CLIMATE RESILIENT PRODUCT DEVELOPMENT FROM SEAWEEDS *KAPPAPHYCUS ALVAREZII, GRACILARIA CORTICATA* AND WATER HYACINTH *EICHHORNIA CRASSIPES*

by

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(2013 - 20 - 105)

THESIS

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DECLARATION

I, Shamiya Hasan (2013 – 20 – 105) hereby declare that this thesis entitled **“climate resilient product development from seaweeds *Kappaphycus alvarezii, Gracilaria corticata* and water hyacinth *Eichhornia crassipes*”** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Shamiya Hasan

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SYMBOLS AND ABBREVIATIONS

AOAC	Association of Analytical Communities
As	Arsenic
Cd	Cadmium
CIFT	Central Institute of Fisheries Technology
CMFRI	Central Marine Fisheries Research Institute
CO_2	Carbon dioxide
Cr	Chromium
CSMCRI	Central Salt and Marine Chemicals Research Institute
Cu	Copper
CUSAT	Cochin University of Science and Technology
EC	Electrical Conductivity
et al	And others
Fe	Iron
FTIR	Fourier Transform Infrared Spectroscopy
GHGs	Green House Gases
IPCC	Intergovernmental Panel on Climate Change
IUCN	International Union for Conservation of Nature
MPa	Mega Pascal
N	Normal
Ni	Nickel
NIO	National Institute of Oceanography
rpm	Rotation Per Minute
Se	Selenium
SEM	Scanning Electron Microscopy
Spp.	Species
STIC	Sophisticated Test and Instrumentation Centre
XRD	X- Ray Diffraction Index
°C	Degree Celsius
<	Less than

INTRODUCTION

CHAPTER 1

INTRODUCTION

Climate change is a global phenomenon and a significant problem (Schmidt *et al,* 2013). Climate change affects the national economy and livelihoods of several populations, especially the coastal inhabitants, which necessities the need to develop resilient strategies. Effective research interventions could negate the climatic impacts. Carbon foot print reduction is one of the targets to mitigate the climatic changes. Depending on emissions and adherence to global agreements such as Paris Climate Agreement (2017), atmospheric Carbon dioxide [CO_2] could reach 540 p-p-m. by 2050 and up to 940 p-p-m. by 2100 (Meinshausen *et al,* 2011). The Paris Agreement advocates nations to move towards development of strategies for low carbon emissions or for carbon sequestration.

Seaweeds are generally known as marine algae. There are mainly three types of seaweeds according to their color; red (Rhodophyta), brown (Phaeophyta), and green (Chlorophyta). As compared to land plants seaweed lack the plant characters like root, shoot, flowers, and pollen etc. seaweeds play a vital role in the marine ecosystem, they provide food, shelter for young fishes and other organisms. The ability of seaweeds for carbon sequestration has been demonstrated in several studies (Ritschard, 1992; Kaladharan *et al.,* 2009; Chung, 2011) and seaweed farming along Indian coastal waters is also identified as prospective climate resilient strategy (Zacharia *et al.,* 2015). The carbon sequestration ability of seaweeds shall have beneficial implication on development of climate resilient products like biofuel. Among the three seaweeds, red seaweeds are capable to produce high amount of carbohydrates and bioethanol (Meinita *et al.,* 2012). Seaweeds could be used for mitigation of ocean acidification as well as for marine pollution. Seaweeds constitute a significant part of the global marine biodiversity and the product development and value addition shall be gainful for conservation strategies as well as for carbon sequestration.

Eichhornia crassipes, commonly known as water hyacinth was introduced into India as an ornamental plant in 1896 from Brazil, but later on become one of the prominent weeds in the water bodies of Kerala. The management of this weed has become one of the serious problems nowadays. Product development from water hyacinth is a sustainable way to utilize the aquatic weed. Biofuel and biochar production from water hyacinth is a step towards climate mitigation strategy. Biochar is a very useful material which can be used for enhancing the carbon sequestration. Water hyacinth can be converted into biochar and applied for agricultural and aquaculture practices. Thus the population of water hyacinth, which is a major cause of the deterioration of the water bodies of Kerala, can be effectively managed.

Most of the world's energy need is met by fossil fuels. The use of fossil fuels results in the emission of Greenhouse Gases such as Carbon dioxide (76%), Methane (13%), Nitrous oxide (6%) and Fluorocarbons (5%) (Dresselhaus and Thomas, 2001). Level of pollutants and the level of probable carcinogens can be reduced by the use of biofuel (Ma and Hanna, 1999). The fossil fuel reserves across the globe are depleting in a fast pace which also points out towards the need to explore alternative energy sources and in this context the present study to use seaweeds and water hyacinth as bioenergy feed-stock is of high significance.

Climate resilient product development from seaweeds shall enhance the feasibility of large scale seaweed farming as well as could be a significant step towards seaweed based biorefinery development across the coastal states. This shall further enhance the economy, industry and livelihoods of the nation. Upon successful commercialization of seaweed products, opportunities shall emerge for coastal fishermen/farmers to take up seaweed farming as a livelihood option. Seaweed could become a key link between energy, environment, and climate change.

The water demand is getting increased due to population growth, industrialization, and civilization. So it is necessary to enhance the water purification technology. Phytoremediation is the process of using plants to clean up polluted soil or water. The biochar and the raw water hyacinth have particular interest for water remediation. The characters like dense fibrous root system of water hyacinth provides an extensive surface area for absorption, adsorption, and for micro-organism attachment. During the conversion of water hyacinth to biochar through pyrolysis, this increases the porous structure and surface area of water hyacinth.

The production pathway of biofuel from seaweed and water hyacinth include the pre-treatment method with hydrogen peroxide and acid hydrolysis with sulphuric acid. The pretreatment with hydrogen peroxide have many benefits over acidic pretreatment. The oxidation of peroxide doesn't leave any environmentally toxic acid wastes or any toxic residues. The low lignin content of seaweeds is an advantage over terrestrial plants, which improves the enzymatic hydrolysis of cellulose. Acid hydrolysis is most widely used for the efficient separation of glucose and other polysaccharides. The high proportion of polysaccharides and the ability of microorganisms (*Saccharomyces cerevisiae*) to convert these sugars into ethanol are factors that drive the use of this co-product for the production of biofuels such as ethanol (Girio *et al.*, 2010). The biochar production from water hyacinth is through pyrolysis at different temperature and at different frequencies. Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and Scanning Electron Microscopy (SEM) are the methods used for the structural analysis of different samples of seaweeds and water hyacinth.

The objectives of the thesis are as listed below

1. To develop seaweed and water hyacinth based products and processes as a climate change adaptation strategy
2 To assess the implications of seaweed and water hyacinths bioproducts in the climate change context

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1. CLIMATE CHANGE AND GLOBAL WARMING

Climate change has a significant impact on both life and natural resources, economic and environmental risk worldwide (Schmidt *et al.,* 2013). Earth's warming in recent decades has been caused mainly by human activities which leads to the increase of greenhouse gases (GHGs) in the atmosphere and rise in global temperature which is termed as global warming (Brevic, 2012).

The concentration of GHGs in the atmosphere has increased considerably in past decades due to human activities. Carbon dioxide (CO_2) is one of the most important greenhouse gas released due to anthropogenic activities and the annual emissions of Carbon dioxide increased by about 80% in the last few decades. Activities such as the burning of fossil fuels, cutting down forests etc. were identified as the main causes of the increase in concentration of CO_2 in atmosphere (Houghton *et al*., 1992).

Carbon dioxide is one of the significant contributors to global warming and climate change, which is 60% of global warming or total greenhouse effect (Rastogi *et al.,* 2002). The percentage-wise distribution of contribution of Green House Gases to global warming are CO_2 (54.7%) methane (30%), nitrous oxide (4.9%), fluorinated gases (0.6%) and other gases (9.8%) (IPCC, 2007).

Carbon dioxide, considered as the primary greenhouse gas, is the major contributors to positive increases in earth's temperature (IPCC, 1996). Since the industrial revolution, the concentration of atmospheric CO_2 and other GHGs has been increasing. The CO2 concentration of the atmosphere has changed significantly since the industrial revolution, increasing from 280 ppm to 390 ppm and it may double by the end of the 21st century if greenhouse gases continue being emitted (Lal, 2003).

2.2. CLIMATE RESILIENT STRATEGIES

The IPCC defines climate resilience is the ability of a social and ecological system to prepare for, mitigate and recover from the stress and changes caused by climate change (IPCC, 2007). Since climate change is a global challenge, it is important to understand its impacts and effect for developing relevant adaptation strategies to reduce the impacts and effects on different sectors in the present climate change scenario (Singh *et al.,* 2013).

The IPCC defines climate change adaptation as the process of adjustment to actual or expected climate and its effects (IPCC, 2014). In this context we should pay more attention on enhancing seaweed production as climate change adaptation as well as climate resilient strategy in view of its potential to provide refugee from ocean acidification and ocean deoxygenation (Duarte *et al*., 2017) and the development of climate resilient products. It cans also have the capacity to support coastal ecosystems like natural kelp forests and macroalgal beds (Smale *et al.,* 2013).

2.2.1. Seaweed farming

Seaweed farming at locations which faces particular risk from climate change impacts like low-lying coastal areas, vulnerable to flooding during storms with increasing sea level, areas prone to exposure to acidified and/or deoxygenation waters, may offer a strategic approach to enhance the benefits of seaweed aquaculture as climate change adaptation (Duarte *et al*., 2017). Seaweeds have high productivity associated with high pH on daytime by photosynthesis. Thus there is a reduction in ocean acidification which enhances the calcification process (Krause-Jensen, 2015) and also reported that the giant kelp forest is supporting the calcification of lobsters, crabs, molluscs, and crustaceans.

Seaweeds are responsible for much of CO_2 capture in marine vegetated habitats as they can generate far more organic matter through photosynthesis than consumed by respiration in the ecosystem (Duarte and Cebrian, 1996).

India has a coastline of about 7,500 km including islands of Andaman & Nicobar and Lakshadweep. Kerala has a coastline of about 580 km, with 9 coastal districts (Jha *et al.*, 2009). In the world mainly there are 42 countries with reports of commercial exploitation of seaweeds and there are ten countries which contribute up to 95% of the world's commercial seaweed utilization. Among them, China holds first rank, followed by North Korea, South Korea, Japan, Philippines, Chile, Norway, Indonesia, USA and India. (Khan and Satam, 2003). Throughout the world about 20,000 species of seaweeds are distributed, of which 221 are commercially utilized, which includes 145 species for food and 110 species for phycocolloid production (Sahoo, 2000). In India, the marine floral diversity includes a total of 844 species of seaweeds (in 217 genera) (Rao, 1970).

The estimated stock of seaweeds in Kerala is 1,000 tonnes, of which about 150 tones are economically important seaweeds (Chennubhotla *et al.*, 2013). The major Indian seaweed species are *Gracilaria, Gelidiella, Ulva, Caulerpa, Sargassum, Enteromorpha, and Codium* (Siddhanta *et al,* 2009).

According to Yadav *et al.* (2015) in the Kerala coast, red seaweeds are dominant (Rhodophyceae) with 19 species (45%), followed by green (Chlorophyceae) with 14 species (33%) and brown (Phaeophyceae) with 9 species (22%). The southern districts (Thiruvanthapuram and Kollam) and the northern districts of the state are suitable for luxuriant growth of seaweeds. As compared to other maritime states in India, Kerala coast have less abundance of the economically important seaweeds. By encouraging the coastal community for seaweed cultivation, Kerala can overcome the present scenario and can establish seaweed based industries and also can support the fishermen/farmers livelihood.

Indian has a vast coastline and has the potential to become seaweed farming zone. Seaweed farming is a low coast technology with less man power requirement, can be met by the fishermen community. It will further enhance the livelihood of fishermen by supplying an additional income. Central Marine Fisheries Research Institute (CMFRI) Cochin, Central Salt and Marine Chemicals Research Institute (CSMCRI) Bhavnagar and National Institute of Oceanography (NIO) Goa are organisations that attempted mariculture of seaweed. In Indian coastal waters the farming of seaweed started with *Gracilaria edulis*, and later *Kappaphycus* farming became popular Seaweed have carbon sequestration potential and and can be used as an alternative source for bioethanol production in a country like India, which have large number of automobiles. Therefore, successful farming of seaweeds have multiple benefits like feedstock for bioethanol production, as means of carbon sequestration and for ocean acidification mitigation and as supportive option for coastal livelihood improvement, etc.(Zacharia *et al.*, 2015). Compared to land based farming, Seaweed can be cultivated in the sea in a large area with little cultivation requirements like water supply and fertilizer application (Buck *et al.*, 2004).

