HYACINTHOL

A PROJECT REPORT

submitted by

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to

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of

Bachelor of Technology
in
Biotechnology

Department of Civil Engineering

SAHRDAYA COLLEGE OF ENGINEERING AND TECHNOLOGY

KODAKARA, THRISSUR - 680684

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DECLARATION

We, undersigned, hereby declare that the project report “HYACINTHOL”, submitted for partial fulfillment of the requirements for the award of degree of Bachelor of Technology of the APJ Abdul Kalam Technological University, Kerala is a bonafide work done by us under the guidance of Ms Ranimol.G, Department of Biotechnology This submission represents our ideas in our own words and where ideas or words of others have been included; We have adequately and accurately cited and referenced the original sources. We also declare that we have adhered to the ethics of academic honesty and integrity and have not misrepresented or fabricated any data or idea or fact or source in my submission. We understand that any violation of the above will be a cause for disciplinary action by the institute and/or the University and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been obtained. This report has not been previously formed the basis for the award of any degree, diploma or similar title of any other University.

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Date of Submission: 06-08-2022

DEPARTMENT OF BIOTECHNOLOGY

SAHRDAYACOLLEGE OF ENGINEERING AND TECHNOLOGY

KODAKARA, THRISSUR

EDUCATION IS DEDICATION

CERTIFICATE

This is to certify that the project report entitled **HYACINTHOL** submitted by **Ashlin Shaju (SHR20BT013), Blessy Mary Joseph (SHR20BT019), Riya Zakkariya (SHR20BT044), Sreerag P (SHR20BT052), Vivina Puthur (SHR20BT060)** to the APJ Abdul Kalam Technological University in partial fulfillment of the requirements for the award of the Degree of Bachelor of Technology in Biotechnology Engineering is a bonafide record of the seminar work carried out by us under our guidance and supervision. This report in any form has not been submitted to any other University of Institute for any purpose.

PROJECT GUIDE

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INSTITUTE VISION

Evolve as a leading technology institute to create high caliber leaders and innovators of global standing with strong ethical values to serve the industry and society.

INSTITUTE MISSION

Provide quality technical education that transforms students to be knowledgeable, skilled, innovative and entrepreneurial professionals.

Collaborate with academia and industry around the globe, to strengthen the education and research ecosystem.

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We at Sahrdaya are committed to provide Quality Technical Education through continual improvement and by inculcating moral &ethical values to mould vibrant engineers with high professional standards.

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DEPARTMENT MISSION

1. Educate the next generation of engineers with a relevant curriculum that will enable them to integrate biological sciences with engineering fundamentals that can create, model, and modify biological systems for industrial applicability.
2. Encourage and facilitate research that would enable students to acquire new knowledge that is industrially and socially relevant.
3. Train students to be leaders who value professional ethics and realize the need for societal contribution and environmental sustainability in their work.

PROGRAM EDUCATIONAL OBJECTIVES (PEOs)

PEO1	To be equipped with the engineering skills and technical concepts enabling them to take up careers in Biotechnology and allied fields globally
PEO2	To instill the passion for lifelong learning and scientific discovery that will allow them to conduct research and innovate technologies keeping them motivated to take up research, entrepreneurial or industrial careers
PEO3	To inculcate entrepreneurial and techno management skills along with professional and ethical responsibility empowering them to be responsible and socially aware citizens

PROGRAM SPECIFIC OUTCOMES (PSOs)

PSO1	To measure, model and manipulate properties of biological systems at the cellular and molecular level so as to produce sustainable products that address environmental and ethical standards
PSO2	To formulate and execute quantitative and design- oriented analysis of biological systems with modern tools and techniques that generate new knowledge at the interface of engineering and biology
PSO3	To transform as socially relevant biological engineers having a professional outlook that would enable them to work as a part of a team in an industrial, research or entrepreneurial set up and sustain a desire for higher learning and research

PROGRAM OUTCOMES (POs)

