of the hydrolysates was adjusted to 7.4 (similar to that of BG11 media), sterilized and used as the growth medium. The optimal concentration obtained was chosen for the further experiments. For the pH optimization study, pH of 6,7,8 and 9 were examined.

5.2.2.2. Microalgal cultivation on banana peels undergoing autoclaving pre-treatment

The maximum glucose concentration for banana peels were obtained after treating them under autoclaving conditions, followed by enzymatic hydrolysis. For the concentration optimization study, the waste peel extract was diluted into different concentrations after hydrolysis i.e. 20%, 25%, 30%, 40% and 50%. The hydrolysate pH was then adjusted to that of the pH of BG11 media (pH 7.4), autoclaved and used as the culture medium. For the next set of experiments, the acquired optimal concentration was chosen. To carry out the optimal pH study, pH of 6,7,8 and 9 were investigated.

5.2.2.3. Microalgal cultivation on mixed peel hydrolysate

For the analysis, peels were mixed in the combinations as per mentioned below,

a) Potato + Banana (1:1 ratio)
b) Banana + Sweet lime (1:1 ratio)
c) Sweet lime + Potato (1:1 ratio)
d) Potato + Sweet lime + Banana (1:1:1 ratio)

For the peel hydrolysate concentration study, the mixed peel hydrolysates were diluted into 20%, 25%, 30%, 40% and 50%. In this study, the pH of the medium was adjusted to 7. Then the medium was sterilized and used for microalgal cultivation.

5.2.3. Statistical analyses

The experiments were carried out in triplicate and the results were expressed as mean ± standard deviation (SD). Origin Pro 9.0 was used for the statistical analysis throughout the experiments.

5.3. Results and Discussion

5.3.1. Hydrolysis of the peels and culture medium preparation

Pre-treatment of the peels breaks its cell wall and makes the carbohydrates more attainable for the cellulose degrading enzymes during the hydrolysis process. Significant amount of glucose is released during the hydrolysis process of the waste peels. Following the optimized conditions through different pre-treatments as mentioned in our previous chapter 3, 46.17 (±0.77) g L^{-1}, 29.84 (±0.57) g L^{-1} and 35.90 (±0.43) g L^{-1} of maximum glucose concentration were acquired from potato (dilute acid treatment followed by enzyme hydrolysis), banana (autoclaving treatment followed by enzyme hydrolysis) and sweet lime (dilute acid treatment followed by enzyme hydrolysis) peel respectively. The resultant hydrolysate was then employed as the culture medium for the cultivation of both the strains to obtain enhanced level of algal biomass and lipid content.

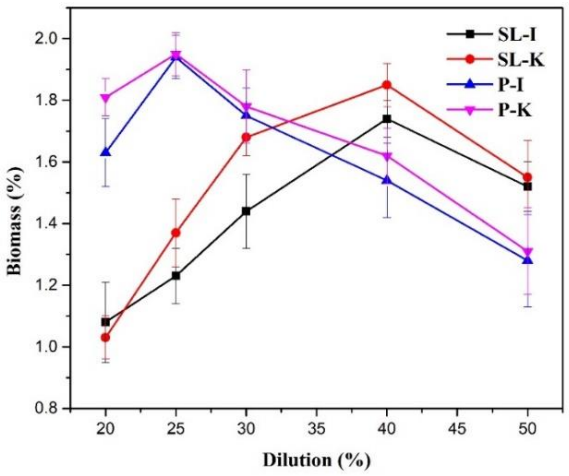
For preparation of the culture medium, the hydrolysates were diluted to reach the desired concentration and autoclaved at 121 ºC for 20 min. After sterilization, the medium was cooled down to room temperature before the inoculation of the microalgae. The medium of growth was neutralized by adjusting the pH.

5.3.2. Effect of varying culture conditions on microalgal growth

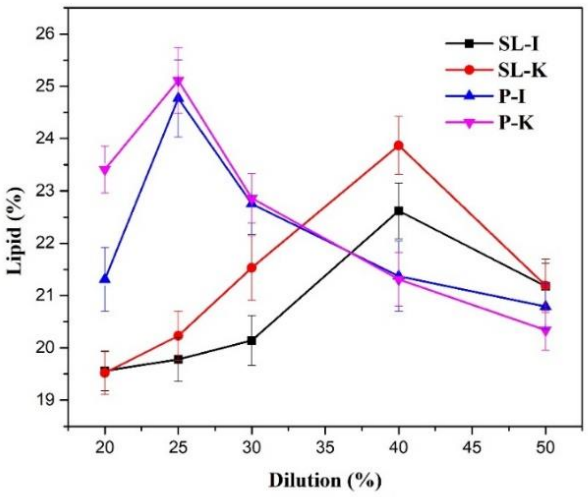
The effect of the peel extracts on the growth and lipid content of the microalgal strains are studied in this study. Different concentrations of hydrolysates were prepared by diluting the peel extracts to reach the desired concentration and used as growth medium after autoclaving to cultivate the strains for a period of fifteen days. The effect of the peel extract medium on the microalgal growth was determined by calculating the specific growth rate and biomass productivity, which varied with the types of extracts and microalgae. Lipid content was determined gravimetrically. BG11 was used as the control against the peel hydrolysate in all the cases.

Since both potato and sweet lime peels showed maximum glucose concentration under acid with autoclaving conditions, the microalgal cultivation on these two strains was compared. For the optimization of the media concentration, 25% and 40% dilution respectively for potato peel and sweet lime were found to support more biomass and lipid yield (Figure 5.1). For the pH optimization study, pH 7 was found to be optimal for the growth of both the strains "I" and "K" (Figure 5.2).

Table 5.1 and 5.2 depicts the specific growth rate (μ, d^{-1}), biomass concentration (g L^{-1}) of DCW, lipid content (%), biomass productivity (g L^{-1} d^{-1}) and lipid productivity (mg L^{-1} d^{-1}) for both the isolates. The strains were found to be a promising source of biomass. It was found that the cells grown in potato hydrolysate and sweet lime hydrolysate support the cost-effective growth of microalgae. They produced significantly higher biomass yield and lipid content while being grown on these extracts. The highest biomass yield of 2.1 g L^{-1} and highest lipid content of 25.87 % were achieved in the strain "K" when cultivated in potato peel hydrolysate. In sweet lime peel hydrolysate, the highest biomass yield of 1.97 g L-1 and highest lipid content of 24.14 % were found in the strain "K".



(A)



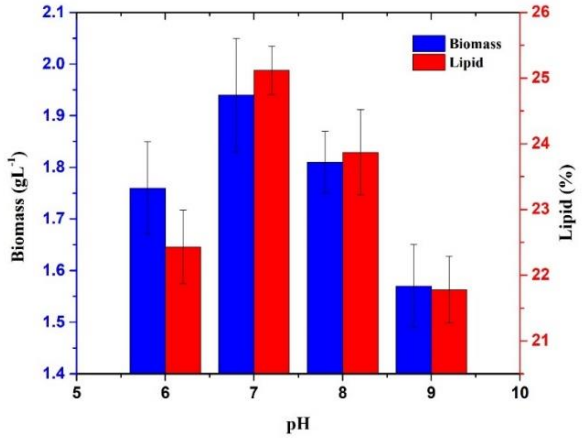
(B)

Figure 5.1. Growth study of the microalgal strains:

(A) Dilution [%] w.r.t Biomass [DCW, g L^{-1}]

(B) Dilution [%] w.r.t Lipid [%].

SL-I: Strain "I" in sweet lime peel, P-I: Strain "I" in potato peel, SL-K: Strain "K" in sweet lime peel, P-K: Strain "K" in potato peel.



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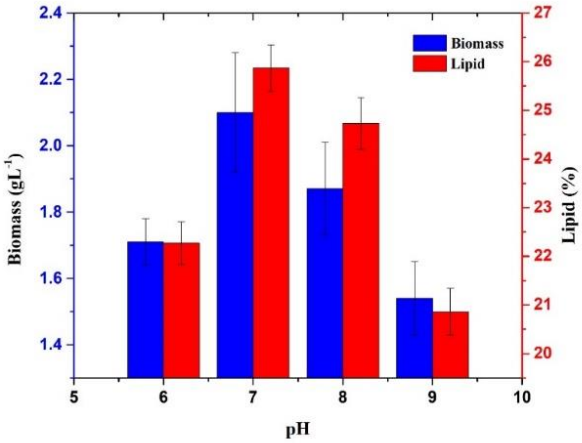

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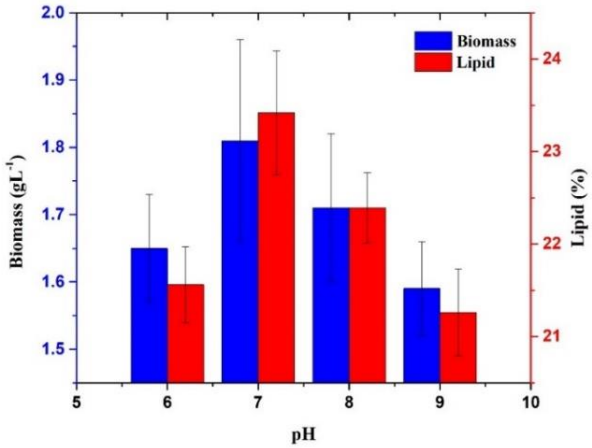

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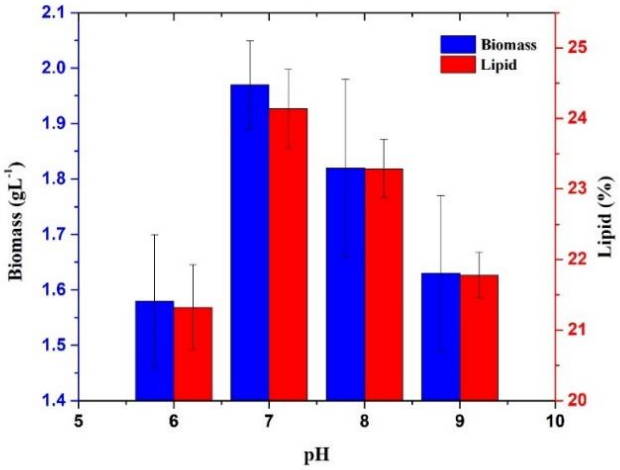


(D)

Figure 5.2. pH optimization study:

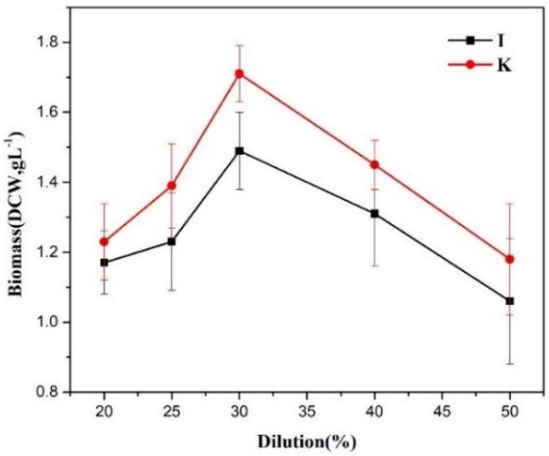
(A) Strain "I" in potato peel

(B) Strain "K" in potato peel

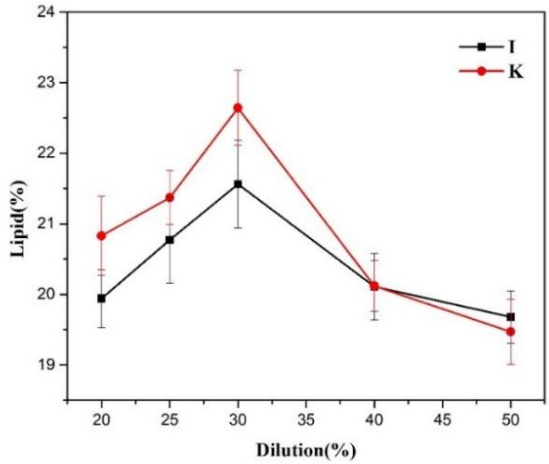
(C) Strain "I" in sweet lime peel

(D) Strain "K" in sweet lime peel.

The effect of the banana peel hydrolysate medium on the growth kinetics of the algal isolates were investigated separately as the maximum glucose concentration for banana peels was achieved under autoclaving conditions. The growth kinetic parameters are reported in Table 5.1 and 5.2 along with the control medium. 30% dilution has been found to support better biomass and lipid yield in the media optimization study (Figure 5.3). Also, pH 7 has been found to be the optimum pH for cultivating both microalgal strains (Figure 5.4). The isolated strains displayed prominent growth in the waste banana peel extract medium. The biomass yield observed in case of strain "K" and strain "I" are 1.72 g L^{-1} and 1.53 g L^{-1} respectively with a lipid content of 22.83 % and 22.17 % respectively for the strains "K" and "I".

