UTILISATION OF AUSTRALIAN PINEAPPLE *(ANANAS COMOSUS)* PROCESSING WASTE FOR THE PRODUCTION OF RENEWABLE ENERGY

A Thesis submitted by

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Abstract

Globally, Pineapple, *Ananas comosus,* is the third most popular tropical fruit grown, after citrus and banana, and is the third most popular juice after orange and apple. The current and increasing consumption of fruit juice and related products has resulted in the growth of the fruit processing industry, which translates into a large generation of waste. Pineapple is processed into canned juice or slices, jam, and glazes so the fruit can be available year-round. Processing generates 40-55% of waste in the form of a crown, outer peel, core, and bottom slice. The waste produced during the processing of the fruit poses significant environmental problems. The debris from the fruit processing industry contains nitrogen and phosphorus along with a high content of carbohydrates and moisture which makes it an ideal substrate for microbes to grow and create an environmental hazard. Until recently, processing waste was considered a liability and mainly sent to animal feed processing plants, composting, or landfills. In recent years, strict government policies on landfill meant that sending processing waste to landfills is a less economic proposition. To reduce the quantity of waste, processors are forced to develop alternative technologies that can either create new products from waste or reduce the environmental impacts of their operations.

This study developed and optimized the effects of different chemical pre-treatments using various concentrations (0.5%, 1%, 3%, 5%, (w/v)) of sulfuric acid (H_2SO_4), phosphoric acid (H_3PO_4), sodium hydroxide (NaOH) or calcium hydroxide $Ca(OH)_2$. The effect of this chemical treatment followed by enzymatic treatment (Cellulase) was investigated and optimized for the production of fermentable sugar, to be converted to bioethanol using yeast. The sample treated with 1% NaOH gave the highest amount of reducing sugars (265.8 ± 4.1 g/L) after 7 days followed by 229.6 ± 5.2 g/L in the untreated raw pineapple waste samples and 0.5% Sulphuric acid with 191 ± 5.2 g/L. Enzymatic hydrolysis using a dosage of 320 µL of commercial cellulase resulted in a 79% increase in reducing sugar concentration after 24 hours, with a maximum of 9.45 ±0.94 g/L ethanol obtained after 48 h of fermentation.

Further to chemical pre-treatment, the effect of physical and combined physical and chemical pre-treatments was studied and optimized using microwave irradiation to shorten the time frame. The effectiveness of microwave-assisted alkaline sodium hydroxide (NaOH) and acidic sulfuric acid (H_2SO_4) was studied using concentrations of 0.5% and 1% at a microwave power level of 100% and 50% for 1 and 2 minutes respectively. The highest concentration of reducing sugars was 298.2g/L using 0.5% sulfuric acid and 100% for 1 minute. Microwave irradiation for a longer time (2 min) led to hydrothermal degradation of sugars and resulted in a lower amount of reducing sugar (231.6 ± 1.9 g/L). 160 µL of commercial cellulase enzyme was used to further hydrolyze the pretreated samples. 1% H_2SO_4 pre-treatment with 2 min microwave heating at 100% power produced the highest amount of phenolic content of 0.918 ± 0.06 mg GAE/mL.

The resulting solid residues of pineapple processing underwent a pyrolysis process with a temperature ramp-up rate of 10 °C per min to three different temperature setpoints of 350 °C, 750 °C, and 900 °C all withholding time of 2 h. The characteristics of these samples were compared using a range of analytical methods, which were used to interpret the mechanism of removal of arsenite [As(III)] from surface water. The samples prepared at 900 °C had the highest removal capacity for As(III) at 9.21 µg/g.

This study confirms that pineapple waste can be readily used for the production of fermentable sugars and conversion to ethanol. The solid residues can be further used in the production of biochar. This biochar can be then utilized as adsorbents for many useful environmental applications.

Certification of Thesis

This Thesis is the work of Mr. Kiran Ramesh Mahale except where otherwise acknowledged, with the majority of the authorship of the papers presented as a Thesis by Publication undertaken by the Student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Principal Superviosr: Dr. Antoine P. Trzcinski

Associate Supervisor: Dr. Mark Lynch

Student's and Supervisors' signatures of endorsement are held at the University of Southern Queensland.