PO1	Engineering knowledge: Apply the knowledge of mathematics, science, engineering fundamentals, and an engineering specialization to the solution of complex engineering problems.
PO2	Problem analysis: Identify, formulate, review research literature, and analyse complex engineering problems reaching substantiated conclusions using first principles of mathematics, natural sciences, and engineering sciences.
PO3	Design/development of solutions: Design solutions for complex engineering problems and design system components or processes that meet the specified needs with appropriate consideration for the public health and safety, and the cultural, societal, and environmental considerations.
PO4	Conduct investigations of complex problems: Use research-based knowledge and research methods including design of experiments, analysis and interpretation of data, and synthesis of the information to provide valid conclusions.
PO5	Modern tool usage: Create, select, and apply appropriate techniques, resources, and modern engineering and IT tools including prediction and modelling to complex engineering activities with an understanding of the limitations.
PO6	The engineer and society: Apply reasoning informed by the contextual knowledge to assess societal, health, safety, legal and cultural issues and the consequent responsibilities relevant to the professional engineering practice.
PO7	Environment and sustainability: Understand the impact of the professional engineering solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.

PO8	Ethics: Apply ethical principles and commit to professional ethics and responsibilities and norms of the engineering practice.
PO9	Individual and team work: Function effectively as an individual, and as a member or leader in diverse teams, and in multidisciplinary settings.
PO10	Communication: Communicate effectively on complex engineering activities with the engineering community and with society at large, such as, being able to comprehend and write effective reports and design documentation, make effective presentations, and give and receive clear instructions.
PO11	Project management and finance: Demonstrate knowledge and understanding of the engineering and management principles and apply these to one's own work, as a member and leader in a team, to manage projects and in multidisciplinary environments.
PO12	Life-long learning: Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change.

COURSE OUTCOMES

CO1	Be able to practice knowledge within the selected area of technology for project development.
CO2	Identify, discuss and justify the technical aspects and design aspects.
CO3	Reproduce, improve and refine technical aspects for engineering.
CO4	Work as a team in development of technical projects
CO5	Communicate and report effectively project related activities and findings.

Abstract

Water Hyacinth (Eichhornia crassipes Martius) is a monocotyledonous freshwater aquatic plant, belonging to the family Pontederiaceae, related to the lily family (Liliaceae) and is a native of Brazil and Equador region. The aquatic weed collected from Irinjalakuda, Thrissur is found to be problematic for many reasons which include increasing evapo-transpiration, providing a refuge for disease-carrying vectors, causing silting of channels, slowing down of the water flow and flooding. Utilization of this weed for energy production seems to be highly beneficial. Enhanced development of bioethanol for its use as a carbon-neutral renewable and clean fuel is ever-increasing as it reduces CO_2 emissions and associated climate change; it is used as fuel mix and octane enhancer in gasoline and improves the ambient air quality. In comparison with conventional agricultural biomass, lignocellulose biomass is a potential and sustainable substrate for bioethanol production. Lignocellulose biomass commonly comes from terrestrial origins, but aquatic plants and weeds like water hyacinth can also provide adequate biomass for bioethanol production. However the biofuel production through water hyacinth needs some processing before the further conversion to ethanol. Pretreatment process does require chemicals which make the process costlier. In this work, comparisons are made between acid and alkaline hydrolysis as pretreatment method for the biomass. The hydrolysis optimization was attempted to decrease the time of fermentation in selected biomass. This report summarizes fermentation mechanism in water hyacinth. The carbohydrate analysis enables the possibility towards ethanol production. The results show the potential of water hyacinth as feedstock for ethanol production however the high moisture content and other chemical analysis also favors the methane production.

Keywords: Water hyacinth, Pretreatment, Bioethanol, lignocellulosic biomass.

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Δ Heating

LIST OF ABBREVIATIONS

WH	Water Hyacinth
S.cer	Saccharomyces cerevisiae
DNSA	Dinitrosalicylic acid

CHAPTER 1

INTRODUCTION

1.1 GENERAL

Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized. Bioenergy is renewable energy and is produced by using various biological organisms. Bioenergy is expected to solve the global warming problem by decreasing the carbon dioxide levels in the atmosphere. A considerable amount of research is currently being conducted on the production of bioenergy due to the increasing demand for fossil fuel and its limited quantities in reserve. Bioethanol is one of the most promising replacements for fossil fuel since it is renewable and emits 85% less green-house gases compared to gasoline. Recently, more research has focused on using non-edible biomass as raw materials including lignocelluloses, celluloses, and marine algae rather than the first generation biomass such as starch and sugar biomass [1]. Lignocellulosic feedstock is considered as an attractive raw material because of its availability in large quantities at low cost not only for the liquid transportation fuel but also for the production of chemicals and materials, i.e. the development of carbohydrate-based biorefineries. Besides terrestrial plants, aquatic plants are also promising renewable resource.