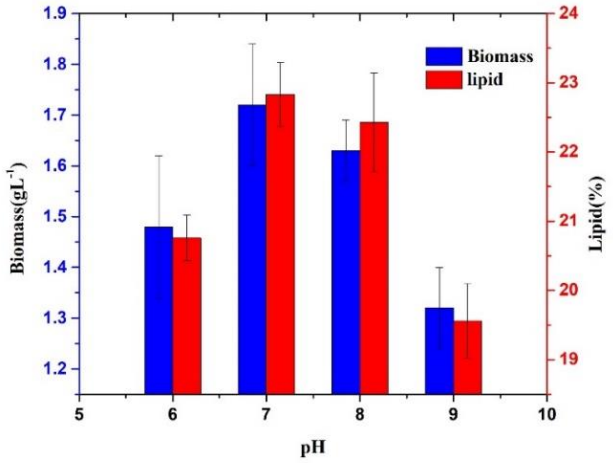


(A)



(B)

Figure 5.3. Growth evaluation of the microalgal strains in banana peel:
(A) Dilution [%] w.r.t Biomass [DCW, g L^{-1}]
(B) Dilution [%] w.r.t Lipid [%].
I: Strain "I", K: Strain "K".



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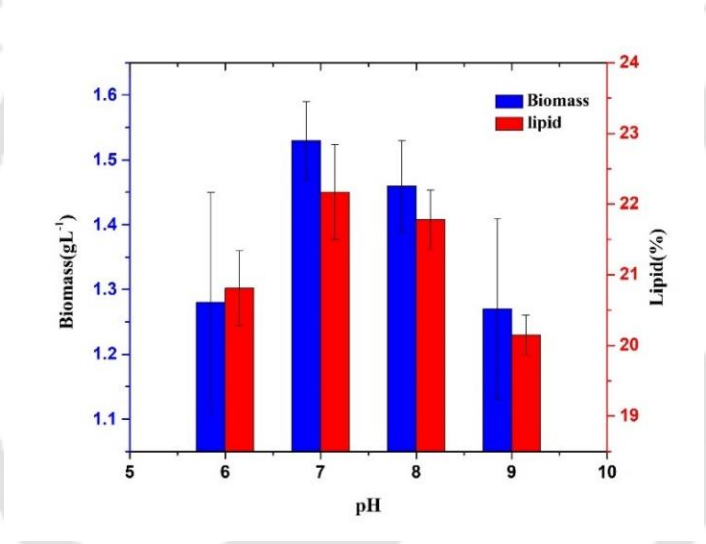


(B)

Figure 5.4. pH optimization study in the banana peel medium:

(A) Strain "I"

(B) Strain "K".

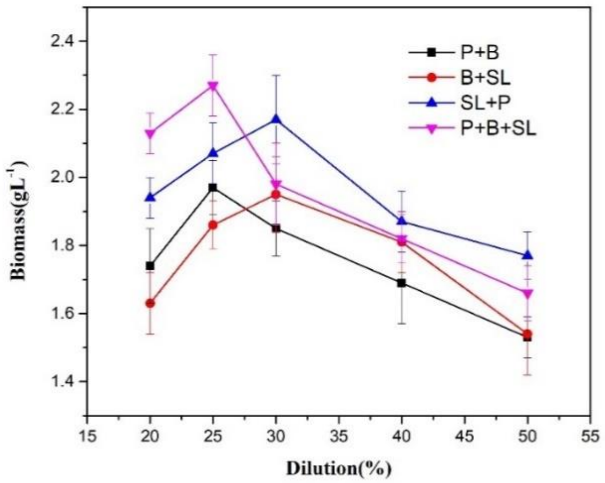
Table 5.1. Growth kinetics of the isolated strain "I" in different peel hydrolysate.

Growth medium	Biomass concentration (DCW, g L^{-1})	Lipid content (%)	Biomass productivity (g L^{-1} d^{-1})	Lipid productivity (mg L^{-1} d^{-1})	Specific growth rate (μ, d^{-1})	Divisions per day (K, d^{-1})	Doubling time/rate (T_D, d^{-1})
BG11	1.59±0.13	21.65±0.51	0.145±.01	31.39±0.56	0.21±0.02	0.30±0.03	3.30±0.27
Potato peel	1.94±0.11	25.12±0.37	0.187±0.03	46.97±0.27	0.25±0.01	0.36±0.07	2.77±0.16
Sweet lime peel	1.81±0.15	23.42±0.67	0.174±0.02	40.75±0.51	0.24±0.03	0.35±0.05	2.89±0.24
Banana peel	1.53±0.06	22.17±0.67	0.156±0.02	34.58±0.47	0.22±0.01	0.32±0.07	3.15±0.33

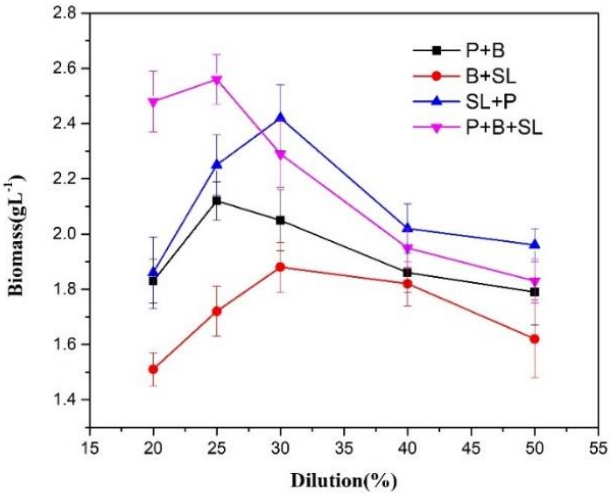
Table 5.2. Growth kinetics of the isolated strain "K" in different peel hydrolysate.

Growth medium	Biomass concentration (DCW, g L^{-1})	Lipid content (%)	Biomass productivity (g L^{-1} d^{-1})	Lipid productivity (mg L^{-1} d^{-1})	Specific growth rate (μ, d^{-1})	Divisions per day (K, d^{-1})	Doubling time/rate (T_D, d^{-1})
BG11	1.65±0.07	21.87±0.38	0.161±0.01	35.21±0.43	0.22±0.01	0.32±0.05	3.15±0.21
Potato peel	2.1±0.18	25.87±0.48	0.193±0.02	49.93±0.34	0.27±0.03	0.39±0.06	2.56±0.18
Sweet lime peel	1.97±0.08	24.14±0.56	0.177±0.03	42.73±0.55	0.26±0.02	0.38±0.04	2.67±0.28
Banana peel	1.72±0.12	22.83±0.46	0.168±0.01	38.35±0.36	0.23±0.02	0.33±0.04	3.01±0.27

Eventually, the effect of the mixed peel hydrolysate was investigated on the growth and lipid yield of the two microalgal strains. The peel extract was diluted to make up to different concentrations so as to reach the desired concentration. Then after sterilization, these extracts were used as the growth medium to cultivate the microalgal strains. A total of four combination investigations were carried out for this analysis. By determining the biomass productivity and the specific growth rate of the microalgal growth of both the strains "I" and "K", the effect of the peel hydrolysate medium was investigated (Table 5.3 and 5.4). The lipid yield and productivity for the strains "I" and "K" are reported in Table 5.3 and 5.4 respectively. It was found that the mixed peel extract of potato, sweet lime and banana produced higher biomass yield (2.56 g L^{-1}) and lipid content (26.34 %) than the other cultivation combinations mentioned in "Section 5.2.2.3", when cultivated with the strain "K". However, it was seen that all the combinations produced significant amount of biomass yield and lipid content. Figure 5.5 and 5.6 represents the growth evaluation through biomass concentration and lipid content respectively for both strains.



(A)



(B)

Figure 5.5. Growth evaluation of the microalgal strains:

(A) Dilution [%] w.r.t Biomass [DCW, g L^{-1}] for the strain "I"

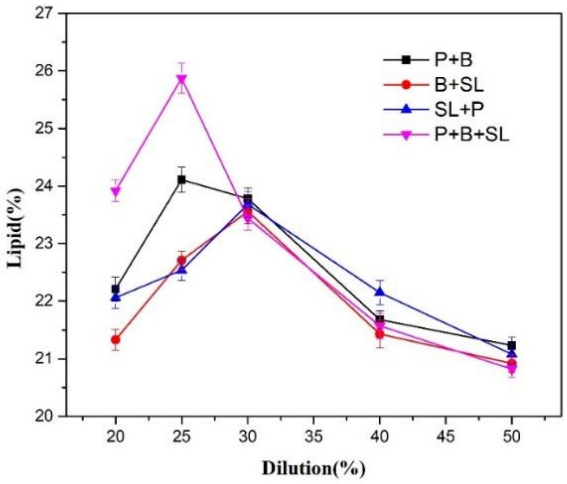
(B) Dilution [%] w.r.t Biomass [DCW, g L^{-1}] for the strain "K".

P+B: Potato & Banana peel

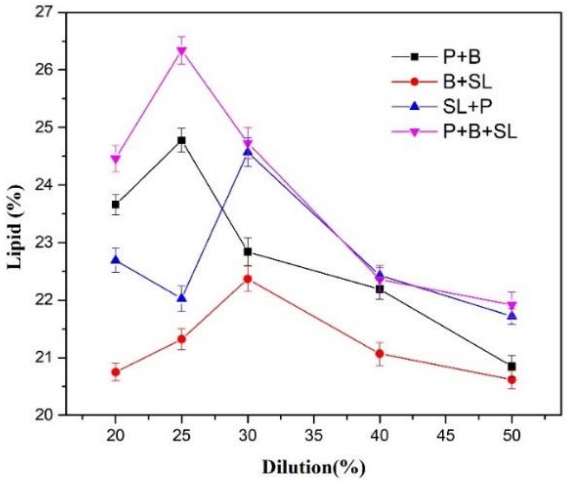
B+SL: Banana & Sweet lime peel

SL+P: Sweet lime & Potato peel

P+B+SL: Potato, Banana & Sweet lime peel.



(A)



(B)

Figure 5.6. Lipid content of the microalgal strains:
(A) Dilution [%] w.r.t Lipid [%] for the strain "I"
(B) Dilution [%] w.r.t Lipid [%] for the strain "K".
P+B: Potato & Banana peel
B+SL: Banana & Sweet lime peel
SL+P: Sweet lime & Potato peel
P+B+SL: Potato, Banana & Sweet lime peel.

Table 5.3. Growth kinetics of the isolated strain "I" in mixed peel hydrolysate.

Growth medium	Biomass concentration (DCW, g L^{-1})	Lipid content (%)	Biomass productivity (g L^{-1} d^{-1})	Lipid productivity (mg L^{-1} d^{-1})	Specific growth rate (μ, d^{-1})	Divisions per day (K, d^{-1})	Doubling time/rate (T_D, d^{-1})
BG11	1.59±0.13	21.65±0.51	0.145±0.01	31.39±0.56	0.21±0.02	0.30±0.03	3.30±0.27
P+B peel	1.97±0.07	24.11±0.22	0.192±0.03	46.29±0.23	0.26±0.01	0.38±0.02	2.67±0.19
B+SL peel	1.95±0.11	23.56±0.22	0.184±0.04	43.35±0.31	0.24±0.02	0.35±0.04	2.89±0.22
SL+P peel	2.17±0.13	23.68±0.22	0.196±0.06	46.41±0.33	0.23±0.03	0.33±0.03	3.01±0.25
P+SL+B peel	2.27±0.09	25.87±0.26	0.206±0.04	53.29±0.26	0.28±0.01	0.40±0.03	2.48±0.18

Table 5.4. Growth kinetics of the isolated strain "K" in mixed peel hydrolysate.

Growth medium	Biomass concentration (DCW, g L^{-1})	Lipid content (%)	Biomass productivity (g L^{-1} d^{-1})	Lipid productivity (mg L^{-1} d^{-1})	Specific growth rate (μ, d^{-1})	Divisions per day (K, d^{-1})	Doubling time/rate (T_D, d^{-1})
BG11	1.65±0.07	21.87±0.38	0.161±0.01	35.21±0.43	0.22±0.01	0.32±0.05	3.15±0.21
P+B peel	2.12±0.07	24.78±0.21	0.205±0.02	50.79±0.24	0.27±0.03	0.39±0.04	2.57±0.17
B+SL peel	1.88±0.09	22.37±0.22	0.192±0.05	42.95±0.32	0.23±0.01	0.33±0.02	3.01±0.18
SL+P peel	2.42±0.12	24.57±025	0.197±0.04	48.40±0.28	0.25±0.03	0.36±0.02	2.77±0.24
P+SL+B peel	2.56±0.09	26.34±0.24	0.225±0.02	59.27±0.31	0.29±0.02	0.42±0.03	2.39±0.22

Alternative economic and cost-effective carbon sources are desirable to make biofuel commercialization more viable. Microalgal cell density and productivity at the end of cultivation are influenced mainly by the nutritional conditions throughout the batch. Lee et al. (1996) reported that utilizing light and organic carbon substrate, microalgal cultivation is the most effective technique for producing algal biomass. Glucose has been considered the best organic source of carbon for *Chlorella* biomass cultivation (Shi et al., 1999). Pre-treatment of vegetable and fruit wastes disrupts the cell wall and makes the carbohydrates more accessible to the cellulose-degrading enzymes (Choi et al., 2013). Hence researchers are trying to investigate alternative cost-effective and economical carbon sources for making the commercialization of algal biofuel more feasible and viable.