Statement of Contribution

The following details are the agreed share of contribution for candidate and co-authors in the presented publications in this thesis:

Paper I: Kiran R. Mahale., Mark Lynch., Antoine P. Trzcinski, and Les Bowtell " Converting Pineapple *(Ananas comosus)* wastes into value-added products."2020. (Prepared for submission to Journal Nutrients; Q1)

Contributions:

- Kiran R. Mahale formulated the existing research gaps, prepared the abstract, and outlined and composed the manuscript. The Contribution made by the candidate is 80%

- Dr. Mark Lynch, Dr. Antoine P. Trzcinski, and Dr. Les Bowtell assisted with the manuscript compilation, editing, and critical revision. Overall, their contribution to this manuscript is 20%.

Paper II: Kiran R. Mahale and Antoine P. Trzcinski, Effects of alkali, acidic and enzymatic pre-treatments on the ethanol fermentation of pineapple (Ananas comosus) processing waste. 2020. **(Submitted to Journal of Biomass Conversion and Biorefinery;)**

Contributions:

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- Dr. Antoine P. Trzcinski assisted with the manuscript compilation, editing, and critical revision. Overall, his contribution to this manuscript is 20%.

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- Kiran R. Mahale prepared the abstract, designed the experiments, collected and analyzed all the data, and composed the content of the manuscript. The contribution made by the candidate is 80%.

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Proceeding II: **Mahale, Kiran R**., and Burey, Paulomi (Polly) (2018) *Emerging technologies for extraction of bioactives and polysaccharides from tropical fruit waste.* In: 2nd ISEKI Food Waste Recovery Workshop 2018, 3-5 July 2018, Stuttgart, Germany

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Paper I:

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Dedication

I dedicate my thesis to My Grandparents, parents,

and

my loving wife Pradnya.

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Abbreviations

2,4-dinitro salicylic acid (DNS)

A. comosus (Ananas comosus)

American Type Culture Collection (ATCC)

Ammonia fiber explosion (AFEX)

Arsenic (As)

Aqueous two-phase system (ATPS)

Bacterial Cellulose (BC)

Biochar (BC)

Biochemical Oxygen Demand (BOD)

Cents per liter (c/L)

Calcium hydroxide $Ca(OH)_2$

Chemical Oxygen Demand (COD)

Distilled water (dH_2O)

Food Waste (FW)

Fourier transform infrared (FTIR)

Furfural (F)

gram/gram (g/g)

Gram/Liter (g/L)

Hydrochloric acid (HCL)

Hydroxymethyl Furfural (HMF)

Million Liters (ML)

Phosphoric acid (H_3PO_4)

Pineapple-On-Farm-Waste (POFW)

Pineapple-Processing-Waste (PPW)

Reducing sugar (RS)

Rotation per minute (RPM)

S. Cerevisiae (Saccharomyces Cerevisiae)

Scanning electron microscope (SEM)

Single-cell protein (SCP)

Sodium Hydroxide (NaOH)

Sulfuric acid (H_2SO_4)

T. Reesei (Trichoderma reesei)

Total sugar (TS)

Total phenolic content (TPC)

United States of America (USA)

Volatile Solids (VS)

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CHAPTER 1: INTRODUCTION TO THESIS

1.1 Introduction

Every year approximately one-third of the total food produced goes wasted throughout the food supply chain starting from agricultural land during harvesting till it gets to the consumer, resulting in loss of resources, economic loss, and impact on environment inform of carbon footprint (fig. 1.1) [1]. Australia produces 5.3 million tonnes of food waste (FW) generates every year [2]. Australian consumers throw away 3.1 million tonnes of edible food and Commercial and Industrial sectors generate 2.2 million tonnes of food waste annually coasting around $ 20 billion each year to the Australian economy [2]. At present FW is categorize as municipal waste and is sent to landfills and incineration plants for disposal. Due to change in government policies and limited space in landfills coast of sending FW to landfills is becoming unaffordable to the processing industry also it has an impact on the environment as pollution of groundwater, soil pollution and emission of toxic and greenhouse gases [1]. To meet the requirement for the food supply of the growing population of the world more and more food production and processing are required which is increasing food waste [3]. Food wastage is considered a serious social, economic, and environmental problem. Underutilized food waste represents a missed opportunity to mitigate climate change, produce cleaner chemicals and fuels, spur local economies, and generate jobs [4]. Currently, a large fraction of pre-and post-consumer organic waste either ends up in landfills and incinerators or is discarded into water bodies. According to recent data, approximately one-third of all food produced for human consumption is lost or wasted, equivalent to 1.6 billion tons per year. Approximately 54% of this loss is caused during food production steps, including postharvest handling and storage, and 46% of the loss occurs during the downstream steps such as processing, distribution, and consumption. On a weight basis, approximately 30% of cereals; 40%–50% of root crops, fruits, and vegetables [4].