Fig 1.1 Water Hyacinth

Biomass-based biofuel is considered a suitable alternative or supplement to fossil fuels. Agricultural wastes, including wheat straw, rice straw, sugarcane bagasse, sugar beet pulp, and so on, are promising sources of second-generation biofuel because of their availability, abundance, and relatively low cost. Moreover, a few aquatic plants,

including duckweed (Lemnoideae), water hyacinth (Eichhornia), and Mimosa, are also ideal resources for the biofuel industry. Water hyacinth (WH) is a perennial, free-floating aquatic plant that is now a highly problematic invasive species due to its fast growth in warm climates. It blocks rivers and channels, eliminates other aquatic plants, and restricts the light and air in the underwater environment [2], although it is useful in the absorption of some heavy metals and organic compounds in water. To reduce the problems caused by WH, much attention has been focused on potential uses of WH for a variety of applications. Recent studies reported that WH is an ideal raw material for animal feed, agricultural fertilizer, and enzyme production, since it contains high amounts of cellulose and hemicellulose and little lignin. Some works also showed that WH is a promising plant for production of fuel ethanol because of its fast growth and low lignin content.

1.2 PROPERTIES

An ideal biomass pre-treatment process should meet the following requirements:

- High rates of hydrolysis and high yields of fermentable sugars
- Minimal degradation of the carbohydrate fractions
- No production of compounds that are inhibitory to microorganisms used in the subsequent fermentation.
- Inexpensive materials of construction
- Mild process conditions to reduce capital costs
- Recycle of chemicals to reduce operating costs
- Minimal wastes

Table 1.1 Average biomass composition of water hyacinth

Sl No.	Components	%Composition
1	Lignin	10
2	Cellulose	25
3	Hemicellulose	35
4	Ash	20
5	Nitrogen	03

Table 1.1 shows the main composition of water hyacinth which is lignin, cellulose and hemicellulose. The carbohydrate polymers in the lignocellulosic material needs to be converted to simple sugars before fermentation, through a process called hydrolysis. There are several possible methods to hydrolyze lignocelluloses. The most commonly applied methods can be classified in two groups: Chemical hydrolysis and enzymatic hydrolysis [3]. Among chemicals acid and alkali are used as pretreatment methods. Acids are predominantly applied in chemical hydrolysis. Acid hydrolysis can be divided into two groups: (a) Concentrated acid hydrolysis and (b) Dilute acid hydrolysis. Concentrated acid process can be operated at low temperature (eg 40°C) however concentration of acid is very high (eg: 30-70 %). Dilute acid hydrolysis is commonly applied. It can be used either as a pretreatment preceding enzymatic hydrolysis or as the actual method of hydrolyzing lignocelluloses to sugars. But one drawback in this is the formation of undesirable products.

The key bottleneck in conversion of lignocellulosic biomass to fermentable sugars is polysaccharide depolymerization (hydrolysis). Chemical hydrolysis (by dilute acid or alkali) and enzymatic hydrolysis are the most commonly applied methods. Chemical hydrolysis can be used either as a pretreatment followed by enzymatic hydrolysis, or as the actual method of hydrolyzing celluloses and hemicelluloses to sugars. A drawback in chemical hydrolysis is the formation of unfavorable by-products, such as furfural, formic, and acetic acid . Biological pretreatment with fungus has advantages, including disrupting the lignin–hemicellulose sheath.

In the current work, we aimed to produce bioethanol from water hyacinth hydrolysate as the sole carbon source.