Various studies have reported that agricultural, food, vegetable, and fruit waste resources have the potential as sustainable cultivation medium for biofuel production (Chokshi et al., 2016). Limbu and Sibi (2017) used mixed fruit waste hydrolysate as a sustainable and cheap medium for microalgae cultivation. They cultivated *Chlorella vulgaris* in the hydrolysate medium pre-treated by acid, enzyme, and aqueous hydrolysis, and obtained the highest specific growth, biomass, and lipid yield of 0.62±0.01 d^{-1}, 47.6±0.10 mg L^{-1} and 12.57±0.08 mg L^{-1}

respectively, in the cells grown in the enzyme-based hydrolysate. When grown in food waste hydrolysate, specific growth rates of *Chlorella vulgaris*, *Chlorella pyrenoidosa* and *Schizochytrium mangrovei* were found to be 0.8 d^{-1}, 1.1±0.41 d^{-1} and 4.7±1.2 d^{-1} respectively (Amit and Ghosh, 2019). *Ettlia oleoabundans*, when grown on agricultural waste effluents, yielded biomass of 2.93 g L^{-1} and lipid content of 9.84% on the 22nd day, and on 41st day of cultivation, it yielded biomass of 2.75 g L^{-1} and lipid content of 12.76% (Yang et al., 2011b). Abreu et al. (2012) reported maximum biomass concentration, biomass productivity and specific growth rate of 3.58±0.12 g L-1, 0.75±0.01 g L-1 d-1 and 0.43 d-1, respectively, when hydrolyzed cheese whey was used for *Chlorella vulgaris* cultivation. In their investigations, Yan et al. (2011) and Pleissner et al. (2013) found that food waste integrated with wastewater or food waste alone, like molasses hydrolysate, can support microalgae growth. Fruit waste hydrolysates prepared from peels, cores and rejected pulps of various fruits were found to boost biomass accumulation of *Chlorella pyrenoidosa* (Wu et al., 2014). *Aurantiochytrium* sp. cultivated in orange peel extract, yielded 4.4 g L^{-1} of biomass (Park et al., 2018). Agwa et al. (2014), reported 5.71 g L^{-1} of biomass content and 40.7 mg L^{-1} d^{-1} of lipid productivity while cultivating microalgae using cassava peels. He stated that this was feasible due to the presence of nutrients in the peels. Nazir et al. (2020), reported biomass yield of 31.87±2.00 g L^{-1} (batch) and 41.5±2.67 g L^{-1} (fed-batch) when *Aurantiochytrium* sp. was grown in Pineapple extract. Rumiani et al. (2018), cultivated *Crypthecodinium cohnii* in date syrup and reported 6.65 g L^{-1} biomass yield and 20 ± 0.8 % lipid content. Katiyar et al. (2019), reported 413.14±1.61 biomass productivity (mg L^{-1} d^{-1}) and lipid productivity (mg L^{-1} d^{-1}) 129.9±2.68 respectively when *Chlorella* sp. was grown using the low-cost feedstock *Citrus limetta* wastes (combination of both discarded peels and pulp). Park et al. (2014), reported 3.4 times magnification in biomass when *Chorella vulgari*s was cultivated in orange peel. Ma et al. (2017), reported a lipid content of 28.9% and lipid productivity of 94.4 mg L^{-1} d^{-1} when cultivating *Scenedesmus* sp. in molasses wastewater. Mu et al. (2015) obtained a maximum biomass and lipid productivity of 4.01 g L^{-1} and 1.19 g L^{-1} $d^{-1,}$ respectively by cultivating *Chlorella protothecoides* on sugarcane bagasse hydrolysate. Microalga *Chlorella pyrenoidosa* yielded a specific growth rate of 1.4 d^{-1} when cultivated in restaurant food and bakery wastes (Pleissner et al., 2015). Lau et al. (2014) mentioned the approach of a "food waste based microalgal biorefinery" from *Chlorella vulgaris* utilizing food waste hydrolysate. They obtained a high specific growth rate of 0.8 d^{-1} and the algal biomass was found to be rich in carbohydrates, lipids and proteins as well. Wang et al. (2020) reported biomass concentration of 6.9 g L^{-1} and

lipid yield of 1.8 g L^{-1} in case of *Chlorella* sp. when cultivated in food waste hydrolysate of unconsumed bread.

Cultivation of microalgae through utilization of food and agricultural waste is considered a sustainably and economically feasible approach. Microalgae utilizes organic carbon sources to enhance the algal biomass and value added bioproducts. The results mentioned in this study demonstrates that glucose could be a preferred carbon source for increased microalgal biomass cultivation. Thus, using glucose-rich feedstock could be considered favorable for *Chlorella* microalga cultivation. The results of this study were compared with previously reported literature and is presented in Table 5.5. Apart from the microalgal cultivation, this process also aids in the reuse and recycling of these raw materials otherwise discarded as wastes and discarded into the environment. This could be a small stepping stone into the global waste management system.

Table 5.5. Relative comparison of the biomass (DCW), specific growth rate, biomass productivity, lipid content (%) and lipid productivity of the isolated strains with previously reported studies.

Strain	Culture medium	Biomass (DCW, g L^{-1})	Biomass productivity	Lipid Content (%)	Lipid productivity	Specific growth (d^{-1})	Reference
Chlorella sorokiniana-I	Potato peel extract	1.94±0.11	0.187±0.03 g L^{-1} d^{-1}	25.12±0.37	46.97±0.27 mg L^{-1} d^{-1}	0.25±0.01	This study
Chlorella sorokiniana-I	Sweet lime peel extract	1.81±0.15	0.174±0.02 g L^{-1} d^{-1}	23.42±0.67	40.75±0.51 mg L^{-1} d^{-1}	0.24±0.03	This study
Chlorella sorokiniana-I	Banana peel extract	1.53±0.06	0.156±0.02 g L^{-1} d^{-1}	22.17±0.67	34.58±0.47 mg L^{-1} d^{-1}	0.22±0.01	This study
Chlorella sorokiniana-K	Potato peel extract	2.1±0.18	0.193±0.02 g L^{-1} d^{-1}	25.87±0.48	49.93±0.34 mg L^{-1} d^{-1}	0.27±0.03	This study

Chlorella sorokiniana-K	Sweet lime peel extract	1.97±0.08	0.177±0.03 g L^{-1} d^{-1}	24.14±0.56	42.73±0.55 mg L^{-1} d^{-1}	0.26±0.02	This study
Chlorella sorokiniana-K	Banana peel extract	1.72±0.12	0.168±0.01 g L^{-1} d^{-1}	22.83±0.46	38.35±0.36 mg L^{-1} d^{-1}	0.23±0.02	This study
Chlorella sorokiniana-I	P+B peel extract	1.97±0.07	0.192±0.03 g L^{-1} d^{-1}	24.11±0.22	46.29±0.23 mg L^{-1} d^{-1}	0.26±0.01	This study
Chlorella sorokiniana-I	B+SL peel extract	1.95±0.11	0.184±0.04 g L^{-1} d^{-1}	23.56±0.22	43.35±0.31 mg L^{-1} d^{-1}	0.24±0.02	This study
Chlorella sorokiniana-I	SL+P peel extract	2.17±0.13	0.196±0.06 g L^{-1} d^{-1}	23.68±0.22	46.41±0.33 mg L^{-1} d^{-1}	0.23±0.03	This study
Chlorella sorokiniana-I	P+SL+B peel extract	2.27±0.09	0.206±0.04 g L^{-1} d^{-1}	25.87±0.26	53.29±0.26 mg L^{-1} d^{-1}	0.28±0.01	This study
Chlorella sorokiniana-K	P+B peel extract	2.12±0.07	0.205±0.02 g L^{-1} d^{-1}	24.78±0.21	50.79±0.24 mg L^{-1} d^{-1}	0.27±0.03	This study
Chlorella sorokiniana-K	B+SL peel extract	1.88±0.09	0.192±0.05 g L^{-1} d^{-1}	22.37±0.22	42.95±0.32 mg L^{-1} d^{-1}	0.23±0.01	This study
Chlorella sorokiniana-K	SL+P peel extract	2.42±0.12	0.197±0.04 g L^{-1} d^{-1}	24.57±0.25	48.40±0.28 mg L^{-1} d^{-1}	0.25±0.03	This study
Chlorella sorokiniana-K	P+SL+B peel extract	2.56±0.09	0.225±0.02 g L^{-1} d^{-1}	26.34±0.24	59.27±0.31 mg L^{-1} d^{-1}	0.29±0.02	This study
Tetraselmis indica	Kinnow peel	0.32± 0.02	22.56± 0.44 mg L^{-1} d^{-1}	-	6.24 mg L^{-1} d^{-1}	0.198± 0.0014	Amit and Ghosh, 2019

Chlorella protothecoides	Sorghum hydrolysate	5.1	-	52.5	535.5 mg L^{-1} d^{-1}	-	Gao et al., 2010
Chlorella vulgaris	Orange peel extract	2.20	183.33 mg L^{-1} d^{-1}	-	-	-	Park et al., 2014
Chlorella protothecoides	Cassava starch hydrolysate	4.26 ± 0.18	0.82 ± 0.03 g L^{-1} d^{-1}	50.2 ± 0.6	0.41 ± 0.02 g L^{-1} d^{-1}	-	Lu et al., 2010
Chlorella pyrenoidosa	Rice straw hydrolysate	2.83 ± 0.04	1.10±0.02 g L^{-1} d^{-1}	56.3±1.4	0.62±0.01 g L^{-1} d^{-1}	-	Li et al., 2011
Schizochytrium limacinum	Sweet sorghum juice	3.08 ±0.02	0.62± 0.01 g L^{-1} d^{-1}	-	0.43±0.03 g L^{-1} d^{-1}	0.51 ±0.01	Liang et al., 2010
Schizochytrium sp.	Hemp hydrolysate	1.8± 0.06	-	16.9± 0.41	-	-	Gupta et al., 2015
Scenedesmus obliquus	Food wastewater	0.41	-	-	13.3 mg L^{-1} d^{-1}	-	Ji et al., 2015
Chlorella vulgaris	Okara waste extract	1.92	-	-	-	0.13	Kamal et al., 2017
Schizochytrium limacinum	Sorghum juice extract	9.38±0.25	1.87±0.05 g L^{-1} d^{-1}	-	1.38±0.02 g L^{-1} d^{-1}	0.91±0.02	Liang et al., 2010
Chlorella sorokiniana	Food waste hydrolysate	9.5	-	26.4	-	-	Haske-Cornelius et al., 2020

Chlorella protothecoides	Waste molasses hydrolysate	14.5	-	48.2	-	-	Yan et al., 2011

5.4. Conclusion

Two indigenous microalgae strains *Chlorella sorokiniana* KMBM_I and *Chlorella sorokiniana* KMBM_K were tested for their growth and adaptability in the peel wastes. Growth kinetic parameters of the strains were analyzed in varying culture conditions. The highest biomass yield of 2.56±0.09 g L^{-1} and lipid content of 26.34±0.24 % was observed when the microalgal strain "K" cultivated in the mixed peel extract of potato, banana and sweet lime. This study showed that these agricultural wastes can be used as a natural, cost effective, eco-friendly, efficient and affordable substrate for the cultivation of the microalgal isolates. By scaling up the process to pilot scale, there is enough future scope for enhancing the biomass and lipid content. Also, the biomass, lipid and de-oiled biomass (after lipid extraction) can be further investigated for potential applications in biofuels, biofertilizers or bio-additives in feed or food industry.

References

Aboul-Enein AM, Salama ZA, Gaafar AA, Aly HF, bou-Elella FA, Ahmed HA (2016) Identification of phenolic compounds from banana peel (*Musa paradaisica* L.) as antioxidant and antimicrobial agents, Journal of Chemical and Pharmaceutical Research 8(4):46-55.

Abreu AP, Fernandes B, Vicente AA, Teixeira J, Dragone G (2012) Mixotrophic cultivation of *Chlorella vulgaris* using industrial dairy waste as organic carbon source, Bioresource Technology 118:61-66.