The environmental concerns, economic crisis on fuel price, and political issues have opened up the opportunities for the development of new technologies to utilize the unused biomass for the production of useful chemicals, materials, sources of energy, and fuels from food waste, which is responsible for the emission of toxic greenhouse gases [5, 6]. The recent trend of consumption of fruit juices and fruit-derived products such as soft drinks, nectars, cordials, and flavored ice-creams have increased the processing of such fruits resulted in a significant increase in the amount of processing waste in the form of peels, seeds, pulp and stones [3, 7, 8]. Disposal of these by-products is a major issue for many processing industries, as the material is prone to microbial spoilage, and sending it to landfills has limitations from limited space and changing government regulations [3, 8-10]. The waste materials from agriculture, industries, and domestic sources are rich sources of phytochemicals [4, 6, 11-13]. FW is mainly composed of carbohydrate polymers (starch, cellulose, and hemicelluloses), lignin, proteins, lipids, organic acids, and a remaining, smaller inorganic part [1, 7, 12]. This composition makes FW perfect biomass to be used in the production of energy using different technologies [5] such as anaerobic digestion [14, 15], fermentation [16, 17], gasification and pyrolysis, and hydrothermal liquefaction [18].

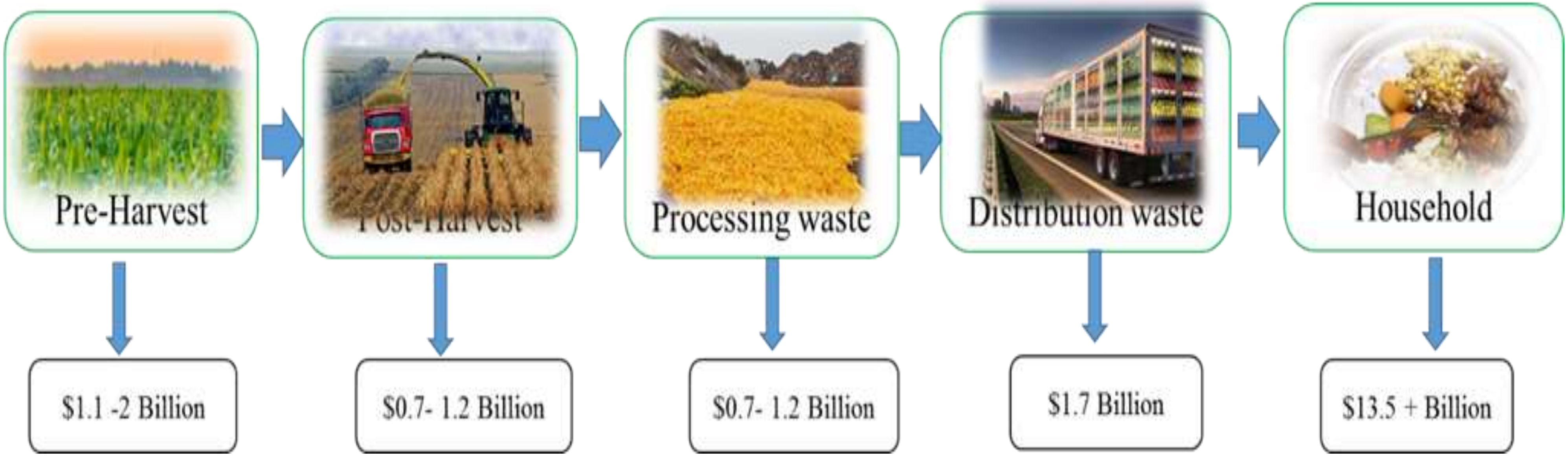

Fig. 1.1 Stages and value of food waste in the food life cycle

1.2 Australia Energy requirements and renewable energy production

Australian energy consumption rose by 2 percent in the year 2015-2016. On average, energy consumption has been growing every year by 0.6 percent a year over the one decade as indicated in table 1.2 The growth in energy consumption was in mining, electricity generation, and transport sectors [19]. Australia is a major importer of crude oil and refined oil products for transport fuel to meet the energy requirement of the country on the other hand Australia is one of the leading exporter of energy in the form of coal [20].