So the bioethanol production from both micro and macroalgae biomass is undoubtedly a sustainable and eco - friendly approach for renewable fuel production (John *et al.*, 2011).

2.2.2. Carbon sequestration through biochar

Eichornia crassipes (water hyacinth) has been identified as one of the 100 most cumbersome invasive species and one of the top 10 worst weeds in the world declared by International Union for Conservation of Nature (IUCN) due to its high reproductive rate and dispersion (Patel, 2012). The aggressive invasion of *E. crassipes* destroying the native biodiversity of many ecologically and economically important water bodies and productive wetlands by starving the oxygen has been documented (Tellez *et al.*, 2008).

The substantial conversion of aquatic weeds (water hyacinth) into climate resilient products such as biochars, biofuels and other value added products, which provides gain on enhanced carbon sequestration. The biomass is converted into bioethanol through acid hydrolysis followed by fermentation and to biochar through pyrolysis process (Rojith and Zacharia, 2015).

Unprocessed or processed all organic waste materials can be utilized as feedstock for biochar production by pyrolysis. Fast growing varieties of aquatic plants can be used as feedstock for biochar production. Biochar can play a role to mitigate the major two aspects of climate change, carbon sequestration and reduction of Green House Gases. Because of its recalcitrant nature, biochar have shown that it has a potential for long-term carbon sequestration (Parmar *et al.,* 2014). The conversion of *Eichornia crassipes* biomass into biochar enhances the carbon stability and also an interesting material to mitigate global climate change (Lehmann, 2007). Biochar derived from macroalgae can be used as a soil ameliorant and a tool for long-term carbon sequestration (Bird *et al.,* 2011).

The conversion of waste material into useful products like biochar, is a promising technology for recycling and discarding of waste materials (Cao and Harris, 2010). Biochar can be used as a powerful technology to reduce carbon emission. Biochar produced through pyrolysis process using different biomasses can be used as soil carbon pools. Therefore sustainable production of biochar can help combat global climate change by displacing fossil fuel use and by carbon sequestration.

2.3. CLIMATE RESILIENT PRODUCTS DEVELOPMENT

2.3.1. Biofuel

The land based crops like maize, corn, sugar, wheat etc. are the main feedstock for bioethanol production. There are chances to arise issues related to increasing the proportion of land use for biomass crops. Marine environment thought to be generating approximately 50% of global biomass. By considering marine biomass as a source of carbon for bioethanol production, these issues are negated and the "food versus fuels" debate is not applicable to marine biomass (Adams *et al.*, 2009). The production of biofuel from seaweed not only reduces the demand of fossil fuel, mitigate the global warming and climate change but also assure the food security (Trivedi *et al.*, 2013).

To overcome fossil fuel depletion and environmental problems seaweeds can be used as a raw material to produce a renewable bio fuel, bio ethanol. Red seaweeds are capable to produce high amount of carbohydrate and bioethanol. *Kappaphycus alvarezii* (also known as cottonii) was identified as the best resource for bioethanol production among 55 species of sea weeds tested. This species is one of the most abundant and easily cultured red seaweeds (Meinita *et al.*, 2012).

According to Kraan (2013), the combustion of fuel leads to the emission of carbon dioxide (CO2), one of the most significant greenhouse gases in the earth's atmosphere. Which will leads to different environmental problems like global warming air quality deterioration, oil spills and acid rain. The level of atmospheric CO2 concentration is predicted to increase to 450 ppm by2020 if no action is taken.

The use of first generation biomass (sugar cane, sugar beet, corn and wheat) for bioethanol production will lead to "food versus fuel feud" (Nigam and Singh, 2011). The second generation biomass (lignocellulosic biomass) is an alternative source for first generation because it overcomes the food versus fuel feud whereas, removal of lignin is one of the problem faced when second generation biomass is used for fuel (Jegannathan *et al.*, 2009). The macroalgae consider as the third generation biomass for bioethanol production will overcome the problems faced in first and second generation and have several advantages over terrestrial plants (Jones *et al.*, 2012). Macroalgae, an abundant third-generation biomass is used in bioenergy production, and carbon–neutral renewable resource (Jang *et al.*, 2012).

Due to the structural differences, macroalgae are capable of producing high yields of material when compared to even the most productive land-based plants. Kelp forests generating large amounts of organic carbon in shallow sub-tidal regions are the most productive communities on earth (EU-Commission, 2003). Several seaweed species, the biomass for bioethanol production contain low lignin or no lignin content and rich in carbohydrates (Wi *et al.*, 2009; Yanagisawa *et al.*, 2011).

According to Meinita *et al.* (2012) carbohydrate the main component of various seaweed species reached a maximum of approximately 64% on a dry weight basis in preliminary experiments. A major carrageenan producer *K.alvarezii*, shows at the highest amount of carbohydrate among the 54 seaweed species tested. Chapman and Chapman (2012), reported that on a carrageenan dry-weight basis, *K. alvarezii* contains more than 50% carbohydrate.

A study by Yoza and Masutan (2013), shows that as compared to terrestrial biomass the lignin content is less in seaweeds. Among the five species of seaweeds studied, *Acanthophora spicifera, Dictyosphaeriaca vernosa, Hypnea cervicornus, Ulva reticulate,* and *Sargassum echinocarpum* the highest lignin percentage was found in *D. cavernosa* (33%). But the chemical composition like carbohydrate, protein, lignin contents in seaweeds are varying based on their environment (Marinho-Soriano *et al.*, 2006), among algal divisions, species, months and sits (Mwalugha *et al.,* 2015; Jayasankar *et al.,* 1999; Kumar, 1993). Also a study by Kumar *et al.* (2015) establishes a relation between the nutritional and mineral values of *Kappaphycus alvarezii* with the environment.

The replacement of non-renewable by renewable energy from ecosystems and agro-ecosystems represents a central strategy to mitigate and to adapt to climate change. Water hyacinth bioconversion is a good example of human adaptation to climatic change by managing carbon export of natural wetlands regarding this climatic change scenarios (Bergier *et al.,* 2012).Water hyacinth have relatively high content of hemicellulose (30–55% of dry weight), which indicates that it could be a good source of hemicellulose for bioconversion as biofuel (Nigam, 2002).

2.3.2. Biochar

Biochar is a carbon rich organic material produced by thermal decomposition of biomass in a closed container with fewer or absence air and at a temperatures less than 700 °C (<700°C) (Lehmann and Joseph, 2015).

A review article by Malik (2007) demonstrated the versatile use and proper management of the invasive noxious aquatic weed, water hyacinth, in different manner. The unique capacity of water hyacinth is to clean up the waste water in different sector and act as “nature’s kidney”. Other options including water hyacinth-based power alcohol, biogas, compost, and animal feed production from the harvested biomass could offer a sustainable system especially in developing countries.

Activated carbon is frequently made from biomass or coal often thought of as a universal adsorbent for removing contaminants from waste water but is costly to make (Mohan and Pittman, 2007). Whereas, the biochar production need less investment as compared to activated carbon and also very easy to get the raw materials for sustainable production (Mohan *et al.,* 2007). Biochar has a high potential for use in water purification and it also replaces the low coast sorbent like coconut shell and wood-based biochars (Marschner *et al.,* 2013).

2.4. BIOMASS TO BIOFUEL CONVERSION

Li *et al.* (2016a), reported that for the higher production of bioethanol from seaweed, pre-treatment with hydrogen peroxide is an attractive method; the optimized conditions for hydrogen peroxide pre-treatment were 0.2% hydrogen peroxide at pH 4, 12 h and at 50 °C. The optimum pH which influences the stability and activity of hydrogen peroxide was pH 4, which results a maximum yield of reducing sugar in the seaweed *Ulva prolifera.* The lowest yield is obtained at a pH of 6, while at pH 8 and 10, a small increase in the glucose yield was observed. The yield of reducing sugar and production of glucose increased when the concentration of hydrogen peroxide increased up to 0.5%. The yield was almost unchanged in the concentrations of 0.1%, 0.2%, and 0.5%. The yield decreased by further increasing of (>0.5%) hydrogen peroxide concentration.

There are lots of pre-treatment methods including dilute acid, liquid hot water, and organic solvent to improve the hydrolysis efficiency of seaweed biomass. As compared to the pre-treatment with hydrogen peroxide above mentioned pre-treatments are performed under high temperature (121- 200°C) and high pressure (15 MPa), with high concentration of chemical solvents (Ge *et al.,* 2011).

The pre-treatment with hydrogen peroxide shows good glucose yield in terrestrial plants and also it can be carried out in moderate temperature and pressure without leave any toxic resides (Rabelo *et al.,* 2014). Li *et al.* (2016b), reported that the appropriate method to remove the lignin content in seaweed was hydrogen peroxide pre-treatment method and the pre-treatment also enhance the bioethanol production. The pre-treatment with hydrogen peroxide have many benefits over acidic pre-treatment. The oxidation of peroxide doesn't leave any environmentally toxic acid wastes or any toxic residues (Yoza and Masutan, 2013).

The red seaweed *Gracilaria* spp. have high carbohydrate content (76.67%) as compared to other macroalgae (50–60 %) which indicates they have high potential to produce bioethanol if efficient scarification were to be developed. Through acid hydrolysis of *Gracilaria* sp. at three concentrations of H_2SO_4 (0.1, 0.3, 0.5 N) relatively high yield of glucose and galactose was seen at a pH of 4.5 and a time period of one hour (Wu *et al.,* 2014).

The successful conversion of *E. crassipes* to liquid fuel was achieved by two-sequential steps, acid hydrolysis with sulphuric acid and yeast fermentation. This can be an appropriate technology for production of renewable energy and a better weed management for developing countries with simple and low cost process (Isarankura-Na-Ayudhya *et al.,* 2007).

In several studies *Saccharomyces cerevisiae* is used for the production of ethanol by fermentation (Borines *et al.,* 2013; Park *et al.,* 2012; Tan and Lee, 2014). For the fermentation of hexose sugar in lignocellulosic hydrolysate *Saccharomyces cerevisiae* is considered as the best yeast (Hahn-HaGerdal *et al.,* 1991).

A study by Borines *et al.* (2013), with fermentation by *Saccharomyces cerevisiae* at 30°C and 40°C, pH of 4.5 and 6.5 for 48 and 72 hours showed maximum ethanol yield at 40°C, pH of 4.5 for 48 hours.

The ethanol fermentation was conducted in a fermentation flask with hydrolysate medium by enriching the yeast by additional nutrition supplementation at a pH range of 5 -6.5 at 30° C (Trivedi *et al.,* 2013; Khambhaty *et al.,* 2012; Chirapart *et al.,* 2014; Kumar *et al.,* 2013).

2.5. BIOMASS TO BIOCHAR CONVERSION

Pyrolysis is a thermo chemical decomposition process in which the organic materials are converted into bio-oil, carbon-rich solids and volatile matter by heating in the absence of oxygen (Demirbas and Arin, 2002). Biochars are carbonaceous residues through pyrolysis of biomass at high temperature in an oxygen limited atmosphere that can be applied for the safe and long term sequestration of carbon in the environment. (Gurwick *et al.,* 2013).

The yield of *E.* biochar decreases with increasing pyrolysis temperature and time, which means that ash content was more at high pyrolysis temperature. The yield sharply decreases at a temperature above 400 °C (Bird *et al.,* 2011). *E. crassipes* biochar yield decreases and stability of biochar carbon increases with increasing pyrolysis temperature. The optimum temperature and residence time for stable organic matter yield was 300-500 °C and 30-40 minutes (Masto *et al.,* 2013). The carbon content of biochar enhances with increasing temperature from 300°C to 700°C, but the oxygen, nitrogen, and hydrogen contents have decreased (Li *et al.,* 2016b).