CHAPTER 2

LITERATURE SURVEY

We had gone through various research papers that emphasized the use of water hyacinth for the production of bio-ethanol. This report is based on an extensive systematic literature review of relevant literature for biofuel synergies, i.e. the handling of by-products between biofuel industries. The systematic literature review process was used due to its applicability in this context to allow for possible exclusion and inclusion of relevant articles based upon a clinical question, in this case finding biofuel synergies. The literature review was carried out in a step-by-step manner in order to exclude non-relevant literature from the abundance literature on the subjects. The first step of the literature review was to review how many articles were available for the topics of biomass, bioethanol, biodiesel and thereafter biofuel and to provide a pool of articles for later analysis. The literature review was carried out using the Science Direct scientific database search engine (reference to science direct). It was apparent that combination words were necessary to narrow the focus of the literature. Combination words were then used, to find relevant articles under each topic for biofuel, i.e. relevant articles for all topics; Water hyacinth, bioethanol, biodiesel and biofuel. The literature review produced a large number of possible synergies to handle external and biofuel by-products. Among the final articles produced, bioethanol synergies seem to be a very popular option for the handling of industrial wastes and biomass. In the production of ethanol, the use of DDGS for various applications is very common and many possible synergies were produced. However, not many further applications for ethanol by-products have been uncovered though several articles deal with the use of different raw materials (which are industrial by-products) for the production of ethanol.

CHAPTER 3
MATERIALS AND METHODS

3.1 Water Hyacinth Collection and Preparation

Fresh WH was collected from an open pond in Irinjalakuda. The samples were washed with tap water carefully to remove dirt, then chopped into pieces (~ 2.0−2.5 cm), dried in sunlight. Many cuts were made in the stalks to dry the stalks and blended to reduce them to small particles (~ 3.0−5.0 mm). The dried biomass was stored in air tight containers at room temperature.

Fig 3.1 Dried water hyacinth powder

3.2 Microorganisms and Cultivation

The fungal species *Saccharomyces cerevisiae* was used for the project. The isolated organisms were maintained on potato dextrose agar slants at 4^0C, and spore suspensions were carried out. It was previously characterized for its ability to ferment and use different types of sugars as sole carbon and energy sources and to produce a high yield of ethanol, was used for fermentation and bioethanol production [4].

3.3 Pretreatment optimization

1. Pretreatment with Acid

Pretreatment was carried out in Erlenmeyer flasks (250 ml) by mixing 3g of the dried water hyacinth with different acids i.e. HCI/H_2SO_4 /HCOOH(2% v/v).

The mixture was autoclaved at 121 °C, 15 Ibs for 15 min and further cooled down to room temperature. The hydrolysate was filtered using Whatman filter paper No.1 to

remove the unhydrolysed material. The filtrate was collected and analyzed for the reducing sugar content by using DNS test.

2. Pretreatment with H_2SO_4 at different Concentrations

On estimation of the reducing sugars content in different acids/alkalis maximum concentation was obtained using 2% H_2SO_4. Therefore, dried water hyacinth was subjected to H_2SO_4 treatment at different concentrations ie. 1%,2%,3%% and 4% (v/v) to obtain a concentration of H_2SO_4; that yielded maximum sugar (Fig 3.2).

Fig 3.2 Pre-treatment with H_2SO_4

3.4 Fungal Hydrolysis

WH (4 g) was moistened with 7.5 ml distilled water in a 250-ml Erlenmeyer flask and then autoclaved at 121˚C for 20 min. 1 ml Spore suspension (≈ 4×10^7 spores/ml) of fungal isolates was added to the WH and incubated at 30˚C for 10 days. After fungal incubation, the solid material was vigorously mixed with 100 ml distilled water for extraction of soluble total reducing sugars. The flasks were then shaken and filtered with a cheese cloth to separate the solid materials. The filtrate was centrifuged at 13,000×g for 10 min, and TRS in the clear supernatant were measured [5].

3.5 Sugar Estimation

Total reducing sugar was estimated by using dinitrosalicylic acid (DNS) reagent. 3ml of DNSA reagent was added to 3ml of hydrolyzed sample in a test tube. The mixture was heated at 90°C for 5-15min to develop the red brown colour. Further 1ml of 40%

Potassium tartarate (Rochelle salt) solution to stabilize the colour. After cooling at room temperature in a cold water bath, record the absorbance with a spectrophotometer at 575 nm.