With the increasing energy demand as shown in table 1.6 and legislation getting stricter, it is getting difficult for landfilling the waste biomass food processing industry needs to consider different options for the utilization of food waste for the production of energy. The Australian biofuel industry and the market is comparatively small than other countries as every state has its law and does not implies to other states. With minimum support from the government biodiesel production and import has been affected since 2014 as the crude oil prices were low and due to drought conditions, the feedstock was expensive. Total production for 2018 is estimated to be 290 million liters (ML), comprised of 250 ML of ethanol and 40 ML of biodiesel [20]. Second-generation biofuels like energy crops, and algae-based fuels, have been successfully tested but are not commercially viable yet. Queensland state government is providing support to in-house industry to develop advanced biofuels for military, aviation, and marine applications [20].

The support for renewable energy in Australia is highly focused on electricity generation using solar panels. More than 16% of total electricity generated in Australia in the year 2016 was generated through renewable sources.

Table 1.1: Australian renewable energy production by fuel type, 2014-15

Source	Renewable energy production (PJ)	Share (%)	2014-15	Average annual growth, 2006-2015 (%)
Biomass	186.7	54.4	6.8	-1.2
Wood, wood waste	81.7	23.8	-2.1	-2.1
Bagasse	102.9	30.0	14.0	-0.6
Biogas	19.1	5.6	17.0	9.9
Biofuels	11.4	3.3	-12.1	23.7
Ethanol	6.7	2.0	-22.7	NA
Biodiesel	4.7	1.4	9.7	NA

(*Source*: Department of Industry and Science (2016), *Australian Energy Statistics*, Canberra)

Table 1.2: Australian renewable energy consumption by fuel type, 2014

Source	Renewable energy production (PJ)	Share (%)	Change since 2013 (%)	Average annual growth, 2006-2015 (%)
Biomass	183	53.0	-1.1	-1.4
Wood, wood waste	93	26.9	3.1	-0.9
Bagasse	90	26.1	-5.1	-2.0
Biogas	16	4.7	17.0	9.1
Biofuels	12	3.6	2.6	24.3
Ethanol	7	2.1	-12.1	NA
Biodiesel	5	1.5	33.5	NA

(*Source*: Department of Industry and Science (2015), *Australian Energy Statistics*, Canberra)

1.3 Australia biofuel production support and policies affecting biofuels

Bioethanol, often shortened as ethanol, is an ecological liquid that can be used in vehicles as fuel derived from biomass. Ethanol is commonly known as ethyl alcohol, grain spirit or moonshine, or even alcohol. Ethanol is now largely used as fuel for vehicles in its purest form as a replacement for gasoline.

1.3.1 Support from state government (mandates)

The government and private companies are now focusing on renewable energy sources as a process of decarbonization and for better fuel options for the future. The first-generation biofuels such as rapeseed biodiesel, wheat ethanol are now getting the attention of the transport sector. The potential of an increase in renewable energy is based on a few factors such as the availability of biomass, resources available. Australia in particular blessed with sustainable biomass resources like specialty grown crops, and bio-products from the agro and agro-processing industry. Municipal solid waste. However, the majority of the bioenergy produced in Australia comes from the combustion of sugarcane bagasse. [21].

The advantages that Australia has that increases the potential to develop the sustainable bioenergy industry are

- Plenty of sunlight, plain land, and climate that suits to grow the dedicated energy plants
- Expertise and availability of world-class facilities
- A strength in natural resources and technological advancement
- First-World economy status and low political risk