2.6. PHYTOREMEDIATION

Phytoremediation is considered as a better green remediation technology. Water hyacinth is one of the ancient technologies that is still used in the modern world. Using invasive plants for the waste water management is one of the future aspects of phytoremediation (Rezania *et al.*, 2015). Water hyacinth has high affinity for the accumulation of several metals. Therefore, it was introduced in wetlands for water phytoremediation as a biomarker (Satyakala and Jamil, 1992).

The conventional waste water treatment plants are expensive to import, install, and operate especially in developing countries; there for the use of aquatic macrophytes for the waste water treatment is cheaper environmentally sustainable and attractive (Ajayi and Ogunbayio, 2012). Kiridi and Ogunlela (2016), suggested that water hyacinth is capable to remediate aquaculture effluent. The remediation rate increased with increasing the remediation period, and also the treated effluent can be re-used for aquaculture if its quality lies within the permissible level. This findings will help the aquaculturists, waste water managers, and environmentalist etc. in waste water management system for farms, commercial aquaculture, domestic and municipal supplies.

The water hyacinth is adaptable to a wide range of environmental factors including electrical conductivity (EC), pH, and temperature. This characteristics the plant weedy and also make it a good plant for remediation (El-Gendy *et al.,* 2004). Aquatic macrophytes, such as water hyacinth is one of the most commonly used plant for waste water treatment due to its characteristics like enormous biomass production rate, its high tolerance to pollution, and heavy metal and nutrient absorption capacities (Singhal and Rai, 2003).

A study done by Ajayi and Ogunbayo (2012), for five week duration with water hyacinth says that, water hyacinth has high efficiency to remove cadmium (Cd), copper (Cu), and iron (Fe) from various waste waters like textile, metallurgical, and pharmaceutical in which it seems to be a good choice for removing Cd but not that much good for Cu and Fe. Also the pH of the water sample had decreased by the end of experiment, the colour and odour also improved. In terms of lead and copper uptake per dried mass of biosorbent, dried roots and aerial part of water hyacinth are better biosorbents then the biomass of the bacterium *Mycobacterium phlei*, yeast *Candida prapsilosis*, fungal *Rizopusoryzae* strains, and acacia bark (Schneider *et al.,* 1995). Priya and Selvan (2017), suggested that water hyacinth has high potential to remove various dye stuffs and heavy metals like iron, cadmium, copper, zinc, mercury, chromium, manganese, and arsenic from aqueous solutions.

A study by Fox *et al.* (2008), demonstrated the potential of water hyacinth to remediate six trace element Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni), and Selenium (Se) under controlled conditions. In this study the accumulation rate of Cd and Cr were high, Se and Cu moderate and, Ni and AS were poor. Cadmium, Cr, Cu, As, and Ni shows accumulation more at roots than shoots, but Se accumulate more at shoots than roots. Therefore, the water hyacinth is an efficient phytoextractor for Cd, Cr, Cu, and Se from waste water.

2.7. BIOCHAR AND WATER TREATMENT

The biochars of different biomasses are good sorbent of heavy metals (Dong *et al.,* 2011; Ho and Ofomaja, 2006), also the *Eichornia* biochar is a promising biosorbent for heavy metal absorption in waste water treatment (Li *et al.,* 2016b).

There are various water treatment technologies in the world; among that adsorption is a fast and universal method because of its significant advantages like the low cost, availability, profitability, ease of operation, efficiency and development of inexpensive adsorbents from waste materials within short time period (Ali and Gupta, 2006).

A study by Beesley *et al.* (2010), reported that biochar has high potentiality to adsorb and remediate heavy metals from waste water and contaminated soils.

The water hyacinth biochar produced through pyrolysis have high removal efficiency of heavy metals like arsenic from aqueous solution, which could also provide an alternative way to manage and utilize this highly problematic invasive species (Zhang *et al.,* 2016).

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1. SAMPLE COLLECTION

The samples of seaweed, *Kappaphycus alvarezii and Gracilaria corticata* of Rhodophyceae (Red algae) were collected from sea off Mandapam, in the Tamil Nadu coast and *Eichhornia crassipes* (water hyacinth) samples were collected from Kadamakkudy backwaters, Ernakulam district, and Kerala coast. The collected seaweed samples were soaked in fresh water for half an hour and washed with running water five to six times to remove debris. The samples were later transported to the laboratory after sun drying and thoroughly washed with tap water three-four times to remove excess salts and other residues. Fresh *E. crassipes* were immediately transported to laboratory and separated into roots and shoots and washed thoroughly with tap water to remove foreign particles. The seaweeds and water hyacinth samples were air dried till the samples get fully dried (Plate 1-3). The samples were cut into small pieces and stored in sealed air tight polythene bags for further processing.

Plate 1: Dried *Kappaphycus alvarezii*

Plate 2: Dried *Gracilaria corticata*

Plate 3: Dried *Eichhornia crassipes*

3.2. COMPOSITIONAL ANALYSIS OF BIOMASS

The proximate composition of seaweeds and water hyacinth were analyzed by AOAC official methods of analysis (AOAC, 2000). For the determination of moisture content 4 g of dried samples were kept in an oven at 105 °C for two hours and weighed before and after oven dry; ash content was determined by combustion of dried samples in a furnace at 600 °C for three hours and weighed. Crude fat was extracted by solvent extraction method using Soxhlet extractor (Plate 4). A 3 g sample was extracted with petroleum ether (60 mL). The extract was oven dried at 100 °C for 2 hours and later the weigh was recorded. Crude protein was determined by micro-Kjeldahl method system at a conversion factor of 6.25. The lignin content was estimated following a procedure by Hill *et al.* (1998) (Plate 5). Cellulose content was determined following Updegroff. (1969) method (Plate 6).

Total soluble sugar content was determined by phenol-sulfuric acid method (Dubois *et al.*, 1956) with minor modification. 100 mg of dried sample was hydrolyzed by keeping in an 80 °C water bath with 25 ml HCl (2.5 N) for one hour (Plate 7). The samples were shaken frequently to ensure complete hydrolysis. After cooling, the samples were centrifuged at 7,000 rpm for 15 minutes. The 0.1 ml of supernatant were collected and diluted with one ml of distilled water. Then one ml of phenol solution (5%) was added to each sample and after shaking, 5 mL of concentrated sulphuric acid (96 %) was added and mixed thoroughly. After shaking the samples continuously for 10 minutes the samples were kept for cooling for another 20 minutes. The same procedure was applied for the standard solutions. The readings were taken at 490 nm using the UV-visible spectrophotometer (Genesis 5, Thermo Spectronic).

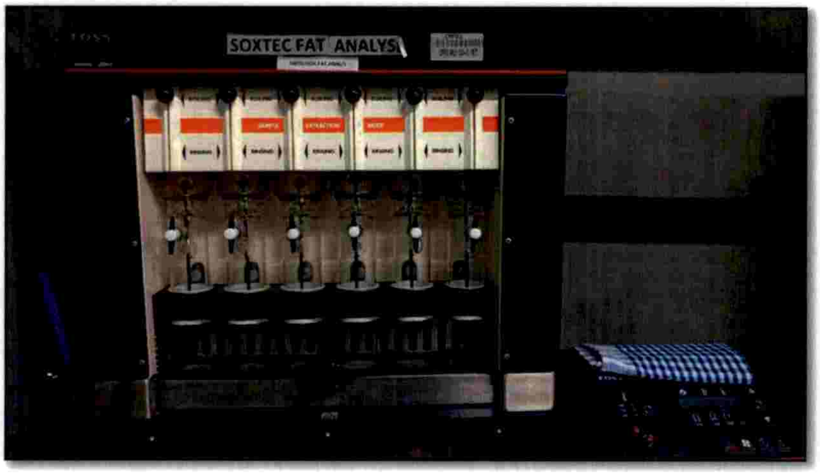


Plate: 4. Soxhlet extractor for fat estimation

Plate: 5. Lignin estimation: mixture of sample with sulfuric acid and phosphoric acid, vacuum filtration by GF/C glass fibre filter paper

Plate: 6. Cellulose estimation: Samples after centrifuged at 8,000 rpm

Plate: 7. Carbohydrate estimation: Hydrolysis of sample with 25mL of HCl at 80 °C water bath

3.3. HYDROGEN PEROXIDE PRE-TREATMENT

The dried seaweed and water hyacinth (1g) samples were pre-treated with pre – optimized concentration of 0.1 % (Li *et al.,* 2016a) of hydrogen peroxide (H_2O_2) for varying pH (4, and 11.5) for 24 h duration at room temperature (Plate 6). After 24 hour the pre-treated residues was thoroughly washed with distilled water and soaked for 4 hours to remove the excess chemical content. The samples were air dried and stored in air tight polythene bags for further treatment. The cellulose and lignin content of pretreated samples were determined by the method mentioned in section 3.2.

Plate: 8. Pretreatment at pH 4: *K.alvarezii, G.coricata,* and *E.crassipes*

Plate: 9. Pre-treatment at pH 11.5: ***K. alvarezii, G. corticata, and E. crassipes***

3.4. STRUCTURAL ANALYSIS

The structural elucidation and characterization studies of untreated (raw), and pretreated samples were done by X-Ray Diffraction (XRD), scanning electron microscope (SEM), and Fourier-transform infrared spectroscopy (FT-IR) at Sophisticated Test and Instrumentation Centre (STIC), Cochin University of Science and Technology (CUSAT), Cochin.

XRD: Rigaku X – Ray diffractometer was used to perform the crystallinity study of the above mentioned samples. The radiation used was of Cuα radiation at a wavelength of 1.5418 A°. The samples were scanned at a scan rate of 1° per minute with scan angle (2θ) from 7 ° to 40°and the sampling rate was 0.02° (2θ).

The cellulose crystallinity index was calculated from the equation (Segal *et al.,* 1959):

$$\mathbf{CrI = (I_{002} - I_{am})/I_{002} * 100}$$

Where CrI indicates the relative degree of crystallinity, **I002** is the maximum intensity (in arbitrary units) of the 002 lattice diffraction, and **Iam** is the intensity of diffraction in the same units at 2θ = 18°.

FTIR: Comparison of structural changes of above mentioned samples were analyzed by Thermo Nicolet, Avatar 370 Fourier Transformation Infrared spectrometer (FTIR). FTIR spectrum of samples was taken with a resolution of $4cm^{-1}$ and 32 scans per sample. The absorbance spectra were recorded at wave numbers from 500 – $4000cm^{-1}$. Comparison of structural changes was done by analysis of characteristic peaks.

SEM: Scanning electron microscope (SEM) analysis was performed on untreated and treated samples to analyze the morphological changes. SEM images were taken at 5, 10 50, and 100 µm range.

3.5. ACID HYDROLYSIS

The dried raw and treated biomass samples (2g) were acid hydrolyzed with pre-optimized concentration of sulfuric acid of 0.1% at 121°C in an autoclave for 60 minutes (Wu *et al.,* 2014) (Plate 10). After the reaction the hydrolyzed samples were centrifuged at 8,000 rpm for 20 minutes. The supernatants were collected and stored at -20 °C. The hydrolysate was used for estimation of reducing sugar by DNS method (Miller, 1959).

Plate: 10 Hydrolysates after acid hydrolysis

3.6. YEAST CULTURING AND FERMENTATION OF THE ALGAL HYDROLYSATE

3.6.1. Yeast culturing

Lyophilized culture of *Saccharomyces cerevisiae* (MTCC No. 170) was procured from Institute of Microbial Technology (IMTech) Chandigarh, India. The culture was activated by introducing into YEPD broth (Yeast extract: 0.3g, Peptone: 1g, Dextrose: 2g, distilled water: 100ml) for 48 hours at 30°C. To check the purity of culture, yeast in nutrient broth was streaked into YEPD agar plate (Yeast extract: 0.3g, Peptone: 1g, Dextrose: 2g, agar: 2g, distilled water: 100ml) and incubated at pH 6.8 for 48 hours at room temperature (30 °C) (Plate 11). The slant of culture was prepared (Yeast extract: 0.15g, Peptone: 0.5g, Dextrose: 1g, Agar: 1g: distilled water: 50ml) and stored for further use (Plate 12).