Agwa OK, Neboh HA, Ossai-Chidi LN, Okoli MC (2014) Cultivation of microalgae using Cassava wastes as a growth media, J Algal Biomass Utln 5(2):8–19.

Amit, Ghosh UK (2019) Utilization of kinnow peel extract with different wastewaters for cultivation of microalgae for potential biodiesel production, Journal of Environmental Chemical Engineering 7:103135.

Arapoglou D, Varzakas Th, Vlyssides A, Israilides C (2010) Ethanol production from potato peel waste (PPW), Waste Management 30:1898–1902.

Ashraful AM, Masjuki HH, Kalam MA, Rizwanul Fattah IM, Imtenan S, Shahir SA, Mobarak HM (2014) Production and comparison of fuel properties, engine performance, and emission characteristics of biodiesel from various non-edible vegetable oils: a review, Energy Convers Manag 80:202-228.

Banerjee J, Singh R, Vijayaraghavan R, MacFarlane D, Patti AF, Arora A (2017) Bioactives from fruit processing wastes: Green approaches to valuable chemicals, Food Chemistry 225:10-22.

Barros HRM, Ferreira TAPC, Genovese MI (2012) Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil, Food Chemistry 134:1892–1898.

Berg P, Boland A (2014) Analysis of ultimate fossil fuel reserves and associated CO_2 emissions in IPCC scenarios, Nat Resour Res 23(1):141-158.

Bhatnagar A, Bhatnagar M, Chinnasamy S, Das KC (2010) *Chlorella minutissima* – a promising fuel alga for cultivation in municipal wastewaters, Appl Biochem Biotechnol 161:523-536.

Chisti Y (2007) Biodiesel from microalgae, Biotechnol Adv 25(3):294-306.

Choi IS, Kim JH, Wi SG, Kim KH, Bae HJ (2013) Bioethanol production from mandarin (*Citrus unshiu*) peel waste using popping pretreatment, Applied Energy 102:204-210.

Chokshi K, Pancha I, Ghosh A, Mishra S (2016) Microalgal biomass generation by phycoremediation of dairy industry wastewater: An integrated approach towards sustainable biofuel production, Bioresource Technology 221:455–460.

Dahiru M, Zango ZU, Haruna MA (2018) Cationic Dyes Removal Using Low-Cost Banana Peel Biosorbent, American Journal of Materials Science 8(2):32-38.

Gao C, Zhai Y, Ding Y, Wu Q (2010) Application of sweet sorghum for biodiesel production by heterotrophic microalga *Chlorella protothecoides*, Applied Energy 87(3):756–761.

Gaurav N, Sivasankari S, Kiran GS, Ninawe A, Selvin J (2017) Utilization of biomass for sustainable biofuels: A Review, Renewable and Sustainable Energy Reviews 73:205-214.

Gupta A, Abraham RE, Barrow CJ, Puri M (2015) Omega-3 fatty acid production from enzyme saccharified hemp hydrolysate using a novel marine thraustochytrid strain, Bioresource Technology 184:373-378.

Hao HN, Karthikeyan OP, Heimann K (2015) Bio-refining of carbohydrate rich food waste for biofuels, Energies 8(7):6350-6364.

Hashem AH, Hasanin MS, AMA, Suleiman WB (2020) Eco-Green Conversion of Watermelon Peels to Single Cell Oils Using a Unique Oleaginous Fungus: *Lichtheimia corymbifera* AH13, Waste and Biomass Valorization 11:5721–5732.

Hashem AH, Suleiman WB, Abu-Elrish GM, El-Sheikh HH (2021) Consolidated Bioprocessing of Sugarcane Bagasse to Microbial Oil by Newly Isolated Oleaginous Fungus: *Mortierella wolfii*, Arabian Journal for Science and Engineering 46:199–211.

Haske-Cornelius O, Vu T, Schmiedhofer C, Vielnascher R, Dielacher M, Sachs V, Grasmug M, Kromus S, Guebitz GM (2020) Cultivation of heterotrophic algae on enzymatically hydrolyzed municipal food waste, Algal Research 50:101993.

Jekayinfa SO, Linke B, Pecenka R (2015) Biogas production from selected crop residues in Nigeria and estimation of its electricity value, Int J Renewable Energy Technology 6(2):101-118.

Ji M-K, Yun H-S, Park S, H Lee, Park Y-T, Bae S, Ham J, Choi J (2015) Effect of food wastewater on biomass production by a green microalga *Scenedesmus obliquus* for bioenergy generation, Bioresource Technology 179:624-628.

Jin M, Slininger PJ, Dien BS, Waghmode S, Moser BR, Orjuela A (2015) Microbial lipid-based lignocellulosic biorefinery: feasibility and challenges, Trends Biotechnol 33:43-54.

Kamal SM, El-Sayed AB, Hassan AA, El-Shazly HAM, Ibrahim MT (2017) Use of Okara waste for algae nutrition, Arab Univ J Agric Sci 25(2):271-279.

Katiyar R, Gurjar BR, Kumar A, Bharti RK, Biswas S, Pruthi V (2019) A novel approach using low-cost *Citrus limetta* waste for mixotrophic cultivation of oleaginous microalgae to augment automotive quality biodiesel production, Environ Sci Pollut Res 26:16115–16124.

Lau KY, Pleissner D, Lin CSK (2014) Recycling of food waste as nutrients in *Chlorella vulgaris* cultivation, Bioresource Technology 170:144–151.

Lee YK, Ding SK, Hoe CH, Low CS (1996) Mixotrophic growth of *Chlorella sorokiniana* in outdoor enclosed photobioreactor, J Appl Phycol 8(2):163-169.

Liang Y, Sarkany N, Cui Y, Yesuf J, Trushenski J, Blackburn JW (2010) Use of sweet sorghum juice for lipid production by *Schizochytrium limacinum* SR21, Bioresource Technology 101:3623-3627.

Li P, Miao X, Li R, Zhong J (2011) In situ biodiesel production from fast-growing and high oil content *Chlorella pyrenoidosa* in rice straw hydrolysate, J Biomed Biotechnol, Research Article ID 141207, doi:10.1155/2011/141207.

Limbu B, Sibi G (2017) Fruit Wastes Hydrolysates as Feedstock: Pre-Treatment Strategies for Cost-Saving and Sustainable Microalgae Cultivation, International Journal of Research in Environmental Science 3(2):35-40.

Lu Y, Zhai Y, Liu M, Wu Q (2010) Biodiesel production from algal oil using cassava (*Manihot esculenta* Crantz) as feedstock, Journal of Applied Phycology 22(5):573–578.

Ma C, Wen H, Xing D, Pei X, Zhu J, Ren N, Liu B (2017) Molasses wastewater treatment and lipid production at low temperature conditions by a microalgal mutant *Scenedesmus* sp. Z-4, Biotechnol Biofuels 10:111.

Mathias DJ, Kumar S, Rangarajan V (2019) An investigation on citrus peel as the lignocellulosic feedstock for optimal reducing sugar synthesis with an additional scope for the production of hydrolytic enzymes from the aqueous extract waste, Biocatalysis and Agricultural Biotechnology 20:101259.

Mu J, Li S, Chen D, Xu H, Han F, Feng B, Li Y (2015) Enhanced biomass and oil production from sugarcane bagasse hydrolysate (SBH) by heterotrophic oleaginous microalga *Chlorella protothecoides*, Bioresource Technology 185:99–105.

Nazir Y, Halim H, Al-Shorgani NKN, Manikan V, Hamid AA, Song Y (2020) Efficient conversion of extracts from low-cost, rejected fruits for high-valued Docosahexaenoic acid production by *Aurantiochytrium* sp. SW1, Algal Research 50:1019772.

Park WK, Moon M, Kwak MS, Jeon S, Choi GG, Yang JW, Lee B (2014) Use of orange peel extract for mixotrophic cultivation of *Chlorella vulgaris*: increased production of biomass and FAMEs, Bioresour Technol 171:343-349.

Park WK, Moon M, Shin SE, Cho JM, Suh WI, Chang YK, Lee B (2018) Economical DHA (Docosahexaenoic acid) production from *Aurantiochytrium* sp. KRS101 using orange peel extract and low cost nitrogen sources, Algal research 29:71-79.

Pleissner D, Lau KY, Schneider R, Venus J, Lin CSK (2015) Fatty acid feedstock preparation and lactic acid production as integrated processes in mixed restaurant food and bakery wastes treatment, Food Research International 73:52–61.

Rumiani LA, Jalili H, Amrane A (2018) Enhanced docosahexaenoic acid production by *Crypthecodinium cohnii* under combined stress in two-stage cultivation with date syrup based medium, Algal Research 34:75–81.

Selvakumar P, Karthik V, Senthil Kumar P, Asaithambi P, Kavitha S, Sivashanmugam P (2021) Enhancement of ultrasound assisted aqueous extraction of polyphenols from waste fruit peel using dimethyl sulfoxide as surfactant: Assessment of kinetic models, Chemosphere 263:128071.

Shi XM, Liu HJ, Zhang XW, Chen F (1999) Production of biomass and lutein by *Chlorella protothecoides* at various glucose concentrations in heterotrophic cultures, Process Biochem 34(4):341-347.

Slade R, Bauen A (2013) Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospect, Biomass Bioenergy 53:29-38.

Wang X, Zhang M-M, Sun Z, Liu S-F, Qin Z-H, Mou J-H, Zhou Z-G, Lin CSK (2020) Sustainable lipid and lutein production from *Chlorella* mixotrophic fermentation by food waste hydrolysate, Journal of Hazardous Materials 400:123258.

Wong MH (1985) Cultivation of microalgae in refuse compost and soy-bean waste extracts, Agric Wastes 12(3):225-233.

Wu S, Xu XR, Sun KF, Li HB (2014) Effects of fruit waste hydrolysates on biomass and chlorophyll a fluorescence parameters of *Chlorella pyrenoidosa*, Int J Environ Bioener 9(2):105-121.

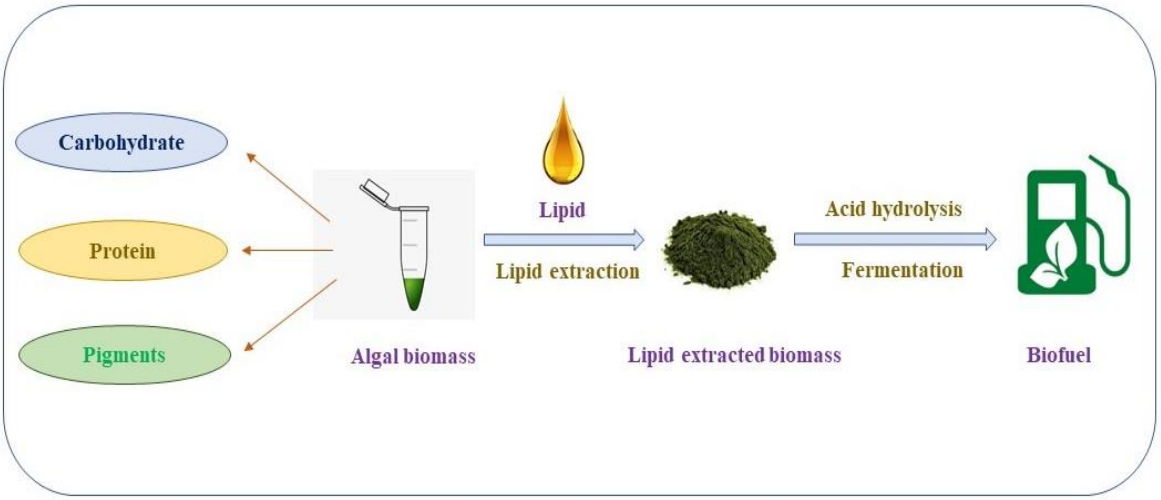
Yan D, Lu Y, Chen YF, Wu Q (2011) Waste molasses alone displaces glucose-based medium for microalgal fermentation towards cost-saving biodiesel production, Bioresour Technol 102:6487-6493.

Yang J, Li X, Hu H, Zhang X, Yu Y, Chen Y (2011a) Growth and lipid accumulation properties of a freshwater microalga, *Chlorella ellipsoidea* YJ1, in domestic secondary effluents, Appl Energy 88:3295–3299.

Yang Y, Xu J, Vail D, Weathers P (2011b) *Ettlia oleoabundans* growth and oil production on agricultural anaerobic waste effluents, Bioresource Technology 102:5076-5082.