1.4 policies affecting biofuels in Australia

- Estimates of subsidies to fossil fuel use in Australia range from 2.2 to 10 billion dollars per year. These estimates include perverse subsidies which increase GHG emissions and reduce economic efficiency and subsidies to motorists — which would still apply if the motorists were running their vehicles on alternative fuels instead of fossil fuels. These need clarification in terms of the categories, values, and beneficiaries across the fossil fuel value chain.
- Assistance currently provided to producers includes (a) a production grant of 38.1 cents per liter (c/L), which fully offsets the excise paid on biofuels; (b) a capital grant for new facilities that effectively provides around 1 c/L in additional assistance over the lifetime of the plant. [22]
- Assistance to biofuels is scheduled to fall to 12.5 c/L for ethanol and 19.1 c/L for biodiesel by 1 July 2015. A banded excise system will impose rates on different fuels, classified into high, medium and low energy groups. This strategy broadly keeps constant the excise payable per kilometer traveled by vehicles using the fuel, with biofuels retaining a 50 % discount on this excise.
- Ethanol imports are subject to both a general tariff of 5 % (zero if imports are from the USA) and the full excise of mid-energy fuels of 38.1 c/L. Between 2011–2015, the net excise payable on ethanol by domestic manufacturers will increase on a sliding scale from 0–12.5 cents per liter. From 2011, the effective excise cost imposed on imported ethanol will be also be reduced to be the same as that faced by domestic manufacturers.
- Recent changes in the Fuel Tax Act 2006 have had a major impact on the biodiesel industry. Since the changes, off-road users of biodiesel blends can no longer claim 38.1 c/L on the biodiesel component of the blend unless the fuel qualifies for the Australian Diesel Standard. [22]

1.4.1 Bio-ethanol production in Australia

Fig. 1.2: Ethanol Plants in Australia. (*Source*: Biofuels Association of Australia; 2012)

Table 1.3: Capacity of Ethanol plants in Australia 2012

Plant Details	Feedstock	Production capacity
Sarina Distillery	Sugar Cane or its by-product molasses	60 Mega Litters
Dalby Bio-Refinery	Red Sorghum	80 Mega Litters
Manildra Ethanol Plant	Waste Starch	300 Mega Litters
Total Production		440 Mega Litters

(*Source*: Biofuels Association of Australia; 2012)

Bio-diesel Production in Australia

Fig. 1.3: Biodiesel Plants in Australia. (*Source*: Biofuels Association of Australia; 2012) [23])

Table 1.4: Capacity of biodiesel plants in Australia 2012

Plant Details	Feedstock	Production capacity
ARF Largs Bay, Adelaide, SA	Tallow, Used cooking oil	45 Mega Litters
ARF Picton, Picton, WA	Tallow, Used cooking oil	45 Mega Litters
BIA Biodiesel Plant, Maitland, NSW	Used cooking oil, Vegetable oil	20 Mega Litters
ARF Barnawatha, Wodonga, VIC	Tallow, Used cooking oil	60 Mega Litters
Consoldated Bio Diesel, Narangba, QLD	Tallow, Used cooking oil	30 Mega Litters
Smorgon Fuels – BioMax Plant Melbourne, VIC	Tallow, Canola Oil and Juncea Oil	100 Mega Litters
Territory Biofuels, Darwin, NT	Palm Oil, Tallow, Used cooking oil.	130 Mega Litters
Macquarie Oil, Tasmania	Poppy oil, waste vegetable oil.	15 Mega Litters

(*Source*: Biofuels Association of Australia; 2012)

1.5 Production of biofuels from Agro-west

Australia produces 5.3 million tonnes of food waste (FW) generates every year [2]. Australian consumers throw away 3.1 million tonnes of edible food and Commercial and Industrial sectors generate 2.2 million tonnes of food waste annually coasting around $ 20 billion each year to the Australian economy [2]. This chapter focuses more on the different pretreatment of pineapple waste for the production of biofuel.

1.6 Pre-treatments of fermentation

Table 1.5 shows the different pre-treatments used in the production of biofuels using food waste as feedstock. Table 1.5 elaborating the different pre-treatments also provides information on the main effect of each pre-treatment and the advantages, and disadvantages of pretreatments. Each chapter of this thesis has investigated the effect of these pretreatments in the production of ethanol using pineapple waste as feedstock.