Plate: 11 Yeast culture at YEPD agar plate

Plate: 12 Slant culture of *Saccharomyces cerevisiae*

3.6.2. Fermentation of the algal hydrolysate

The fermentation was carried out in 250 mL conical flasks. Each hydrolysate volume was adjusted into 100 ml and was supplemented with 0.5 g of peptone and 0.3 g yeast extract. The pH was brought to 6.5 with 10 N NaOH. The flasks were autoclaved at 121 °C for 15 minutes. The inoculum for fermentation was prepared from slant by inoculating a loop full of yeast cells in sterile YEPD broth (Yeast extract: 0.3g, Peptone: 1g, Dextrose: 2g, distilled water: 100ml) followed by incubation at 30°C for 48 hours.

For ethanol fermentation, 10 mL of YEPD broth containing *S. cerevisiae* was added to the previously autoclaved fermentation media. Fermentation was conducted at 30 °C with agitation at 120 rpm for 48 hours (Plate 13). After fermentation, the media centrifuged at 8,000 rpm for 15 minutes. The supernatants thus obtained were subjected for ethanol estimation by High-performance liquid chromatography (HPLC) at Cochin University of Science and Technology (CUSAT), Cochin.

Plate: 13 Fermentation

3.7. BIOCHAR PREPARATION AND HEAVY METAL TREATMENT

3.7.1. Biochar preparation from *E. crassipes*

Known quantity of air dried material of *E. crassipes* was taken and pyrolyzed at 300°C for 30min in a muffle furnace. The sample material was pyrolysis at a rate of 10°C/min until the desired values reached. Samples after pyrolysis were cooled in desiccators and used for further analysis.

3.7.2. Measurement of biochar pH

Biochar pH was measured according to Ahmedna *et al.* (1997). The method consisted of preparing a 1% (wt/wt) suspension of biochar in deionized water. The suspension was heated to about 90 °C and stirred for 20 minutes to allow dissolution of the soluble biochar components. After cooling to room temperature, the pH of the biochar suspension was measured using a pH meter.

3.7.3. Biochar heavy metal analysis

Water mixed with known concentration of Zn (2ppm in 100ml) [permissible level of Zn is 0.12ppm by US EPA] from Zinc sulfate ($ZnSO_4$) stock solution was taken as control and another set of 10g biochar was added (Plate 14). Treatment efficacy was assessed after 72 hours at an agitation of 100 rpm at room temperature. After 72 hours the sample is centrifuged (4,000rpm, 15 minutes) and stored by adding concentrated HNO_3 (2ml/l) for further analysis. Initial and final pH of water sample was recorded by pH meter. The heavy metal analysis of water samples were done by inductively coupled plasma-optical emission spectroscope (ICP-OES, model: optima 2000 DV) from Central Institutes of Fisheries Technology (CIFT), Cochin.

Plate: 14 Heavy metal analysis: control experimental setup, heavy metal with biochar

3.8. PHYTOREMEDIATION OF HEAVY METAL USING WATER HYACINTH

Water hyacinth, *E. crassipes* collected from Kadamakkudi were used for phytoremediation of heavy metal using Zinc (Zn) as the test metal. Medium sized plants were fixed in a 100 L tank containing 50 L of water. And 0.2ppm Zn prepared from stock solution of $ZnSO_4$ (Plate 15). The experiment was continued for one month and water samples were collected weekly. For analysis of dissolved oxygen and alkalinity Standard analytical procedures were followed. The pH was measured using pH meter. The heavy metal water samples are stored in concentrated HNO3 (2ml/l) until use. The heavy metal analysis of water samples were done by inductively coupled plasma-optical emission spectroscope (ICP-OES, model: optima 2000 DV) from Central Institutes of Fisheries Technology (CIFT), Cochin.

Plate: 15 Phytoremediation: Fresh *Eichhornia crassipes*, phytoremediation experimental setup

RESULT AND DISCUSSION

CHAPTER 4
RESULTS AND DISCUSSION

The experimentation using seaweeds and water hyacinth as substrates resulted in bioethanol production and instrumental analysis elucidates the structural changes induced by the treatment process. The efficacy of water hyacinth biochar produced through pyrolysis for heavy metal adsorption was also found to be positive. The phytoremediation potential of fresh rooted water hyacinth for heavy metal removal was also established.

4.1. PROXIMATE ANALYSIS OF BIOMASS

The composition of substrates seaweeds *Kappaphycus alvarezii, Gracillaria corticata,* and *Eichhornia crassipes* are as tabulated below.

Table 1: Compositional Analysis of Seaweeds and Water Hyacinth

Parameters (%)	Samples		
	K. alvarezii	*G. corticata*	*E. crassipes*
Moisture	17.51	13.00	13.00
Crude fat	0.52	0.36	1.42
Crude ash	24.04	20.25	19.63
Acid insoluble ash	0.44	4.30	0.00
Protein	13.59	19.51	19.08
Total soluble sugar	46.18	36.54	32.69
Cellulose	17.86	25.71	32.14
Lignin	0.20	0.19	0.31

Among the two seaweed sample, high amount of total soluble sugar (46.18%) was estimated in *Kappaphycus alvarezii* and high amount of cellulose (25.71%) was found in *Gracillaria corticata. E. crassipes* was found to be with highest percentage of both total soluble sugar (32.69%) and cellulose (32.14%). As compared to terrestrial plants, the lignin content is observed to be very low in these three aquatic substrates. The high amount of total soluble sugar, cellulose contents and low lignin in biomass are indicative of prospective biofuel production potential of the substrates as the total sugars including cellulose could be hydrolysed into fermentable sugars. Low lignin content is beneficial, as lignin and lignocellulosic binding are major barriers in biomass to bioenergy conversion process (Jegannathan *et al.*, 2009). The results of compositional analysis reflects that the selected seaweed and water hyacinth biomass could be prospective substrates for bioethanol production, as it contains high content of total soluble sugar, cellulose, and low lignin content.

Owing to the low lignin content, the macroalgae (seaweed) considered as the third generation biomass for bioethanol production could overcomes the problems faced by the second generation (lignocellulosic) biomass. However, the chemical composition like carbohydrate, protein, lignin contents in seaweeds vary based on their environment (Marinho-Soriano *et al.*, 2006), algal divisions, species, and months (Mwalugha *et al.*, 2015; Jayasankar *et al.*, 1999; Kumar, 1992).

Similarly, water hyacinth also contain high amount of soluble sugar and cellulose. As compare to other lignocellulosic sources of bioethanol production, water hyacinth is a fast growing weed easily available at tropical regions (Singh and Bishnoi, 2012) which hinders the aquaculture practises and reduces the oxygen content in the water bodies. The biofuel development from water hyacinth (Isarankura-Na-Ayudhya *et al.*, 2007; Mishima *et al.*, 2007) is a sustainable way to utilize the aquatic weed and is in line with the waste to energy generation.

4.2. HYDROGEN PEROXIDE PRE-TREATMENT

Bleaching of the substrate occurred due to the hydrogen peroxide treatment and the substrate colour change to slight white was observed after oxidative treatment (Plate 16). This can be attributed to the bleaching ability of hydrogen peroxide which converts the coloured carbonyl groups in lignin to non- chromosphoric components. The lignocellulosic compositional changes induced by hydrogen peroxide pre-treatment are detailed in Table 2.

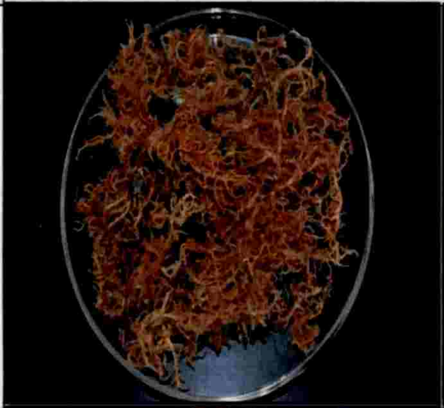
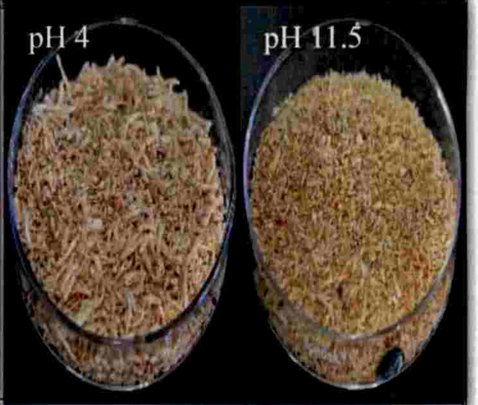
Samples	Untreated Samples	Hydrogen Peroxide treated samples
Kappaphycus alvarezii		
Gracillaria corticata		
Eichhornia crassipes		

Plate. 16 Bleaching of samples due to hydrogen peroxide pre-treatment

Table 2: The compositional changes in cellulose and lignin content of substrate before and after pre-treatment

Samples	Pre-treatment pH	Cellulose content (%)	Lignin (%)
K. alvarezii	Untreated	17.86	0.20
	4	14.64	0.09
	11.5	10.71	0.07
G. corticata	Untreated	25.71	0.19
	4	21.43	0.18
	11.5	18.21	0.12
E. crassipes	Untreated	32.14	0.31
	4	28.57	0.22
	11.5	23.21	0.14

The cellulose and lignin content get reduced due to pre-treatment. In *K. alvarezii* cellulose and lignin content was reduced from 17.86 % to 14.64 % and 0.20% to 0.09 % respectively at pH 4. At pH 11.5 the cellulose get reduced to 10.71% and lignin to 0.07%. In *G. corticata* cellulose gets reduced from 25.71% to 21.73% and further to 18.21%, respectively for untreated, pH 4 and pH 11.5 pre-treatment. For *G. corticata* lignin content varied from 0.19% to 0.18% and further to 0.12% for untreated, pH 4 and 11.5 pre-treatment respectively. *E. crassipes* cellulose reduced from 32.14% (untreated) to 28.57 % (pH 4) and 23.21 % (pH 4) with reduction in lignin content from 0.31% (untreated) to 0.22% (pH 4) and 0.14% (pH 11.5).

Higher range of reduction in lignin and cellulose content was observed in hydrogen peroxide pre-treatment at pH 11.5 in all three samples. Hydrogen peroxide pre-treatment is an applicable method to reduce the lignin content in seaweeds and similar results reported by Li *et al.* (2016b).

Oxidative delignification process is the chemistry behind hydrogen peroxide pre-treatment. Delignification process leads to the solubilisation of lignin and its derivative into the filtrate (Rojith and Singh, 2012). Oxidative delignification may induce lignocellulosic structural changes, which is explained in the FTIR analysis section. Hydrogen peroxide pre-treatment may also result in cellulose crystallinity change, which was evident from XRD analysis. Loss of cellulosic component due to hydrogen peroxide retreatment is of concern. However the effectiveness of the pre-treatment could be weighed based on the reducing sugar yield after the saccharification stage.

4.3. STRUCTURAL ANALYSIS

The XRD, SEM and FTIR analysis confirms the cellulose crystallinity changes, surface changes and functional group changes induced to seaweeds and water hyacinth samples due to the pre-treatment process.

4.3.1. XRD

The XRD patterns of pre-treated and untreated samples are as shown in Fig.1-9. From the graph, the 2 theta peak values around 22° (highest) and 18° (valley) were identified and substituted in the crystallinity index equation (mentioned in the methodology) and accordingly the crystallinity index (CrI) values were calculated. The estimated crystallinity index values of pre-treated and untreated samples are shown in Table 3.

Table 3: Crystallinity index values (CrI)

	K. alvarezii	*G. corticata*	*E. crassipes*
Untreated	34.72	23.47	61.50
Treated at pH 4	64.05	44.73	83.35
Treated at pH 11.5	95.89	47.88	83.82

From the table values, it could be inferred that the cellulose crystallinity index was found to increase in the pre-treated samples (pH 4 and pH 11.5) as compared with the untreated sample of seaweed and water hyacinth. Comparatively higher cellulose crystallinity index was observed in all samples for pre-treatment at pH 11.5. After pre-treatment process, the cellulose may be either in amorphous or crystalline form and X-ray diffraction is a commonly used technique to analyse the cellulose crystallinity of the pre-treated samples. In this study also XRD analysis helped us to identify the cellulose crystallinity index change occurred due to the hydrogen peroxide pre-treatment of seaweed and water hyacinth samples.