CHAPTER 6

INTEGRATED BIOREFINERY APPROACH: SIMULTANEOUS GENERATION OF MULTIPLE PRODUCTS FROM THE MICROALGAE



6.1. Overview

At present, the global energy dependence is quenched by non-renewable fossil fuels. However due to the adverse environmental repercussions of the fossil resources, sole dependence on them is not sustainable. Primarily produced from biomass, biofuels can be either in gaseous or liquid form. They can be used in addition to petrol, diesel or other fossil fuels or can replace the existing fossil fuels for mainly transport and other applications. Biofuels being renewable and eco-friendly, has the potential to replace conventional energy resources as they also have lower greenhouse gas (GHG) emission profiles (Deeba et al., 2020). But the excessive price for the commercial production of biofuels as compared to traditional fuels is a major bottleneck. Therefore, researchers worldwide are trying to transform economic resources into biofuels from an environment-friendly and cost-effective perspective. Microalgae have been considered as an efficient producer of biofuel for transportation purposes. Microalgae are can adapt well to various stressful environments.

Due to their metabolic versatility, microalgae are treated as a potential and viable energy feedstock. They have a fast growth rate and can manufacture significant oil. Still the microalgal biofuel system endures many bottlenecks during their downstream processing. A considerable amount of de-oiled lipid extracted microalgal biomass residue is generated as a waste product during the biofuel production. Valorization of these de-oiled microalgal residue into value-added products through the microalgal biorefinery concept is possible to offset the biofuel production cost (Maurya et al., 2016). Biorefineries follows the concept of zero waste production and are very energy efficient. Biorefineries aids in taking advantage of the huge potential of microalgae by recovering more value-added products from them and by enhancing their production by utilizing an ecological engineering approach (Cho et al., 2015). During microalgal cultivation, the pilot scale biorefineries can integrate the novel harvesting techniques and methodologies with various biomass valorization pathways. These biorefinery techniques are considered to be phenomenal in the future generation of valuable bio-based products from microalgae through an integrated approach. Different nutritional modes like autotrophic, mixotrophic and heterotrophic exist in microalgal cultivation. Microalgae demonstrates an extensive network of extremely integrated upstream and downstream processes for biofuel production. Major restraints in downstream processing like biomass harvesting and dewatering are cost and intensive processes; however, there is a significant development in recent times in the field of biofuel production. Since the autotrophic mode of

cultivation yields significantly low biomass, hence the algal bioprocesses have shifted the focus towards heterotrophic and mixotrophic cultivation approaches by implementing biorefinery approach through multiple product strategies (Yen et al., 2013). Microalgae exploits photosynthesis to convert solar/light energy into valuable bio-based products (Leu and Boussiba, 2014). In the recent times, microalgal biorefineries which can fabricate biofuels and value added bioproducts simultaneously, are vigorously probed and explored. Microalgae have the budding potential of being the producer of various kinds of biofuels and biomaterials as well.

As the waste peel biomass contains nutrients and organic carbons, their potential as an alternative to traditional and conventional culture medium is investigated for oleaginous microalga *Chlorella sorokiniana*. The culture conditions were optimized for the waste molasses to cultivate the microalga. Waste peels can be used as an economical and advantageous substrate to lower the costs for microalgal culture. It is a promising substrate for cheap production of microalgal based biofuel. Biomass yield and lipid content can be improvised through optimized culture conditions (Yan et al., 2011). This study further exploited the full potential of the usage of waste peels to replace the conventional medium for growth and lipid accumulation in *Chlorella sorokiniana*.

For microalgal based bioethanol production, those microalgae which contain carbohydrates in high quantity specially glucose are considered to be the most favorable feedstock (Gerardo et al., 2014). The defatted biomass after lipid extraction was hydrolyzed and the obtained hydrolysate was fermented to generate bioethanol. This is a significant step in the production of sustainable biofuels through reuse and recycling of spent microalgal biomass. This study demonstrates the potential of indigenous native microalgae species for a biorefinery that generates lipids, bioethanol and various value added bioproducts.

The microalgal biomass is a complex network of compounds like carbohydrates, proteins, pigments and lipids. The amount of these potentially valuable molecules could vary as a result of the selected algal species and strains; and their adopted cultivation techniques. However, if we consider a conservative quantity of 25% oil extractable (Chisti, 2007), from the microalgal biomass, then from every 100% of biomass, three times (75%) the quantity of lipid extracted microalgal biomass residue (LEMBR) will be generated. To make the process more sustainable, valorizing such biomass for different applications is very important, thus balancing the price of biodiesel production. The different applications of LEMBRs includes its

fermentation to bioethanol and biomethane; nutrient source as fish feed; as fertilizers; as bio-sorbents for removal of dye and heavy metals from wastewater. Depending on the microalgal species, the content may vary but the LEMBRs are rich in nitrogen and protein content, essential macro and micro nutrients. However, they cannot be directly utilized for biomethane and bioethanol generation, but can be used as fertilizer; nutrient feed for animals and fish (Maurya et al., 2016).

This study investigated new prospects in the framework of biorefinery by using waste peels as renewable feedstock to produce biofuels and value-added products, and also by trying to manage the environmental issues regarding organic waste residues.

6.2. Materials and Methods

6.2.1 Ultimate and proximate analysis

6.2.1.1. Ultimate analysis

Using EuroEA elemental analyzer, the elemental analysis of the sample for the determination of CHNS(O) composition was carried out.

The high heating value (HHV, MJ Kg^{-1}) or gross calorific value (GCV) and the low heating value (LHV, MJ Kg^{-1}) or net calorific value (NCV) based on the CHNS(O) elemental analysis of the biomass was determined using the following equations (Pandey et al, 2019),

$$GCV \text{ or } HHV = 0.383 \times C + 1.422 \times (H - O/8) \quad (1)$$

$$NCV \text{ or } LHV = HHV - 2.447 \times (H/100) \times 9.011 \quad (2)$$

Where C, H, and O are the weight in percentages (%) of Carbon, Hydrogen and Oxygen in the biomass, respectively.

6.2.1.2. Proximate analysis

To determine the Moisture content, Ash, and Volatile matter (Proximate analysis) of the three peel feedstocks, the National Renewable Energy Laboratory (NREL) method (2008) was used.

The content of Fixed carbon (FC) has been calculated by deducting the values of the moisture (M), volatile matter (VM), and ash (A_S) content from 100 (Artemio et al., 2018).

$$FC = 100 - (M + VM + A_S) \quad (3)$$

6.2.2. Biochemical analysis of the microalgae

6.2.2.1. Total Carbohydrate

The previously reported Phenol-Sulphuric acid method was employed for carbohydrate estimation (DuBois et al., 1956).

6.2.2.2. Total Protein

The total content of Nitrogen (N) of the biomass was measured using a CHNS(O) elemental analyzer (EuroEA Elemental Analyzer). For the estimation and calculation of the crude protein in the biomass, nitrogen to a protein conversion factor of 6.25 was employed through the following equation (Becker, 1994),

$$\text{Total protein (\%)} = 6.25 \times N\ (\%) \quad (4)$$

Where "N" is the nitrogen content.

6.2.2.3. Pigments: Chlorophyll and Carotenoid content

Based on a previously reported spectrophotometric method by Pruvost et al. (2011), the pigments in the sample were extracted using pure methanol. The below mentioned Arnon's equations were utilized to calculate the chlorophyll and carotenoid concentration in the extract (Lichtenthaler, 1987).

$$\text{Chlorophyll a (chl-a)}: 16.72 \times A_{665} - 9.16 \times A_{652} \quad (5)$$

$$\text{Chlorophyll b (chl-b)}: 34.09 \times A_{652} - 15.28 \times A_{665} \quad (6)$$

$$\text{Total Chlorophyll } (\mu g\ mL^{-1}) = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (7)$$

$$\text{Carotenoids } (\mu g\ mL^{-1}): [1000 \times A_{470} - 1.63 \times \text{chl-a} - 104.9 \times \text{chl-b}] / 221 \quad (8)$$

6.2.3. Fourier transform infrared (FTIR) spectrophotometer analysis

FTIR (PerkinElmer, Spectrum 2) analysis was carried out to analyze the functional groups present in the algal biomass and lipid. Infrared spectra in the range 400 cm^{-1} to 4000 cm^{-1} was read during the analysis.

6.2.4. Biorefinery approach through the utilization of LEMBR for Bioethanol production

Algal biomass has acquired considerable recognition owing to its great potential as a sustainable substitute to fossil fuels. Depending on their cultivation environment and conditions, they can cumulate various amounts of carbohydrates, lipids, proteins and pigments. Hence, they can represent a rich origin of fermentable sugars for production of third-generation biofuel i.e. bioethanol.

Three prime steps are involved during the bioethanol production from utilization of microalgal carbohydrates. They are: (i) pre-treatment (ii) saccharification (iii) fermentation. In order to increase the accessibility to intracellular sugars, pre-treatment of the biomass is carried out, which plays a significant part in enhancing the overall efficacy of the bioethanol production procedure (Velazquez-Lucio et al., 2018).

Because of its environment friendliness and biochemical framework, microalgae are often considered to be the future of renewable biofuel. Hence it is very important to focus on generating promising value-added products in revenue from the microalgal biomass. This could act as amplifying the economics of microalgal biofuel production. A biorefinery approach which aims in the utilization of the spent microalgal biomass after the lipid extraction, is therefore well sought after. The present study produces bioethanol from the microalgal residue after the lipid extraction. The lipid extracted microalgal biomass is subjected to acid hydrolysis and subsequent fermentation employing yeast to produce bioethanol.

Biorefinery approach aids in the generation of secondary product through utilizing the spent biomass after lipid extraction from the microalgal biomass. In the present study, lipids, pigments, proteins, and carbohydrates are produced along with bioethanol. The LEMBR (Lipid extracted microalgal biomass residue) was used as the substrate for hydrolysis and fermentation for bioethanol production, thus enhancing its prospects and eligibility as a precursor for bioethanol.

Acid-Thermal hydrolysis was performed on the spent biomass to convert the complex polysaccharides into simple sugars. The leftover biomass after lipid extraction was treated with sulphuric acid (H_2SO_4) to undergo acid hydrolysis for one hour at 121°C and 20 psi pressure in an autoclave (Equitron PAD). The bioethanol fermentation utilizing *Saccharomyces cerevisiae* (Baker's yeast) was done as per the method suggested by Chakrabarthy et al. (2014). A 3% yeast inoculum was cultured in LB medium for 24 h at 30°C and incubated in a shaker at 200 rpm. The yeast cells were then harvested by centrifugation, washed and added to the

algal hydrolysate in air tight bottles in dark for 4 days in 200 rpm orbital shaking at 30°C. The bioethanol produced was estimated by the protocol described by Pilone (1985) where the standard potassium dichromate method was employed. In this method, 1.5 mL of the potassium dichromate reagent was added with the 150 µL of the sample and incubated for 30 minutes at 30°C. Then the sample's optical density (OD) was measured at 590 nm and the ethanol concentration (% v/v) was estimated using a standard curve.

6.2.5. Statistical analysis

All the trials were carried out in triplicate and the results were indicated as mean ± SD (standard deviation).

6.3. Results and Discussion

6.3.1. Biochemical, pigment, ultimate and proximate analysis of the microalgae

The microalgal biomass was a significant producer of pigments like chl-a, chl-b and carotenoids (Table 6.1). Carotenoids and chlorophyll have significant application in the field of pharmaceuticals, cosmetics, nutraceuticals and food supplements (Miazek et al., 2017). The Carbohydrate and protein analysis results were presented in Table 6.1. These results were found to be in accordance with the results reported by Arif et al. (2021). Microalgae have the potential to create biomass with significant quantity of carbohydrates and lipid, that can be further processed into produce biofuels (Chokshi et al., 2016). The proximate and ultimate results of the microalgal biomass were presented in Table 6.2. The GCV for the strains "I" and "K" are 18.15 MJ Kg^{-1} and 20.30 MJ Kg^{-1} respectively and NCV for the strains are "I" and "K" are 16.57 MJ Kg^{-1} and 18.61 MJ Kg^{-1} respectively. The proximate analysis, ultimate analysis, GCV and NCV in the both strains were found to be at par with the values reported by Phukan et al. (2011) during the analysis of *Chlorella* sp. An increase in lipid content is linked to the increase in calorific value in case of algae. The high protein content in the strains can be contributed to its high nitrogen content. The high carbon, high hydrogen content, high HHV and no Sulphur content of both the strains make them a prospective contender for biofuel generation. The high protein and carbohydrate content make the microalgal strains a significant feedstock for the generation of bioethanol (Arif et al., 2021).

No Sulphur detection in microalgal biomass aids in the production of FAME (Fatty acid methyl esters) during the exploitation of lipids (Yew et al., 2020). However, it is not a limited

product from the exploitation of the lipids from microalgal biomass. Rather, the de-oiled residue after lipid extraction reopens enticements of different biofuels like bioethanol, biohydrogen etc. Also, the defatted biomass is also rich in value-added products like carbohydrates, vitamins, proteins, and pigments. This lipid-extracted microalgal biomass also has its application as a fertilizer and fish feed (Jaiswal et al., 2021).