3.5 Bioethanol Production

Batch bioethanol production was performed using shake-flask fermentation. The fermentation process was carried out in a medium with the following ingredients [6]: 10 g/l peptone, 2 g/l KH_2PO_4, 1 g/l $MgSO_4 \cdot 7H_2O$ and sugar hydrolyzates obtained from WH. The medium pH was adjusted to 5.5, and it was sterilized. In aseptic conditions, the bioreactor was inoculated with 10% (v/v) yeast suspension ($\approx 10^5$ cells/ml). The batch fermentation was conducted for 60 h at 30°C, and then the ethanol produced was determined.

Fig 3.3 Production of Bio-ethanol in Shake-flask fermentation

CHAPTER 4
RESULTS AND DISCUSSION

Production of bioethanol from water hyacinth was confirmed.

Bioethanol was produced from water hyacinth through series of steps. Sulphuric acid gave best results for the yield of sugars as compared to other acids and alkalis which was higher amount of sugar per gram of water hyacinth sample when treated with 4% (v/v) of H_2SO_4. Two confirmation tests were done in order to make sure that the bioethanol is formed [7].

- 0.2 ml of ethanol is mixed with 5ml of 20% H_2SO_4 and 1 ml of 5% $K_2Cr_2O_7$ and heated in water bath to obtain bluish-green colour.
- Test for confirmation of bioethanol was adding iodine and sodium hydroxide, a pale yellow precipitate of iodoform or triiodomethane is formed.

These tests gives us the confirmation that bioethanol is formed from water hyacinth.

Fig 4.1 Bluish – green colour obtained in $K_2Cr_2O_7$ Test

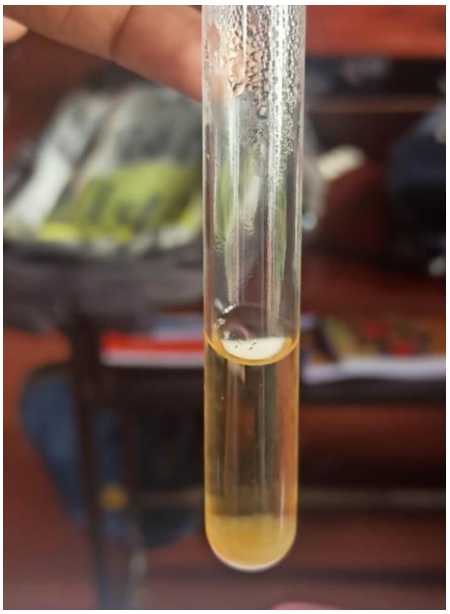

Fig 4.2 Pale yellow precipitate obtained in Iodoform Test

CHAPTER 5

CONCLUSION

Lignocellulose-to-ethanol bioconversion holds great potential as the substrate is abundant and relatively of low cost. However, the integration of low cost pre-treatments with advanced ethanol-producing microorganisms may play a crucial role in lowering the cost of biomass bioconversion processes. As water hyacinth is a pollutant and gradually converting this to bioethanol would help us in reducing pollution and also it can be used as a biofuel. Saccharification of lignocellulosic biomass increases the amount of available sugars and thus reduces bioethanol production costs [8]. Bio-ethanol feed stocks can be divided into three major groups: (1) sucrose-containing feed stocks (eg. sugar cane, sugar beet, sweet sorghum), (2) starchy materials (e.g. corn, wheat, rice, potatoes, cassava, sweet potatoes and barley), and 3) lignocellulosic biomass (e.g. wood, straw, and grasses). Biofuel/Bioethanol production from agricultural products has been in practice for the past 80 years which has created a direct competition between food and energy sector. Limited fraction of the biomass can be converted with known enzymatic technology today for production of cellulosic ethanol. Bioethanol production is renewable, as the CO_2 emitted from burning is recycled by plants during their photosynthesis. Bioethanol production from weed biomass is promising and cheaper, as their cellulosic substrates do not need extra additional economic effort to grow on waste land or water bodies. Due to the difference in their structure and cell wall components from other plant species, the digestion in the bioconversion process is affected during bioethanol production. The most potential fraction of the cell wall for the production of bioethanol is cellulose and hemicelluloses, whereas through acidic and enzymatic (hemicellulase/cellulase) reactions, carbohydrate is possible to be transformed to fermentable monomeric sugars.

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