XRD graph of untreated samples

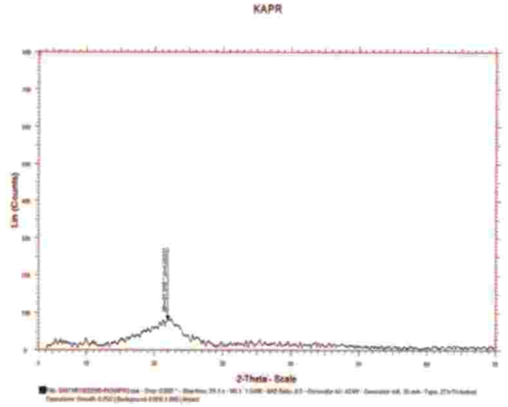


Fig. 1 XRD graph of untreated *K. alvarezii*

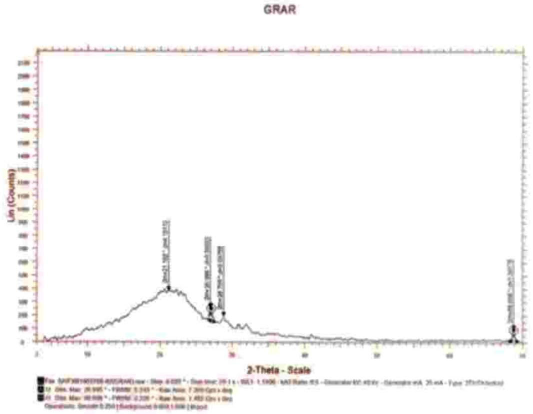


Fig.2 XRD graph of untreated *G.corticata*

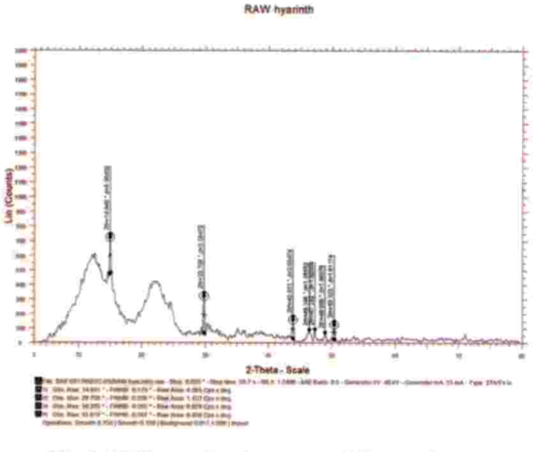


Fig.3 XRD graph of untreated *E.crassipes*

XRD graph of pre-treated samples at pH 4 and 11.5

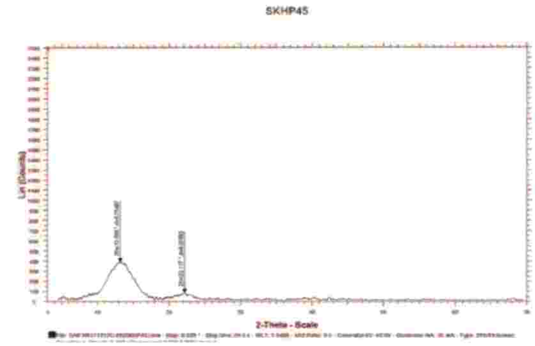


Fig. 4 XRD graph of treated *K. alvarezii* at pH 4

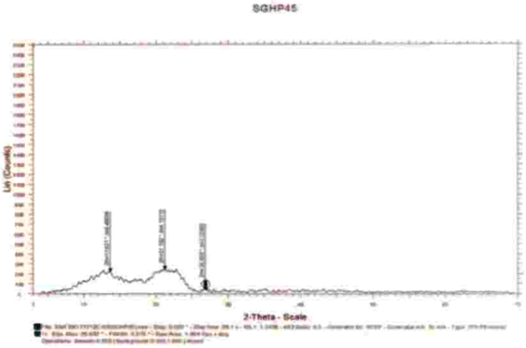


Fig.5 XRD graph of treated *G.corticata* at pH 4

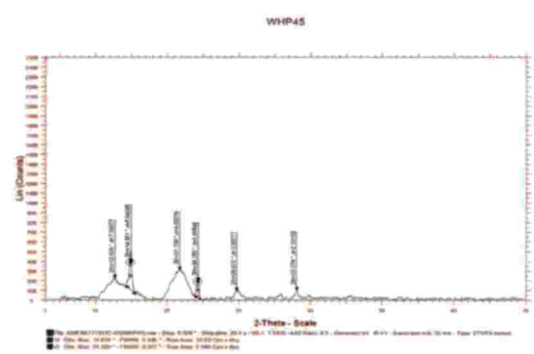


Fig.6 XRD graph of treated *E.crassipes* at pH 4

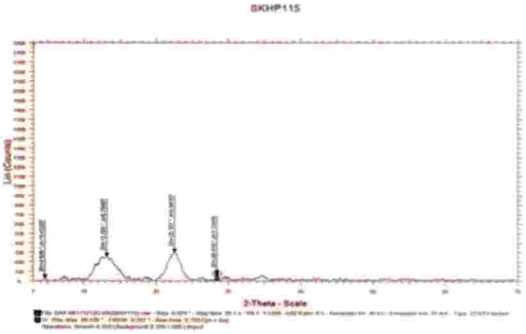


Fig.7 XRD graph of treated *K. alvarezii* at pH 11.5

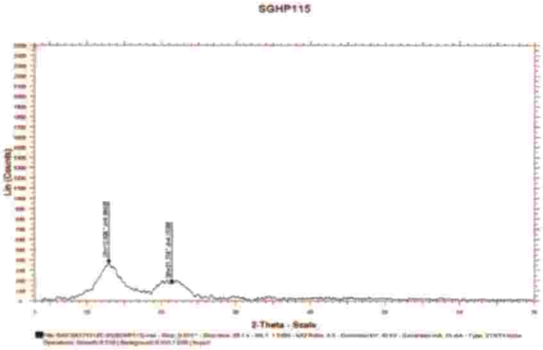


Fig.8 XRD graph of treated *G.corticata* at pH

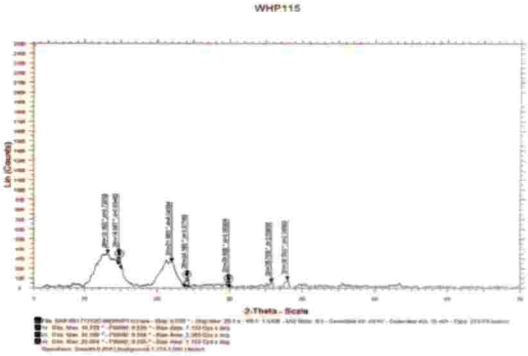


Fig.9 XRD graph of treated *E.crassipes* at pH 11.5

Cellulose crystallinity index is an important parameter, which indicates whether enzymatic hydrolysis or chemical hydrolysis should be further followed. Higher cellulose crystallinity makes the substrate unfit for microbial and enzymatic hydrolysis route. The enzymatic saccharification could be effectively attained only in the case of amorphous cellulose. The XRD analysis inferences helped us to choose acid hydrolysis route for further bioenergy conversion pathway.

4.3.2. SEM Analysis

SEM analysis reveals the surface modifications induced by the hydrogen peroxide pre-treatment. The SEM images of untreated and treated substrates taken at 10, 50, 100 μm are as shown in Fig.10-13, which confirms morphological changes. The surface of treated samples become rugged as compare to raw. Increase in porosity and surface area of treated samples occurred than untreated samples. The raw structure was broken into several fragments as a result of pre-treatment.

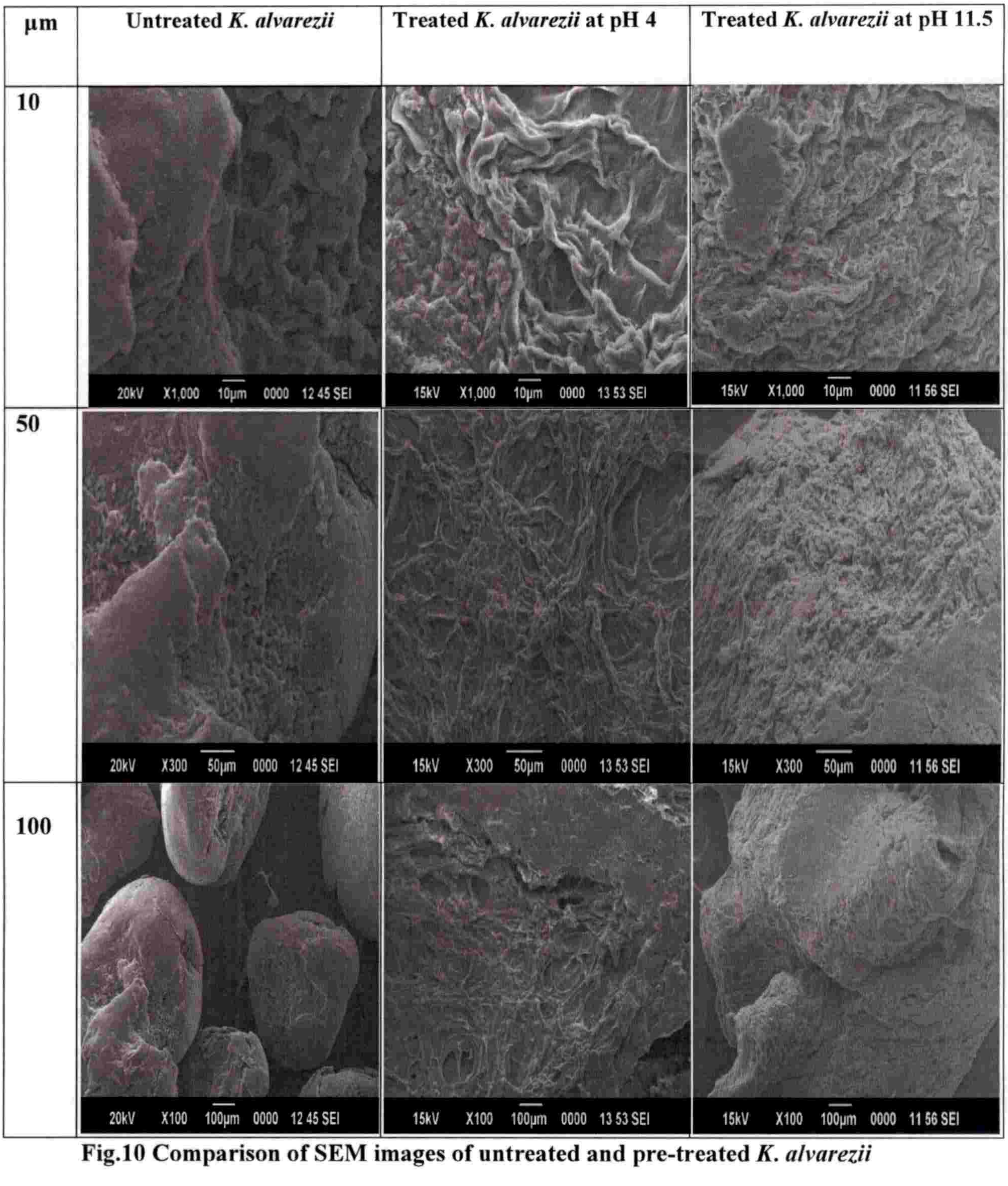


Fig.10 Comparison of SEM images of untreated and pre-treated *K. alvarezii*

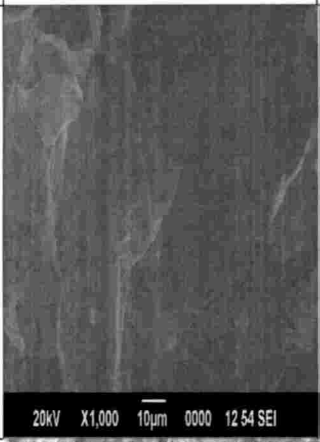
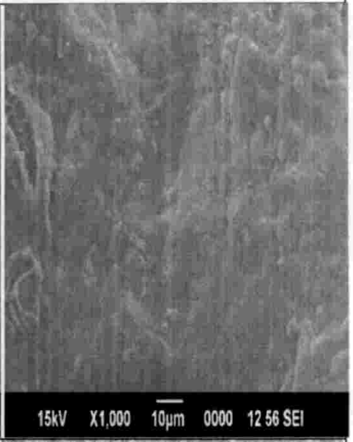
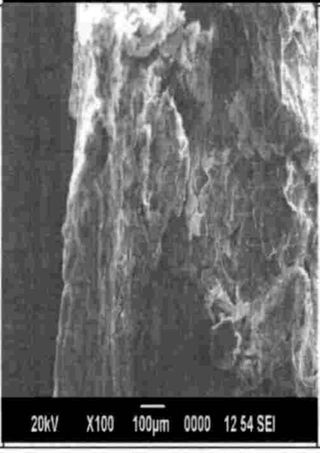
μm	Untreated *G. corticata*	Treated *G. corticata* at pH 4	Treated *G. corticata* at pH 11.5
10			
50			
100			