6.3.2. FTIR analysis

Based on previously published studies, The FTIR peaks were assigned to a specific molecular group (Mecozzi et al., 2011) and were presented in Table 6.3. The FTIR spectra is able to identify the functional groups of the phytochemicals present. The findings suggest that the microalgal strains "I" and "K" biomass contain biochemical compounds like proteins, polysaccharides, lipids, and pigments. The growth conditions during the microalgal cultivation process may influence the presence or absence of any particular chemical constituent in the algal biomass. The FTIR spectra of the microalgal biomass were according to the results depicted in previously reported studies (Olasehinde et al., 2019).

Table 6.1. Biochemical and pigment analysis of the microalgae.

Microalgal strains	Carbohydrate (%)	Protein (%)	Chlorophyll (µg mL^{-1})		Carotenoids (µg mL^{-1})
			Chl-a	Chl-b	
I	28.34±0.48	42.13	10.86±0.67	6.11±0.45	2.71±0.18
K	31.17±0.63	50.89	12.23±0.77	7.67±0.38	3.25±0.29

Table 6.2. Proximate and Ultimate analysis of the microalgal biomass.

Parameters	I	K
	Proximate analysis	
Moisture content (%)	7.93±0.63	6.76±0.71
Volatile matter (%)	76.28±1.11	79.34±1.46
Ash (%)	7.95±0.42	7.61±0.77
Fixed carbon (%)	7.84±0.56	6.29±0.64
	Ultimate analysis	
C (%)	38.64	41.17
H (%)	7.17	7.65
N (%)	7.74	8.14
O (%)	38.50	35.43
C/N (%)	4.99	5.06
O/C (%)	0.99	0.86
HHV or GCV (MJ Kg^{-1})	18.15	20.30
LHV or NCV (MJ Kg^{-1})	16.57	18.61

Table 6.3. FTIR analysis of the microalgal strains.

Wavenumber range (cm^{-1})	Respective group	Respective biomolecules	Peaks obtained (wavenumber cm^{-1})	
			I	K
1120-1160	C-O-C	Carbohydrates as Polysaccharides	1117.12, 1164.09	1123.81, 1150.87
1230-1240	P=O and C-N bending	Phospholipids of nucleic acids and proteins (Amide III band)	1237.42	1242.46
1540-1550	C-N stretching and N-H bending	Proteins (Amide II band)	1538.51	1544.66
1610-1685	C=O stretching	Proteins (Amide I band)	1634.99	1635.07
1730-1745	C=O stretching	Lipids (esters of fatty acids) and carbonyl groups of chlorophyll pigments	1735.43	1740.32
2100-2200	C-C=C-C=C and CΞC	Lipids, chlorophyll and carotenoid pigments	2131.76	2131.67
2850	CH2 stretching	Aliphatic groups of lipids and proteins	2852.14	2859.45
2950	CH3 stretching	Aliphatic groups of lipids and proteins	2934.78	2931.56
3300-3400	OH stretching	Carbohydrates, proteins, lipids (sterols and fatty acids), nucleic acids	3338.90	3338.95

6.3.3. Bioethanol production from LEMBR through biorefinery approach

The present study describes a thorough protocol for microalgal biorefinery – with lipid as the main product, and pigments, proteins and carbohydrates as the side products, and bioethanol as the secondary product. The biorefinery approach presented in this study is viable and can be executed for amplifying the economy and revenue of the microalgal industry.

The lipid extracted microalgal biomass residue (LEMBR) has various applications like biogas, biochar and bioethanol production, pigments, animal and fish feed, and fertilizer. Further the simultaneous production of microalgal biomass along with lipid and carbohydrate content, which are used to produce biofuel, is considered to be an integrated biorefinery approach (Chokshi et al., 2016). Value added applications like these aids in lessening the overall cost of biofuel production and microalgal cultivation. To develop a sustainable biorefinery and enhance the economics of the process, scaling up of the cultivation process; processing of value-added products like proteins, pigments, bioethanol, cosmetics etc; high efficiency bioreactor designing is of utmost requirement.

This study reports a meticulous protocol for the production of lipids along with value added products and bioethanol from the biomass residue left after lipid extraction, following a biorefinery approach. The green microalgal strain *"*K" produced a substantial amount of maximum lipid (26 wt % approx.) and left behind algal biomass residue of approximately 74 wt %, which was used to produce bioethanol. The biorefinery approach depicted in the present study can therefore be considered substantially feasible and could be administered to magnify the algal industry's economy and revenue.

The strain "K" LEMBR biomass produced 26.34±0.24 % lipid along with 7.16±0.43 g L^{-1} bioethanol. The microalgal biomass was also rich in value-added bioproducts like pigments, proteins, and carbohydrates. The bioethanol content was in accordance with Olia et al. (2022), as they achieved maximum ethanol concentration of 8.31±0.18 g L^{-1} by treating the biomass of *Chlorella* sp. with sulphuric acid for 20 min at 121°C and a fermentation period of 48 h. Hence it can be concluded from their findings that the *Chlorella* LEMBR hydrolysate can be a feasible promoter of bioethanol, which may be industrially applied.

The experimental set up for the bioethanol preparation process is depicted in Figure 6.1.

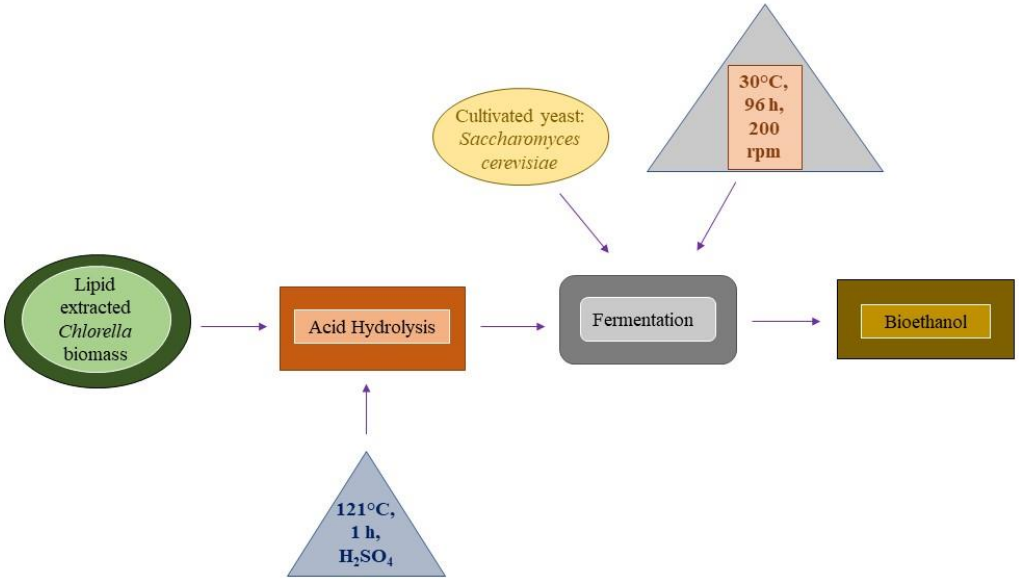


Figure 6.1. Experimental set up for the bioethanol preparation process.

6.4. Conclusion

This study highlights the potential of a bio-based refinery of two novel microalgal isolates in producing lipid, bioethanol, pigments, etc. The isolates demonstrated significant amount of carbohydrate, protein and lipid. The strains also exhibited significant pigments like chlorophyll-a, chlorophyll-b and carotenoids, allowing their utilization in food, cosmetic and pharmaceutical industries. The GCV for the strains "I" and "K" are 18.15 MJ Kg^{-1} and 20.30 MJ Kg^{-1} respectively and NCV for the strains are "I" and "K" are 16.57 MJ Kg^{-1} and 18.61 MJ Kg^{-1} respectively.

The spent biomass after extraction of lipids were reused and recycled for sustainable biofuel synthesis. Experimental results indicated that a waste-based refinery could lead to efficient production of value-added products from microalgae utilizing the organic wastes, in turn contributing to establishing a "green society". The lipid-extracted biomass residue of strain "K" generated 7.16±0.43 gL^{-1}.

References

Arif M, Li Y, El-Dalatony MM, Zhang C, Li X, Salama E-S (2021) A complete characterization of microalgal biomass through FTIR/TGA/CHNS analysis: An approach for biofuel generation and nutrients removal, Renewable Energy 163:1973-1982.

Artemio CP, Maginot NH, Serafin CU, Rahim FP, Guadalupe RQJ, Fermin CM (2018) Physical, mechanical and energy characterization of wood pellets obtained from three common tropical species, PeerJ 6:5504.

Becker EW (1994) Microalgae: Biotechnology and Microbiology, Cambridge university press, Cambridge.

Chakravarthy K, Veeramuthu A, Senthil C, Sailendra B, Ramasamy R (2014) Pretreatment of lipid extracted *Botryococcus braunii* spent biomass for bioethanol production, Int J Curr Biotechnol 2(1):11-18.

Chisti Y (2007) Biodiesel from microalgae. Biotechnology Advances, 25:294 – 306.

Cho DH, Ramanan R, Heo J, Kang Z, Kim BH, Ahn CY, Oh HM, Kim HS (2015) Organic carbon, influent microbial diversity and temperature strongly influence algal diversity and biomass in raceway ponds treating raw municipal wastewater, Bioresource Technol 191:481–487.

Chokshi K, Pancha I, Ghosh A, Mishra S (2016) Microalgal biomass generation by phycoremediation of dairy industry wastewater: An integrated approach towards sustainable biofuel production, Bioresource Technology 221:455–460.

Deeba F, Kumar B, Arora N, Singh S, Kumar A, Han SS, Negi YS (2020) Novel bio-based solid acid catalyst derived from waste yeast residue for biodiesel production, Renew Energy 159:127–139.

DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric Method for Determination of Sugars and Related Substances, Analytical Chemistry 28 (3):350–356.

Gerardo ML, Oatley-Radcliffe DL, Lovitt RW (2014) Integration of membrane technology in microalgae biorefineries, J Membr Sci 464:86–99.

Jaiswal KK, Kumar V, Vlaskin MS, Nanda M **(**2021) Impact of pyrene (polycyclic aromatic hydrocarbons) pollutant on metabolites and lipid induction in microalgae *Chlorella sorokiniana* (UUIND6) to produce renewable biodiesel, Chemosphere 285:131482.

Leu S, Boussiba S (2014) Advances in the production of high-value products by microalgae, Ind Biotechnol 10(3):169–183.

Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes, Methods in Enzymology 148:350-882.

Maurya R, Chokshi K, Ghosh T, Trivedi K, Pancha I, Kubavat D, Mishra S, Ghosh A (2016) Lipid extracted microalgal biomass residue as a fertilizer substitute for Zea mays L., Front Plant Sci 6:1266.

Mecozzi M, Pietroletti M, Tornambè A (2011) Molecular and structural characteristics in toxic algae cultures of *Ostreopsis ovata* and *Ostreopsis* spp. evidenced by FTIR and FTNIR spectroscopy, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 78:1572–1580.

Miazek K, Remacle C, Richel A, Goffin D (2017) Beech wood Fagus sylvatica dilute-acid hydrolysate as a feedstock to support *Chlorella sorokiniana* biomass, fatty acid and pigment production, Bioresource Technology 230:122–131.

National renewable energy laboratory (NREL) 2008.

Olasehinde TA, Odjadjare EC, Mabinya LV, Olaniran AO, Okoh AI (2019) *Chlorella sorokiniana* and *Chlorella minutissima* exhibit antioxidant potentials, inhibit cholinesterases and modulate disaggregation of β-amyloid fibrils, Electronic Journal of Biotechnology 40:1–9.

Olia MSJ, Azin M, Moazami N (2022) Application of a statistical design to evaluate bioethanol production from *Chlorella* S4 biomass after acid - Thermal pretreatment, Renewable Energy 182:60-68.

Pandey A, Srivastava S, Kumar S (2019) Isolation, screening and comprehensive characterization of candidate microalgae for biofuel feedstock production and dairy effluent treatment: A sustainable approach, Bioresource Technology 293:121998.

Pilone GJ (1985) Determination of ethanol in wine by titrimetric and spectrophotometric dichromate methods: collaborative study, J Assoc Off Anal Chem 68 (2):188-190.

Phukan MM, Chutia RS, Konwar BK, Kataki R (2011) Microalgae *Chlorella* as a potential bio-energy feedstock, Applied Energy 88:3307–3312.

Pruvost J, Van Vooren G, Le Gouic B, Couzinet-Mossion A, Legrand J. (2011) Systematic investigation of biomass and lipid productivity by microalgae in photobioreactors for biodiesel application, Bioresour Technol 102:150– 158.