Fig.11 Comparison of SEM images of untreated and pre-treated *G. corticata*

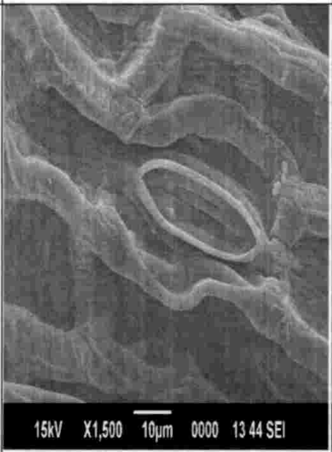
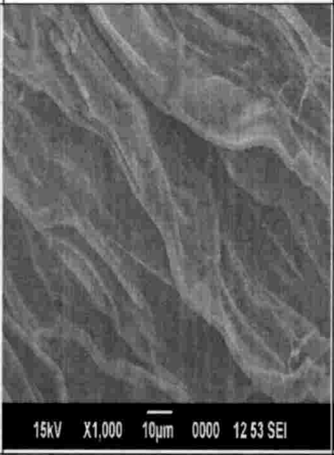
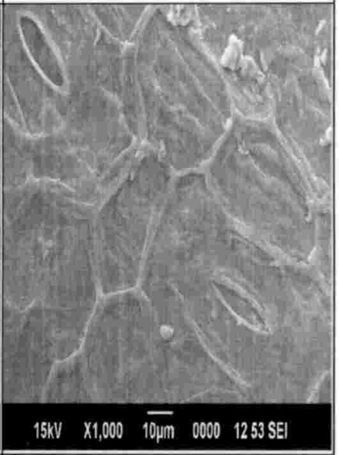
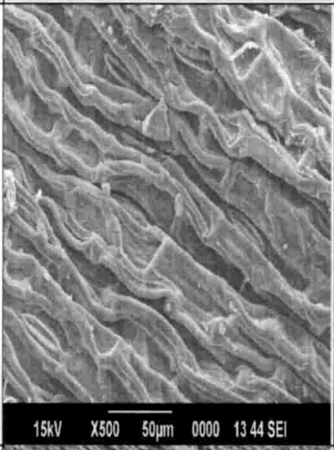
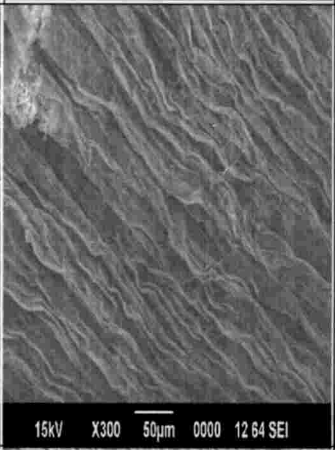
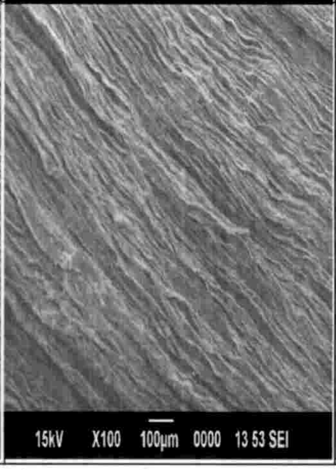
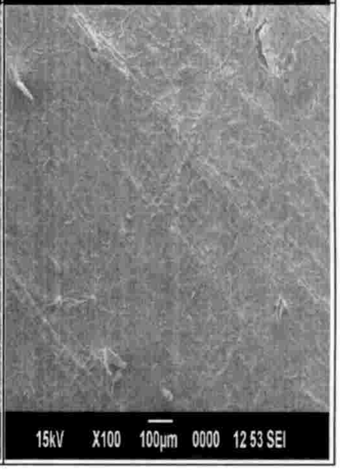
μm	**Untreated *E. crassipes***	**Treated *E. crassipes* at pH 4**	**Treated *E. crassipes* at pH 11.5**
10			
50			
100			

Fig. 12 Comparison of SEM images of untreated and pre-treated *E. crassipes*

4.3.3. FTIR Analysis

Comparison of FTIR images of the untreated and pre-treated samples lead to the inference that, structural changes occurred due to the pre-treatment. Changes in characteristic peaks indicate lignocellulosic compositional changes. FTIR spectra also help identification of functional groups, which is evident by several peak shifts and disappearance of several peaks. The peak shift and disappearances indicates the chemical transformations due to pre-treatment process. The FTIR image of untreated and pre-treated samples are shown in Fig. 13-15. Prominent characteristic peaks and its associated functional groups are as shown in Table 4.

The peaks 3420, 1650, 1622, 1600, 1260, 1250, 1125, 1070, 1060 cm^{-1} indicates the main functional groups of cellulose and hemicellulose. Peak 3420 cm^{-1} represent the O – H stretching present in water and saccharide, hydroxyl group in phenolic and aliphatic structures (typical absorption peaks of cellulose or hemicelluloses). The peaks 1622, 1600 cm^{-1} indicates the presence of hemicellulose. C – O stretch band around the peaks 1260, 1250, 1125, 1070, 1060 cm^{-1} usually represents the hemicellulose and cellulose. The peaks 1505, 1501, 1374, 1258, 1246 cm^{-1} represents the functional groups of lignin. Aromatic skeletal vibration band around the peak 1505 and 1501 cm^{-1} represents measure for lignin content. S = O stretching at the peaks 1374, 1258, 1246 cm^{-1} indicates the presence of lignin.

In seaweed *K. alvarezii* substrate the peak 1070 cm^{-1} was observed as shifted to 1124 cm^{-1}.A shoulder peak was observed between 1260 cm^{-1} and 1124.92 cm^{-1} peaks for untreated sample but it vanished for treated samples. In untreated sample at 1600cm^{-1} only one peak existed. However, a new peak was observed at 1658 cm^{-1} for the sample treated at 11.5. At 3420 cm^{-1} there is a sharpen peak for untreated sample, but for treated it is seen as broad one (indicates crystallinity). The peak changes at 1505 1374 cm^{-1} and 1374 cm^{-1} indicates the structural changes of lignin.

In *G. corticata* sample, peak at 1060 cm^{-1} of untreated sample is observed as shifted in samples pre-treated at pH 4 and 11.5. Peak at 1250 cm^{-1} is found to be disappeared in sample treated at pH 11.5. Also valley peak is not seen in between the peaks 1370 cm^{-1} and 1258 cm^{-1}. For pH 11.5 treated sample 1650 cm^{-1} peak is observed as shifted. In pH 4 treated sample, peak change occurred in 2920 cm^{-1} and 1650 cm^{-1} and also the peak at 2920 cm^{-1} is sharper than the other two samples. A peak shift to 1125 cm^{-1} in both *K. alvarezii* and *G. corticata* indicates the C – O stretching.

In *E. crassipes* shifts of 3420, 2920, 1622, 1501, 1374, and 1318 cm^{-1} for raw and treated samples is observed. The lignin peaks at 1501, and1374 cm^{-1} peaks is found to disappear which could be attributed to the delignification process. The peaks changes at 1112 cm^{-1} indicates the hydrogen bonded CH – OH angle coupled to phenyl ring vibration.

A prominent peak at 618 cm^{-1} were observed in the pre-treated samples of *K. alvarezii, G. corticata,* and *E. crassipes*, which indicates the C – H bend. Also a peak shift around 1060 cm^{-1} into 1125 cm^{-1} is found in both pre-treated (pH 4 and 11.5) samples of seaweeds and into 1112 cm^{-1} in pre-treated water hyacinth sample, which indicates the structural changes in cellulose and hemicellulose. Several characteristic peak shifts and peak disappearances are observed which lead to infer that structural changes occurred due to delignification process.

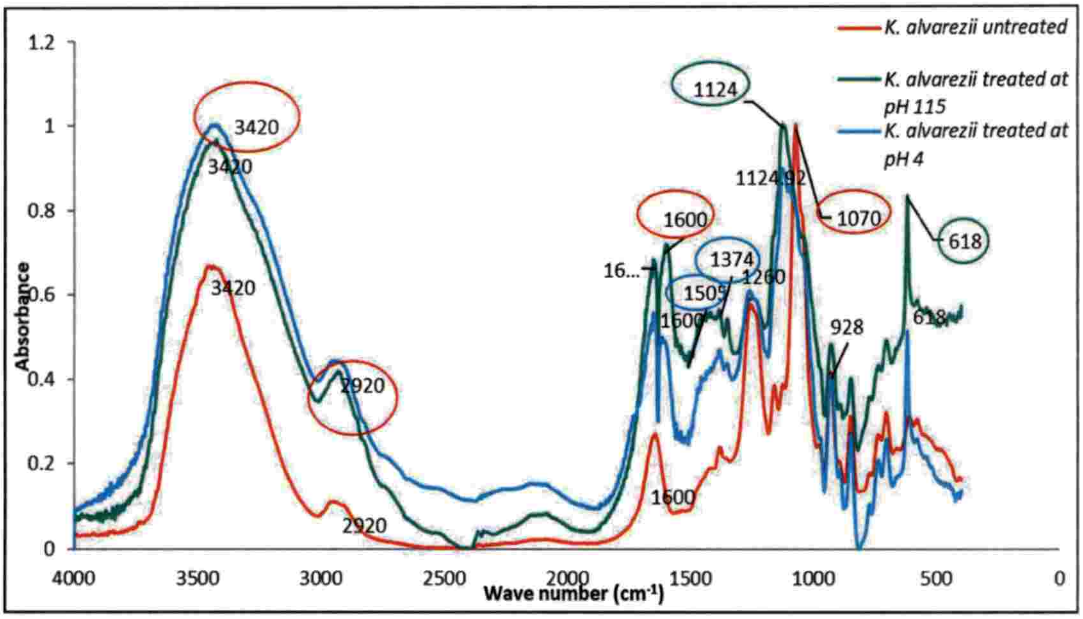


Fig.13 Comparison of FTIR images of untreated and pre-treated *K. alvarezii*

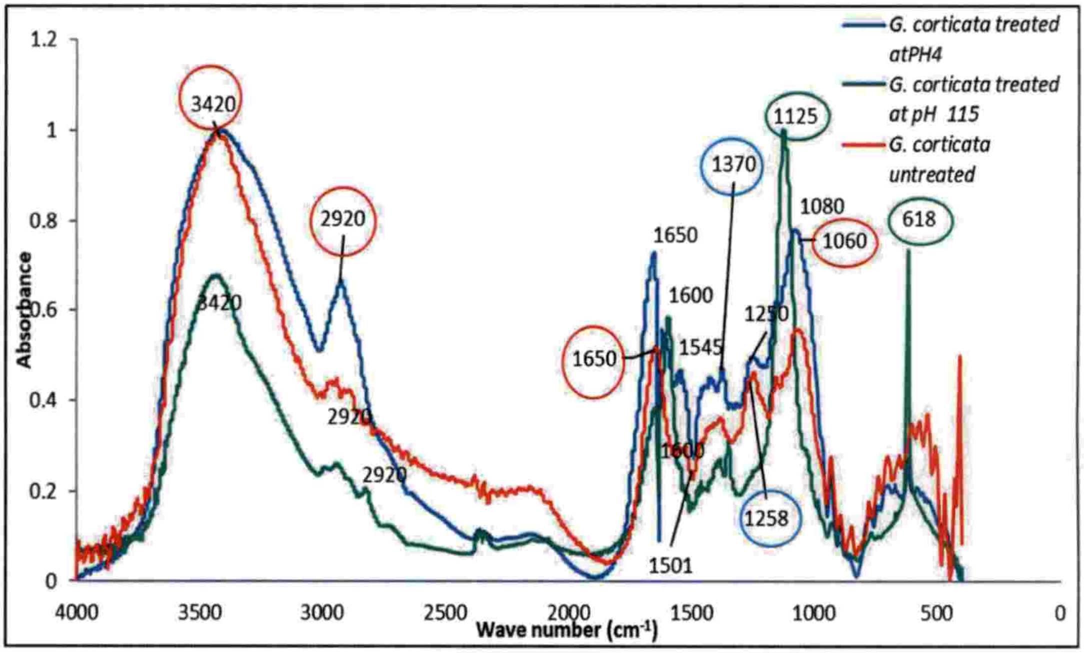


Fig.14 Comparison of FTIR images of untreated and pre-treated *G. corticata*

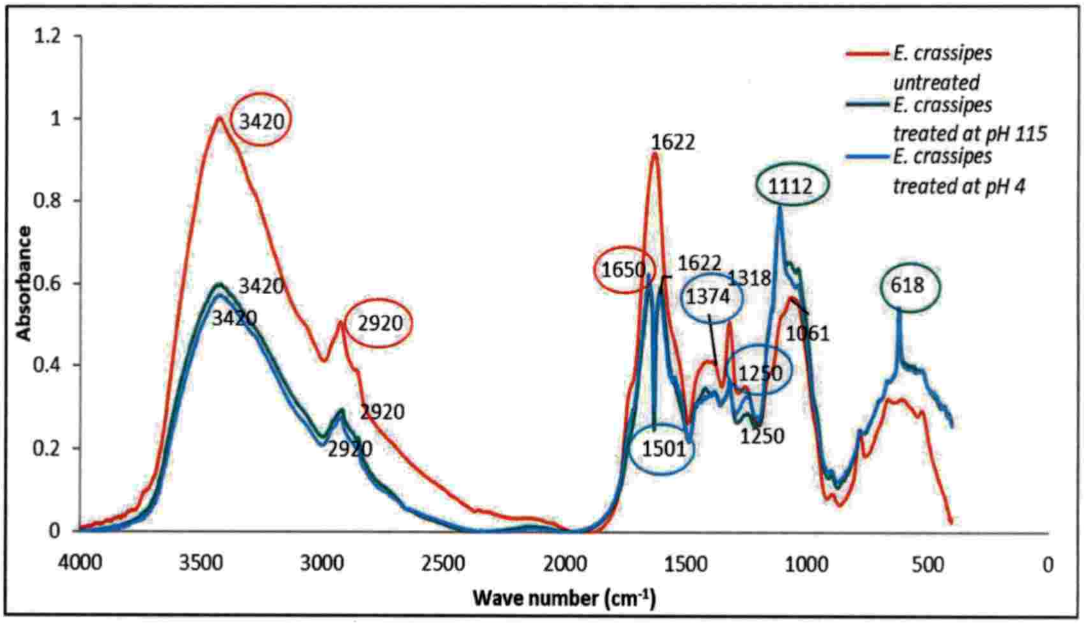


Fig.15 Comparison of FTIR images of untreated and pre-treated *E. crassipes*

Table: 4 FTIR peaks and corresponding Functional groups

Peaks (cm^{-1})	Functional groups
3420	O – H stretching present in water and saccharide, hydroxyl group in phenolic and aliphatic structures (typical absorption peaks of cellulose or hemicelluloses)
2920	CH stretching in aromatic methoxyl group
1650	C = C stretch
1658	amide C=O stretching band
1622,1600	Presence of hemicelluloses
1620	C=C stretching
1542- 1545	Aromatic C=C stretching
1505, 1501	Aromatic skeletal vibration indicates presence of lignin
1374,1258,1246	S = O stretching, presence of lignin
1370 – 1390	CH_3 CH bend
1318	Carboxyl stretch
1260, 1250, 1125,1070,1060	C – O stretch (cellulose and hemicellulose)
1070 (CH stretch)	C – O stretching.
1112	Hydrogen bonded CH – OH angle coupled to phenyl ring vibration
1080	C – O – C stretch
1061	C – N stretch
618	C – H bend

4.4. ACID HYDROLYSIS AND REDUCING SUGAR ESTIMATION

Acid hydrolysis by sulphuric acid was found to be appropriate in converting polysaccharides of seaweed and water hyacinth substrates into fermentable sugars. After acid hydrolysis, the reducing sugar content was estimated and is as tabulated in Table 5. High reducing sugar content was observed in the samples pre-treated at pH 4 and lower reducing sugar content was observed in the sample pre-treated at pH 11.5. A study by Li *et al.* (2016b) in green seaweed *Ulva prolifera* reports high amount of reducing sugar through the pre-treatment with hydrogen peroxide at a pH 4 and also the yield of reducing sugar was almost unchanged in the concentrations of 0.1%, 0.2%, and 0.5%. Reducing sugar content is of significance for further fermentation pathways and hence hydrolysis stage needs to focus on maximum reducing sugar yield. The high reducing content enhances further processes of bioethanol production.

Table: 5 Reducing sugar content of seaweed and water hyacinth samples

Sample	*K. alvarezii*	*G. corticata*	*E. crassipes*
Untreated	36.14	27.51	30.62
Treated at pH 4	47.53	43.04	57.28
Treated at pH 11.5	21.47	24.92	25.78

4.5. Bioethanol Production

Yeast *Saccharomyces cerevisiae* was found to be efficient in fermentable sugar to bioethanol conversion. Sub culturing of yeast and further inoculation to broth were successful followed by ethanol yield. Fermentation process was carried out for 48 hours and bioethanol presence was detected in crude sample analysis by HPLC. Bioethanol presence was detected in water hyacinth sample and also in seaweed *G. corticata* sample. However ethanol presence was not detected for seaweed sample *K. alvarezii.* Inhibitory effect of hydrolysate and fermentation process needs to be investigated for seaweed sample *K. alvarezii.* Chromatograph of pure ethanol standard matches with that of crude samples of untreated *G. corticata*, *G. corticata* pre-treated at pH 4, *E.crassipes* pre-treated at pH 4, and *E.crassipes* pre-treated at pH 11.5 (Fig.16-20).

This work could establish bioethanol production from seaweed *G. corticata* sample and water hyacinth *E.crassipes*. Process optimisation could yield higher ethanol production.