Velazquez-Lucio J, Colla LM, Rodríguez-Jasso RM, Sáenz-Galindo A, Cervantes-Cisneros DE, Aguilar CN, Fernandes BD, Ruiz HA (2018) Microalgal biomass pretreatment for bioethanol production: a review, Biofuel Research Journal 17:780-791.

Yan D, Lu Y, Chen YF, Wu Q (2011) Waste molasses alone displaces glucose-based medium for microalgal fermentation towards cost-saving biodiesel production, Bioresour Technol 102:6487-6493.

Yen HW, Hu IC, Chen CY, Ho SH, Lee DJ, Chang JS (2013) Microalgae based biorefinery–from biofuels to natural products, Bioresource Technol 135:166–174.

Yew GY, Khoo KS, Chia WY, Ho YC, Law CL, Leong HY, Show PL (2020) A novel lipids recovery strategy for biofuels generation on microalgae *Chlorella* cultivation with waste molasses, Journal of Water Process Engineering 38:101665.

CHAPTER 7

CONCLUSION AND FUTURE SCOPE

7.1. Overall conclusion

Rise in worldwide energy demand and environmental concerns regarding the usage of fossil fuels has forced the world to look for other suitable replaceable fuels from sustainable and non-food materials. To address this concern, the potent utilization of microalgae for producing fuels and value-added bio products is deemed to play a key role. Microalgal biomass as a renewable feedstock has received substantial attention for the development of a sustainable energy system that can replace depleting fossil fuels in the area of producing energy. Biofuels formed from these renewable resources could aid in decreasing the world's dependence on conventional oil, and lessen the fossil fuel burning and CO_2 emission.

In this study, an integrated process for the production of lipid and bioethanol from microalgae cultivated using waste peel hydrolysate was presented. This entire process consisted of the following steps:

(1) Pre-treatment and hydrolysis of the waste peels

(2) Characterization of the waste peels.

(3) Isolation and identification of indigenous microalgae

(4) Microalgal cultivation in the peel waste hydrolysate

(5) Lipid extraction from the algal biomass

(6) Biochemical analysis of the microalgae.

(7) Bioethanol generation from the defatted LEMBR.

Two novel wild indigenous microalgae *Chlorella sorokiniana* KMBM_I (Strain "I") and *Chlorella sorokiniana* KMBM_K (Strain "K") were isolated and are considered as the model organism for this study. The study demonstrated sustainable bioenergy production with simultaneous value added bioproducts generation from microalgae through waste peel valorization. This bioconversion facilitates the way for emergence and creation of algae-based biorefinery through the production of biofuels and bioproducts. Further, this waste peels-based microalgal biorefinery concept could aid in establishing "zero waste" strategies with proper scale-up techniques.

Undergoing the optimized conditions through different pre-treatments, 46.17 (±0.77) g L^{-1}, 29.84 (±0.57) g L^{-1} and 35.90 (±0.43) g L^{-1} of maximum glucose concentration were acquired from potato (dilute acid treatment followed by enzyme hydrolysis), banana (autoclaving treatment followed by enzyme hydrolysis) and sweet lime (dilute acid treatment followed by enzyme hydrolysis) peel respectively. The resultant extract was then utilized as the culture medium for the cultivation of both strains to attain enhanced level of microalgal biomass and lipid content.

When the effect of varying culture conditions were investigated utilizing the different peel hydrolysates, the highest biomass yield of 2.56±0.09 g L^{-1} was observed when *Chlorella sorokiniana* KMBM_K was cultivated in the mixed peel extract of potato, banana and sweet lime.

Chlorella sorokiniana KMBM_K contains maximum lipid content of 26.34±0.24 % which can be further processed to produce biodiesel while the spent defatted LEMBR was utilized to produce bioethanol of 7.16±0.43 g L^{-1}.

While the previous studies were concentrated primarily on the isolation of potential microalgae and mainly analyzed the lipid content for production of FAME (Fatty acid methyl esters). However, this study focusses on all the major biocomponents such as carbohydrates for biofuel generation, proteins, pigments, and lipid content as well.

For our experiments, the substrate (peels) was procured from inside the IIT Guwahati campus and were of zero value. However, the power consumption of the autoclave (portable Equitron autoclave) was 0.8 units per cycle. Since we used 40 minutes of autoclaving for the banana peels, two cycles were used, as one cycle equals 20 minutes. Hence a total of 1.6 units (0.8 + 0.8= 1.6) of power consumption happened throughout the pre-treatment process. The potato and sweet lime peels were acid hydrolysed with 1% Sulphuric acid for 80 minutes in the autoclave. Hence a total of 3.2 units (0.8+0.8+0.8+0.8= 3.2) of power consumption happened throughout the pre-treatment process for these two waste peels. The Cost of Laboratory grade Sulphuric acid was Rs. 1092 for 2.5 L, which is approximately 13.19 USD.

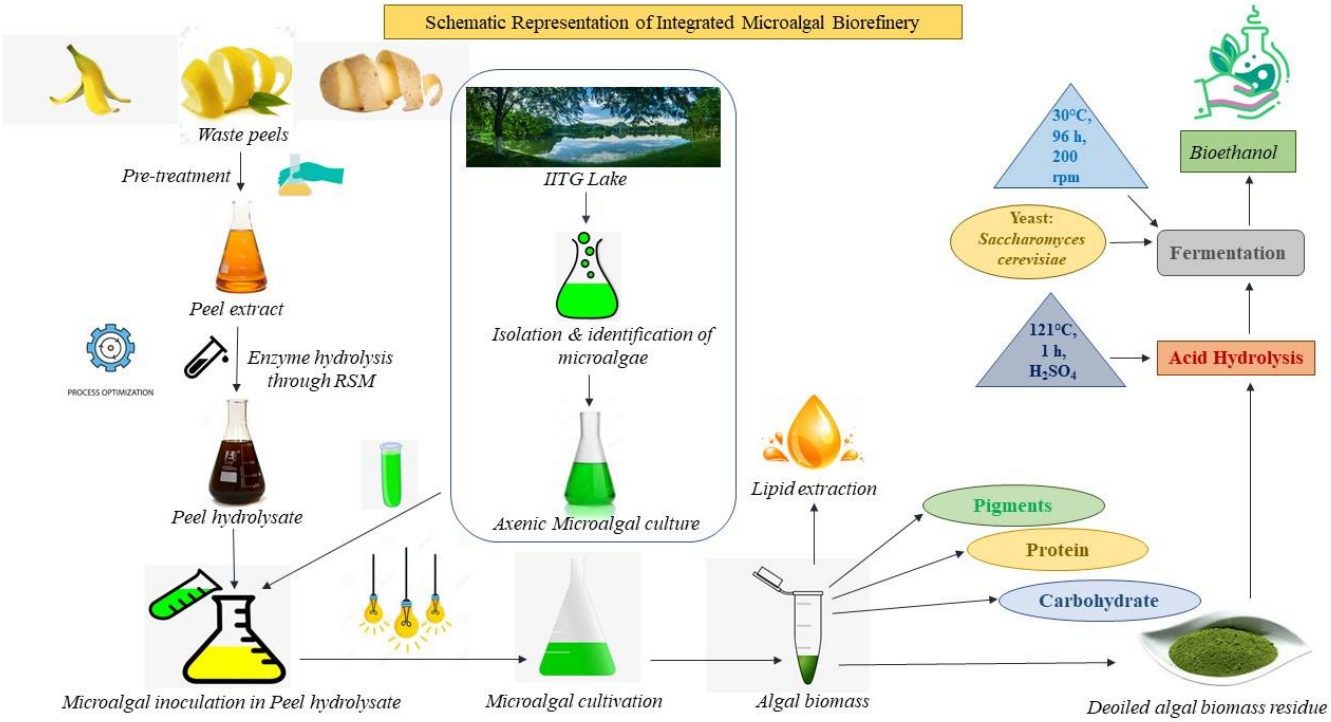


Figure 7.1. Schematic Representation of the Integrated Microalgal Biorefinery.

7.2. Future Prospects

The present findings had made it evident that the two wild microalgae are excellent aspirants for a sustainable biorefinery producing biomass, lipid, biofuel and other value-added products. However, the current study was limited to a lab scale. Therefore, the research can be extended to support the commercial implementation of the proposed biorefinery model.

- Scale up studies through open raceway ponds and closed large photobioreactors for mass biomass production. This will help study the effect of both the open and closed system on the microalgal growth and composition and the scale up effect on the microalgal cultivation.
- To determine the sustainability of the overall process, technoeconomic analysis and life cycle studies can be carried out.
- Genetic modification or alterations of the isolated strains can be carried out for enhanced biomass and lipid content.
- Development of an energy efficient process for the direct conversion of the wet biomass to bio-oil.

- This study employed the lipid extracted microalgal biomass residue (LEMBR) to obtain bioethanol. It can be further analyzed to check its other potential applications.

List of Publications

JOURNAL (From Thesis):

1. Malakar B, Das D, Mohanty K (2020) Optimization of glucose yield from potato and sweet lime peel waste through different pre-treatment techniques along with enzyme assisted hydrolysis towards liquid biofuel. **Renewable Energy** 145:2723-2732. https://doi.org/10.1016/j.renene.2019.08.037.
2. Malakar B, Das D, Mohanty K (2022) Utilization of waste peel extract for cultivation of microalgal isolates: a study of lipid productivity and growth kinetics. **Biomass Conversion and Biorefinery**. https://doi.org/10.1007/s13399-022-02313-7.
3. Malakar B, Das D, Mohanty K (2022) Evaluation of banana peel hydrolysate as alternate and cheaper growth medium for growth of microalgae *Chlorella sorokiniana*. **Biomass Conversion and Biorefinery**. https://doi.org/10.1007/s13399-022-03106-8.
4. Malakar B, Das D, Mohanty K (2023) Utilization of Chlorella Biomass Grown in Waste Peels-Based Substrate for Simultaneous Production of Biofuel and Value-Added Products Under Microalgal Biorefinery Approach. **Waste and Biomass Valorization**. https://doi.org/10.1007/s12649-023-02058-y.

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1. Sarma PJ, Malakar B*****, Mohanty K (2023) Self-sustaining bioelectricity generation in plant-based microbial fuel cells (PMFCs) with microalgae-assisted oxygen-reducing biocathode. **Biomass Conversion and Biorefinery**. https://doi.org/10.1007/s13399-023-03848-z. ***Equal first author contribution**.

BOOK CHAPTER:

1. **Malakar B**, Mohanty K (2020) The budding potential of algae in cosmetics *Algae (Springer)* 181-199. https://doi.org/10.1007/978-981-15-7518-1_8.

CONFERENCES:

1. B Malakar, D Das, K Mohanty, Comparison of glucose yield from potato and banana peels using different pre-treatment conditions with respect to various time profiles, Indian chemical engineering congress CHEMCON, Indian Institute of Technology Guwahati, 27-30 December, 2015.

2. B Malakar, D Das, K Mohanty, Study on glucose yield from sweet lime peels using different pre-treatment conditions with respect to various time profiles, International conference on Waste management (RECYCLE), Indian Institute of Technology Guwahati, 1-2 April, 2016.

3. B Malakar, D Das, K Mohanty, Comparative study on Enzyme assisted different pre-treatments on various biomasses, Indo Japan bilateral symposium on Future prospect of bioresource utilization in northeast India, Indian Institute of Technology Guwahati, 1-4 February, 2018.

4. B Malakar, K Mohanty, Optimization of glucose yield from autoclaved and enzyme hydrolyzed banana peels, International conference on Biotechnological research and innovation for sustainable development, Indian Institute of Chemical Technology Hyderabad, 22-25 November 2018.

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Optimization of glucose yield from potato and sweet lime peel waste through different pre-treatment techniques along with enzyme assisted hydrolysis towards liquid biofuel

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ABSTRACT

In this work, potato and sweet lime peels otherwise considered as waste were used as substrates for producing glucose. Different pre-treatment processes were carried out and the best conditions yielding higher amount of glucose concentration were further hydrolysed by enzyme. The pre-treated extract at 80 min in case of dilute acid hydrolysis with autoclaving conditions gave higher glucose concentration amongst all the pre-treatment processes for both the peels and hence considered for further analysis. Rationale for the next step of the experiment was designed based on a central composite statistical design (CCD). To obtain high glucose yield, response surface methodology (RSM) was used to optimize the hydrolysis conditions. The three parameters chosen for the study were; time (h), temperature (^{o}C) and the rotation frequency of the incubator (revolutions per minute i.e. RPM). The optimum conditions in case of potato peel were found to be 56 h, 68 °C and 144 RPM and in case of sweet lime peel, 56 h, 68 °C and 167 RPM. Under these conditions, 46.17 (±0.77) gL^{-1} and 35.90 (±0.43) gL^{-1} of glucose were obtained for potato and sweet lime peels respectively. The optimum factors acquired from the statistical model were further confirmed using the experimental results.