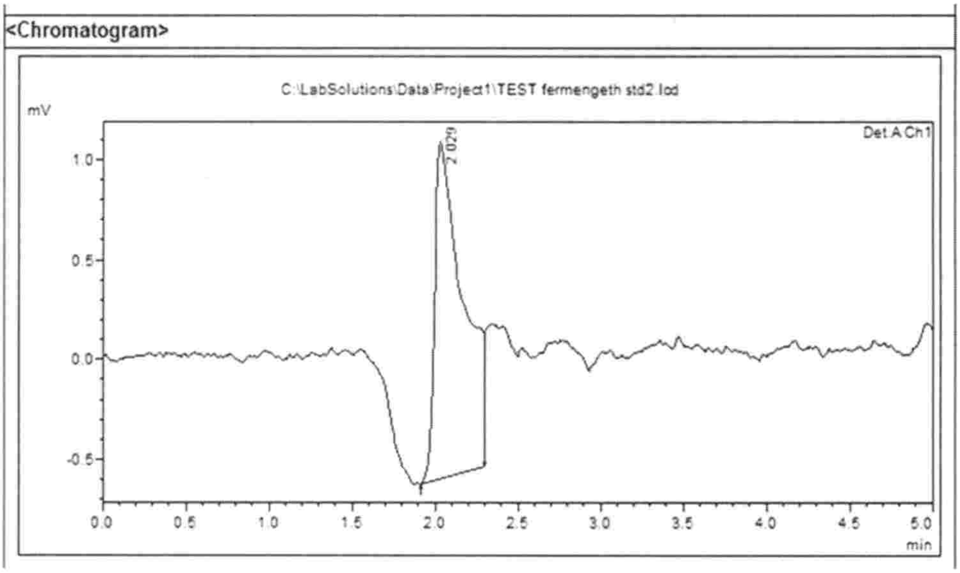


Fig. 16 Chromatograph of standard ethanol

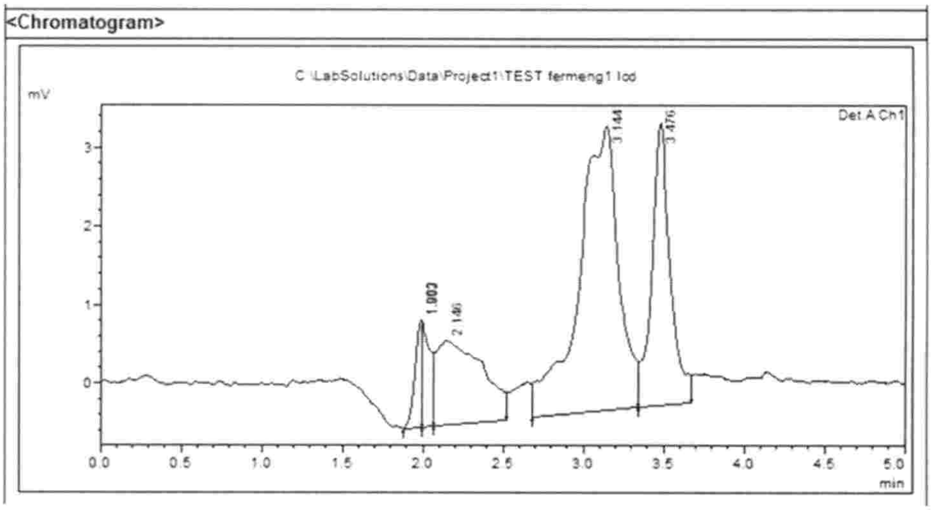


Fig. 17 Chromatograph of *G. corticata* untreated

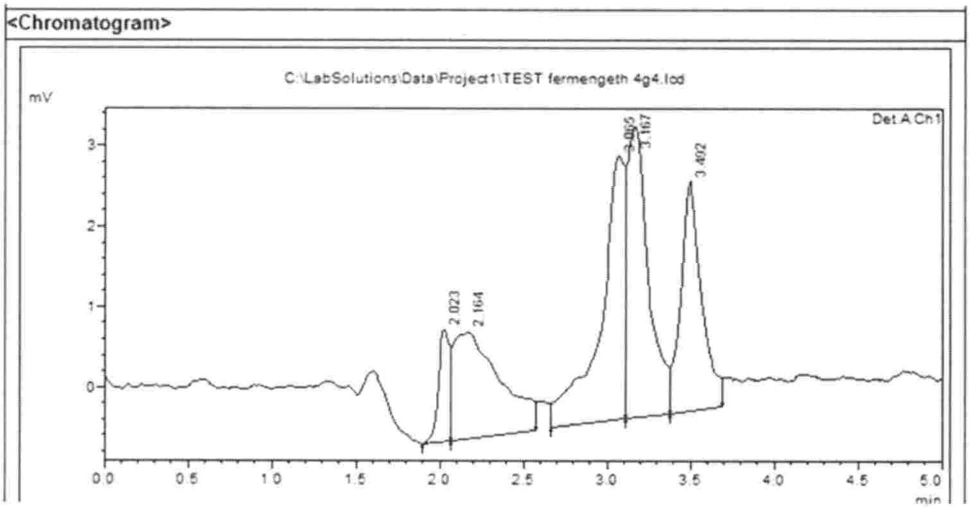


Fig. 18 Chromatograph *G. corticata* pre-treated at pH 4

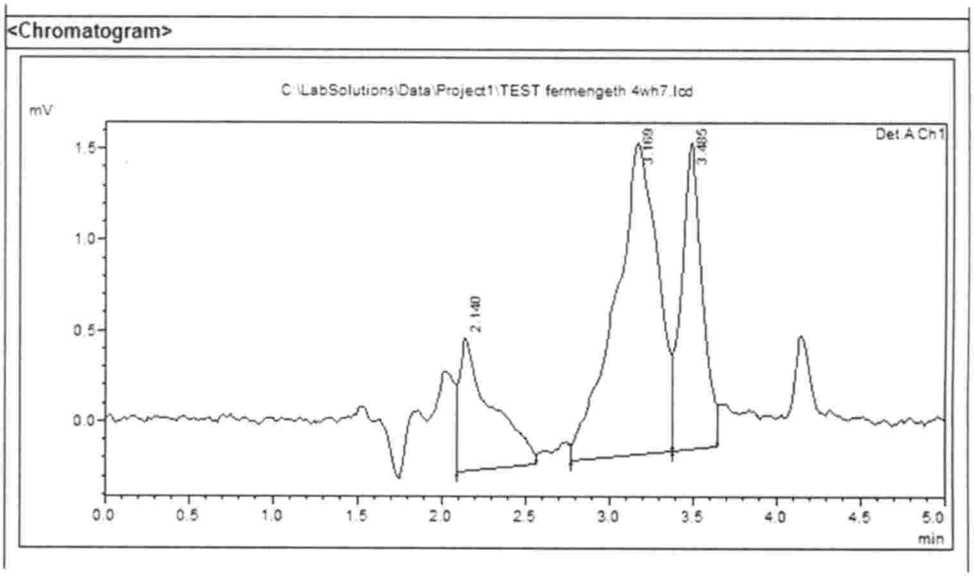


Fig. 19 Chromatograph *E. crassipes* pre-treated at pH 4

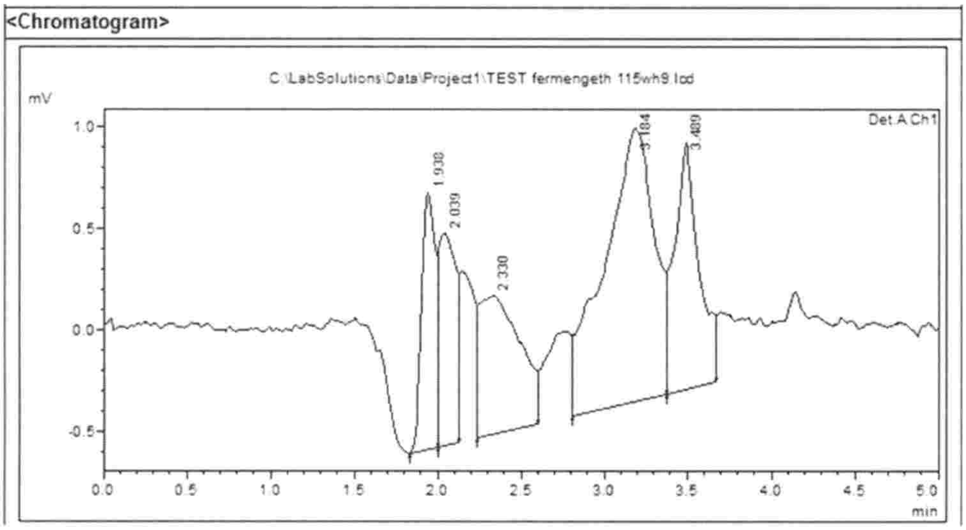


Fig. 20 Chromatograph *E. crassipes* pre-treated at pH 11.5

4.6. PHYTOREMEDIATION BY WATER HYACINTH *E. CRASSIPES*

Phytoremediation experiment was done for four week and the water quality data collected for each week are as tabulated Table 6. Dissolved Oxygen content was observed to be reducing weekly and other parameters remains the same. The dissolved oxygen content reduction could be attributed to the over growth and spread of *E. crassipes.* Through aeration we can improve the Dissolved Oxygen content and also by controlling the over growth of water hyacinth. Phytoremediation potential of water hyacinth could be well established as the concentration of zinc was decreased from 0.2 ppm to 0.0135 ppm within first week. Analysis carried out in further weeks indicated absence of zinc concentration, which implies that the remaining zinc was also absorbed by water hyacinth. Accordingly it could be inferred that the fresh rooted water hyacinth is an effective material for remediation of zinc. Similar results were reported in a study conducted by Ajayi and Ogunbayo. (2012) for five weeks in Copper, cadmium, and iron and also for in Fox *et al.* (2008).

Table: 6 Weekly data of water quality

Parameters	Week I	Week two II	Week III	Week IV
Temperature	26.1	26.5	26	25.4
pH	7.5	7.4	7.4	7.3
Alkalinity (mg/L $CaCO_3$)	70	70	70	70
Dissolved oxygen (mg/L)	3.2	3.2	2.4	1.2
Heavy metal (Zn) Initial concentration (0.2ppm)	0.0135	0	0	0

4.7. Biochar water treatment

Conversion of water hyacinth into biochar was attempted as a means to enhance the carbon sequestration and also to convert the biomass to bioethanol conversion residues into biochar. The work could successfully demonstrate the potential of biochar for water treatment application. The pH of prepared biochar was observed as 6.4. Amendment of biochar to the sample shows slight increase in pH. Biochar could successfully reduce the amount of zinc in the water and could be attributed to the adsorptive property of biochar. The amount of zinc get reduced from 2ppm to 1.0265 ppm after 3 days experiment and the values are as shown in Table 7. The zinc content was reduced from 2ppm to 1.928 ppm within one day and into 1.0265ppm within three days. The biochar produced from water hyacinth have high rate of adsorption capacity. It could be inferred that the water hyacinth biochar is an effective biosorbent for the heavy metal zinc. This is in line with the similar results reported in a study by Zhang *et al.*, (2016) for arsenic.

Table: 7 Biochar water treatment

Biochar water treatment	**0 hour**	**24 hours**	**48 hours**	**72 hours**
pH	6.5	6.9	7	7
Zn (ppm)	2	1.928	-	1.0265

4.8. IMPLICATIONS OF SEAWEED AND WATER HYACINTH BASED BIOPRODUCTS IN THE CLIMATE CHANGE CONTEXT

Climate change affects the national economy and livelihoods of several populations which necessities the need to develop resilient strategies. In the climate change context National Policy on Biofuels advocates mixing of 30% bioethanol in transportation fuel through alternative energy sources. This work envisages bioethanol production from aquatic vegetation. Besides carbon sequestration ability, seaweed is a non-food grain and is independent of the land and fertilizer requirements for cultivation, which makes the substrate more advantageous as compared to other terrestrial food grain crops used for biofuel production. Large scale seaweed farming along the coastal states could contribute significantly towards climate change mitigation and income generation. However farmers cannot venture to seaweed farming for climate benefits alone and to enable it research interventions towards new product development should happen. Beyond existing industrial demand for seaweed substrates, biofuel based products shall open new horizons. To enhance seaweed farming as a sustainable option, integration of bio-refinery approach needs to be focused. This shall further enhance the economy, industry and livelihoods of the nation. Upon successful commercialization of seaweed products, opportunities shall emerge for coastal fishermen/farmers to take up seaweed farming as a livelihood option. Seaweed could become as a key link between energy, environment, and climate change.

Waste to energy conversion is a sustainable concept and water hyacinth based biofuel gains significance in this context. Water hyacinth is a menace to aquatic water bodies of the nation and it also adversely affects the regional small scale fishing activities. Biofuel is a climate resilient product, which reduces the burden on fossil fuel combustion and utilizing waste water hyacinth as a biofuel substrate is a sustainable resilient strategy. Biochar is another climate resilient product which is often considered as carbon neutral or carbon negative product. Biomass after decaying may emit carbon dioxide to atmosphere. However if the biomass is converted to biochar, the biomass carbon shall be transformed into more stable form that may not decompose for several decades. The biochar could be applied to agriculture and aquaculture practices. Our work envisages conversion of biomass to biofuel process residues into biochar which shall have carbon sequestration implications. Also this work could demonstrate the application of biochar in water treatment, and accordingly the biomass to biochar conversion gains more significance.

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SUMMARY

CHAPTER 5
SUMMARY

The seaweeds *Kappaphycus alvarezii, Gracillaria corticata,* and water hyacinth *Eichhornia crassipes* were used for the production of climate resilient products biofuel and biochar. The compositional analysis of these three substrate revealed good quantity of total soluble sugar including cellulosic components along with low lignin content, which indicates suitability of the substrates as prospective bioenergy source. The hydrogen peroxide pre-treatment at varying pH (4 and 11.5) were attempted on the substrates, which resulted in substrate colour change along with lignocellulosic structural changes. The observed color change could be attributed to the bleaching ability of hydrogen peroxide. Reduction in lignin and cellulosic component were estimated. The lignocellulosic changes due to the pre-treatment was confirmed using XRD, SEM, and FTIR, analysis. The XRD analysis helped to infer the cellulose crystallinity change whereas SEM analysis confirmed the morphological changes like surface area, porosity improvement and FTIR analysis helped to identify the lignocellulosic changes through analyzing the FTIR peaks and corresponding functional group before and after the pre-treatment with hydrogen peroxide.

The cellulose crystallinity was found to increase by hydrogen peroxide pre-treatment and accordingly chemical hydrolysis was chosen instead of enzymatic hydrolysis. The hydrolysis was done with sulfuric acid, which was effective in releasing reducing sugars from the pre-treated and untreated samples. Based on reducing sugar yield, hydrogen peroxide pre-treatment at pH 4 was found to be better. During fermentation the reducing sugar gets converted into biofuel through fermentation. The sub cultured yeast *Saccharomyces cerevisiae* was inoculated into the hydrolysate enriched with supplementary nutrients and fermentation was carried out for 48 hours. High performance liquid chromatography (HPLC) was used to analyze the presence of bioethanol. Among the 9 samples the chromatographs of four samples matches with the standard ethanol chromatograph. The samples detected with presence of bioethanol are untreated *G. corticata*, *G. corticata* pre-treated at pH 4, *E.crassipes* pretreated at pH 4 and 11.5. Further investigations on the inhibitory effect of pretreatment methods and also optimization of parameters in each stages of bioethanol production may yield higher ethanol.

Biochar is considered as carbon neutral or negative product and this work applied the concept of waste to resource conversion with demonstration of the product application for heavy metal removal. Pyrolysis technique was effective in producing biochar from Water Hyacinth in a muffle furnace at 300°C and 30 minutes. The biochar produced was used for treatment of water containing heavy metal zinc. The heavy metal analysis revealed reduction in zinc which could be attributed to the adsorption ability of water hyacinth based biochar. The work output could be extended further for large scale conversion of water hyacinth into biochar and its application for heavy metal remediation from aquaculture fields. Phytoremediation with fresh rooted water hyacinth was also attempted for zinc and further analysis confirmed complete removal of heavy metal within two week. However in case of fresh rooted water hyacinth application decrease in dissolved oxygen level was observed and should take necessary aeration steps in upscaling to large scale applications. Climate variability induced rainfall, runoffs, storm water and floods bring lots of pollutant loads including toxic heavy metals to aquatic bodies. Phytoremediation using fresh rooted water hyacinth and also heavy metal removal using climate resilient product biochar gains significance in this context.

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CHAPTER 6

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CLIMATE RESILIENT PRODUCT DEVELOPMENT FROM SEAWEEDS *KAPPAPHYCUS ALVAREZII, GRACILARIA CORTICATA* AND WATER HYACINTH *EICHHORNIA CRASSIPES*

by

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THESIS ABSTRACT

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CHAPTER 7

ABSTRACT

The work focuses on development of climate resilient products Biofuel and Biochar from Seaweeds and Water hyacinth as substrates. Compositional analysis including carbohydrate and cellulose content of *Kappaphycus alvarezii, Gracilaria corticata* and water hyacinth *Eichhornia crassipes* were done followed by pretreatment, hydrolysis and fermentation. Pretreatment was done with a concentration of 0.1% of hydrogen peroxide at two varying pH (4, 11.5), for a duration of 24 h at room temperature. Structural characterisation using FTIR, XRD and SEM were done to assess the effect of pretreatment. Acid hydrolysis was performed on the pretreated substrates and reducing sugars were further estimated. Fermentation of the substrates was carried out using yeast strain *Saccharomyces cerevisiae.* Bioethanol yield was estimated using HPLC analysis. The bioethanol content were detected in four samples (untreated *G.corticata, G.corticata* and *E.crassipes* treated at pH 4, and treated *E. crassipes* at pH 11.5). Further process optimization may provide optimum ethanol yield.

Water hyacinth was converted into biochar in a muffle furnace in a pre-optimized condition of 300°C at 30 minute. The produced biochar was attempted for heavy metal remediation under lab conditions. The heavy metal analysis indicates efficacy of water hyacinth biochar in heavy metal removal. Additionally, phytoremediation of heavy metal was carried out using fresh rooted water hyacinth followed by analysis. The results indicates higher efficacy of water hyacinth in heavy metal removal from water. Implications of seaweed and water hyacinth based products have also been described through the work.

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