1. Introduction

The most abundant renewable energy resource available in the world is biomass, which is composed of cellulose, hemicelluloses and lignin [1]. Fruit and vegetable peels are generated in huge amounts in industries, hostels, juice centres and households and are mostly dumped along with other wastes without segregation making it unfit for further use. This study is an attempt to utilise these waste peels for the recovery of sugars towards producing biofuels as well as betterment of the environment. Potato is accepted as the staple food and is a main source of carbohydrate in many parts of the world and an integral part of much of the world's food supply. Sweet lime (mosambi or musambi) is a juicy fruit which is a leading juice substrate in all the juice based industries. A favourite among the juice lovers, sweet lime, is a rich source of vitamins and minerals. Potato dominates the chips producing industries the same way sweet lime dominates the juice and jam-jelly making industries. India is a major producer of both potatoes and sweet limes. The potato and sweet lime peels can be regarded as an agricultural waste product. The solid peel, discarded in the industries is zero value by-product, collected in large amounts depending on the type of peeling in these industries after their processing. Mostly abrasion peeling and steam peeling are employed in these food processing industries [2]. The crude composition of potato peel (*Solanum tuberosum*) and sweet lime (*Citrus limetta*) peel has been reported as: 55.25% cellulose, 11.71% hemicelluloses, 14.24% lignin [3] and 18.3% cellulose, 26.2% hemicelluloses, 8.9% lignin [4] for potato and sweet lime peel respectively.

Biomass cellulose is composed of crystalline and amorphous components and the amorphous component is more easily digested by enzymes than the crystalline component [5]. Highly specific cellulose enzyme carries out enzymatic hydrolysis and reducing sugars like glucose is the product of such hydrolysis. For enhancing the hydrolysis of cellulose, it is required to pre-treat the biomass which in turn decreases the crystallinity of cellulose present in it. Hence, before enzymatic hydrolysis it is crucial to pre-treat the biomass [6]. Different pre-treatment techniques have been

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ORIGINAL ARTICLE

Utilization of waste peel extract for cultivation of microalgal isolates: a study of lipid productivity and growth kinetics

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Abstract
The excessive price of nutrients needed for microalgal cultivation is a vital constraint, although mass production is essential for attaining economically viable production of algal fuels and value-added products. Increasing energy demand has prompted the world to look for alternative oil resources. There is a crucial demand for sustainable and economically viable source of energy due to the reduction in conventional fossil fuel supplies. In this study, potato peel and sweet lime peels were exploited in place of conventional growth mediums for the cultivation of microalgal cells. These wastes are zero-value material, which contains vital nutrients and elements for the cultivation of oleaginous microorganisms like microalgae. The growth of the isolated strains *Chlorella sorokiniana* KMBM_K and KMBM_I in the pretreated peel hydrolysates was investigated. The biomass and lipid extracted can be further investigated for potential applications in biofuel, feed, and food additives. A new insight can be obtained with this study as it integrates the concept of lipid extracted microalgal biomass utilization (LEMB) approach along with waste disposal, thereby serving in the management of these agricultural wastes. While performing the optimization studies, pH 7 and 25% and 40% dilution, respectively, for potato peel and sweet lime were found to support more biomass and lipid yield. The highest biomass yield of 2.1 g L^{-1} and highest lipid productivity of 49.93 mg L^{-1} day^{-1} are obtained in *Chlorella sorokiniana* KMBM_K when cultivated in potato peel extract. This study showed that these agricultural wastes can be used as a natural, cost effective, ecofriendly, efficient, and affordable substrate for the cultivation of the microalgal isolates.

Keywords Peel · *Chlorella sorokiniana* · Lipid·mass cultivation · Biorefinery · Food processing waste

Highlights
- Potato and sweet lime peel hydrolysate were used for microalgal cultivation.
- Microalgal strains were isolated from biodiversity-rich waterbodies of IIT Guwahati, Assam.
- The isolated strains were identified as *Chlorella sorokiniana* KMBM_K and KMBM_I.
- The peel hydrolysates support the cultivation of the isolated microalgae.

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1 Introduction

Microalgal fuels have the budding possibility to aid the sector of renewable energy, although the nutrient and water dependence are seen as crucial issues for mass cultivation. Growing them on a commercial scale seems even more expensive. It has been reported that per year, US needs more than 85% of the annual freshwater to create one billion gallons of microalgal biodiesel [1]. The continuous usage of fossil fuels has paved its way into one of the most vital world-wide environmental challenge, i.e., global warming. The decrease in oil deposits and increase in fuel rates have forced the mankind in search of alternative sustainable energy resources as a substitute for conventional fuels. Biomass such as microalgae is a suitable candidate for that and has gathered more interest than land plants and crops because they have faster growth rate, higher photosynthetic efficacy, high oil content, and sustainable biomass production [2]. Oleaginous microbes like microalgae, fungi, and

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ORIGINAL ARTICLE

Evaluation of banana peel hydrolysate as alternate and cheaper growth medium for growth of microalgae *Chlorella sorokiniana*

Barasa Malakar[1] · **Debasish Das**[1,2] · **Kaustubha Mohanty**[1,3]

Received: 9 May 2022 / Revised: 14 July 2022 / Accepted: 17 July 2022

Abstract
Continuously escalating energy demand and depletion of conventional fossil fuels has challenged and urged our globe to search for alternative resources which are economically viable, renewable and sustainable. Microalgae has emerged as an excellent resource in this regard but the excessive cost of nutrients is a vital restriction for producing economically viable algal fuels. In this study, waste banana peels were used as the feedstock for cultivation medium for microalgae after proper pre-treatment and hydrolysis. Various pre-treatment techniques were executed and the best conditions (40 min, autoclaving) furnishing the higher glucose concentration were chosen for further investigation. Rationale for the enzyme hydrolysis of the pre-treated peels was created through central composite design (CCD) using response surface methodology (RSM) to attain higher concentration of glucose to optimize the hydrolysis parameters. Temperature (°C), time (h) and the rotation frequency or agitation speed (revolutions per min, RPM) of the incubator were the three chosen parameters. 29.84 ± 0.57 g L^{-1} glucose was obtained from the banana peels. The peel extract after hydrolysis was used as the medium for microalgal growth, and the growth kinetics of the two microalgal isolates (*Chlorella sorokiniana* KMBM_I and *Chlorella sorokiniana* KMBM_K) cultivated in the waste peel hydrolysate were evaluated. *Chlorella sorokiniana* KMBM_K and *Chlorella sorokiniana* KMBM_I displayed a biomass yield of respectively 1.72 and 1.53 g L^{-1} with a lipid content of 22.83% and 22.17% respectively. This study demonstrated that this organic peel waste can act as an effective, eco-friendly, efficient and natural substrate for cost effective microalgal cultivation.

Keywords Banana peel · *Chlorella sorokiniana* · Extract · Pre-treatment · Hydrolysis

1 Introduction

The world today is majorly dependent on fossil resources as the main energy supply. However, the gradual depletion of these resources has resulted in a global concern. As the existing fuel reserves are left only for few more decades, hence development of alternative fuel resources is the need of the hour. Biofuels derived from plants and microbial origin acts as an alternative to fossil derived fuels due to similar properties [1, 2]. However, sustainability of biofuel depends on the water supply, land use, demand of nutrients and fertilizer as well as competition with food crops. Due to their rapid biomass production and high oil content, microalgae are considered advantageous than terrestrial plants [3, 4]. Despite having various advantages, commercialization of microalgal fuels has been considered expensive as its cultivation depends on costly growth medium. To reduce this drawback, substitution of chemical medium with low-cost medium appears to be a solution. To produce commercially viable biofuel, organic waste as feedstock for microalgal growth can be considered an effective solution, as these organic resources are rich in sugars, carbohydrates and nutrients. In this regard, waste peels of vegetables and peels could act as a good feedstock. Hydrolytic treatment on these wastes alters its physiochemical properties resulting in release of carbohydrates and mineral nutrients [5, 6]. Selection of a suitable pre-treatment is required to produce an appropriate growth medium for algae cultivation as growth medium could influence the growth rate, biomass and lipid

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Waste and Biomass Valorization
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ORIGINAL PAPER

Utilization of *Chlorella* Biomass Grown in Waste Peels-Based Substrate for Simultaneous Production of Biofuel and Value-Added Products Under Microalgal Biorefinery Approach

Barasa Malakar[1] · Debasish Das[1,2] · Kaustubha Mohanty[1,3]

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Abstract
Two indigenous microalgae strains *Chlorella sorokiniana* KMBM_I and *Chlorella sorokiniana* KMBM_K were tested for their growth and adaptability in the peel wastes. Growth kinetic parameters of the strains were analyzed in varying culture conditions. This study highlights the potential of a bio-based refinery of novel microalgal isolates in producing lipid, bioethanol and pigments etc. The isolates demonstrated significant amount of lipid, protein, carbohydrate and pigments like chlorophyll-a, chlorophyll-b and carotenoids. The spent biomass after extraction of lipids were reused and recycled for sustainable biofuel synthesis. Experimental results indicated that a waste-based refinery could lead to efficient production of value-added products from microalgae utilizing the organic wastes, in turn contributing to the establishment of a 'green society'. The highest biomass yield of 2.56 ± 0.09 g L^{-1} and lipid content of $26.34 \pm 0.24\%$ was observed when the microalgal strain 'K' was cultivated in the mixed peel extract of potato, banana and sweet lime. Also, 7.16 ± 0.43 g L^{-1} bioethanol was derived from its spent biomass after the lipid extraction.

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ORIGINAL ARTICLE

Self-sustaining bioelectricity generation in plant-based microbial fuel cells (PMFCs) with microalgae-assisted oxygen-reducing biocathode

Pranab Jyoti Sarma[1] · **Barasa Malakar**[1] · **Kaustubha Mohanty**[1,2]

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Abstract
Microalgae-based biocathode development was studied for the first time in a three-chamber PMFC incorporating *Chlorella sorokiniana.* It represents an efficient, cost-effective and eco-friendly strategy for increase oxygen availability to the cathode. Three different indoor plants viz., *Epipremnum aureum*, *Philodendron erubescens* and *Anthurium andreanum* were chosen for this study. Among all the PMFCs, *P. erubescens* showed the highest performance, with the power density and current density of 32.21 mW m^{-2} and 63 mA m^{-2} respectively. This represents a 31% increase with respect to previous studied bacterial biocathode and is amongst the best in PMFC research reported till date. Cyclic voltammetry (CV), dissolved oxygen (DO) and Field emission scanning electron microscopy (FESEM) analysis confirmed microalgal biocathode development. The presence of *C. sorokiniana* in the cathode increases the DO concentration and the oxygen reduction rate with a higher reduction current density of −16.2 mA m^{-2} as compared to mechanical aeration (−5.6 mA m^{-2}). Anodic microbial community structure showed a diverse bacterial consortium dominated by Firmicutes (56.84%) and Bacteroidetes (38.45%), which are known for their ability to generate bioelectricity.

Keywords Bioelectricity · PMFC · Biocathode · Microalgae

1 Introduction

The demand for renewable energy has been consistently rising in recent times due to concerns of the rapid exhaustion of our fossil fuel resources and the rise in global warming in the world. In the arena of bioelectricity generation, the microbial fuel cells (MFCs) technology has gained widespread attention as a potential bioelectrochemical system (BES) to generate bioelectricity by active microbial action on wastes. As an alternative to the traditional MFC system, the plant microbial fuel cell (PMFC) technology has the potential to explore new arenas to the already existing BES technology [1, 2]. It has evolved as a self-sustainable, eco-friendly, and economically viable option for nutrient requirements for microbes [3, 4]. In a PMFC, plants grown in the anodic chamber carry out photosynthesis by utilizing sunlight and produces organic substances. In this process, the atmospheric CO_2 is fixed, which does help in biomass development in plants. However, the quantity of organic substances produced is not fully utilized by the plant itself, and a portion always remains unutilized and is laid off through roots as rhizodeposits [5]. Near the root surfaces, the living anaerobic microbial consortium utilizes the organic matters released by plants and disintegrates them into protons and electrons. These can further be harnessed by electrodes placed near the root surfaces to generate bioelectricity [6]. In this way, PMFCs have become a source of bioelectricity in the utmost sustainable way as the nutrient requirements for microbes are met continuously. The plants need to be taken care of and maintained under healthy conditions. In this way, it turns out to be an economically viable and sustainable nutrient requirement option as organic compounds need not be added externally at repeated intervals. Moreover, growing plants in a PMFC provides multiple benefits of purifying air from harmful chemicals and energy generation. Various studies have discussed the fundamental principle of a PMFC in detail [3, 6–8].

Pranab Jyoti Sarma and Barasa Malakar share equal authorship.

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The Budding Potential of Algae in Cosmetics

8

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