Table 1.5: Different pre-treatments used in the production of biofuels

Pre-treatment method	Main effects	Advantages	Disadvantage/limitations	Reference
Alkali	• Removes lignin and hemicelluloses. Increases accessible surface area.	• High digestibility High lignin removal	• Long resistance time Irrecoverable salts formation	[24]
Biological pre-treatment	Degrades lignin and hemicelluloses	• Low energy consumption Cost effective	• Low rate of hydrolysis	[25] [26]
Concentrated acid	Hydrolyses both hemicelluloses and cellulose	• High glucose yield • Reduction in the operational costs due to moderate operation temperature • Low formation of degradation products • No enzymes are required	• Acid recovery is mandatory • Equipment corrosion • Generation of inhibitory compounds	
Diluted acid	• Hydrolyses hemicelluloses • Renders cellulose more amenable for a further enzymatic treatment Alters lignin structure	• Less corrosion problems than concentrated acid • Low formation of inhibitors	• Generation of degradation products due to high temperature • Low sugar concentration exit stream	[27] [28] [29]

Ionic liquids	• Reduces cellulose crystallinity Removes lignin	• High digestibility • Green solvents	• Large-scale application still under investigation	[30]
Mechanical	• Reduces cellulose crystallinity	• No formation of inhibitors	• High power and energy consumption	[31]
Supercritical fluid technology	• Increases accessible surface area	• Cost effective • No formation of inhibitors	• Does not affect lignin and hemicelluloses. • Very high pressure requirements	[32]
Organic Solvents	• Hydrolyses lignin and hemicelluloses	• Pure lignin recovery • High digestibility	• High cost • Solvents need to be drained and recycled	[33] [30]
Microwave Irradiation	• The electromagnetic waves that interact with polar molecules and ions in material and result in thermal and non-thermal effect.	• Low energy consumption • Cost effective • No formation of inhibitors	• Large-scale application still under investigation • Higher cost of instrument •	[34]

1.7 Research gaps

1. So far, no research has been conducted on producing bioethanol from Australian grown pineapple and pineapple processing waste ethanol
2. No Studies have elaborated on the efficacy of different alkaline and acidic treatment at various concentration and for a long duration
3. No studies have found comparing the efficacy of alkaline and acidic treatment together
4. No evidence was found on microwave-assisted acidic pretreatment of pineapple peel hydrolysis for bioethanol production
5. No clear evidence of utilization of solid residues left-over from bioethanol fermentation apart from AD.
6. So far, no research has been conducted on producing pineapple biochar at different temperature ranges to study its effect on As removal from water, specifically, As(III)
7. No studies have been conducted on the performance of coating Zeolite powder on biochar on As removal from water, specifically, As(III)

1.8 Research questions

1. What is the potential of Australian grown pineapple and pineapple processing waste in the production of biofuels?
2. What are the effects of different pre-treatments on pineapple waste for higher production of reducing sugars?
3. Doe's enzymatic hydrolysis will enhance the production of fermentable sugars after chemical pretreatments?
4. What is the potential for pineapple waste to be converted into an economically valuable resource, such as biochar and its composite, for water treatment?
5. Effect of zeolite powder as a coating agent on biochar for As (III) removal?

1.9 Research aims and objectives:

The overall aim of the project is to investigate the efficiency of different pretreatments on pineapple waste for the production of biofuels and the utilization of solid residues for the removal of As (III) using Pineapple biochar. The main objectives are as follows

1. Studying the effect of different chemical pre-treatments on the pineapple waste for the production of reducing sugars for fermentation of biofuels.
2. Optimization of different pre-treatments on the pineapple waste for the production of reducing sugars for fermentation of biofuels.
3. Studying the effect of different Physical pre-treatments on the pineapple waste for the production of reducing sugars for fermentation of biofuels.
4. The study combines the effects of chemical and physical pre-treatments on the pineapple waste for the production of reducing sugars for fermentation of biofuels.
5. Studying the effect of charring temperature on the sorbent properties and adsorption performance of the biochar.

1.10 References

Australia, BAo 2012, *Biodiesel Plants in Australia*, Biofuels Association of Australia, viewed 08/02/2019, <http://www.biofuelsassociation.com.au/~biofuels/images/stories/pdf/biodieselmap.pdf>.

da Silva, ARG, Ortega, CET & Rong, B 2016, 'Techno-economic analysis of different pretreatment processes for lignocellulosic-based bioethanol production', *Bioresource Technology*, vol. 218, pp. 561-70.

Energy, DotEa 2018, *Tackling australia's food waste: Deapartment of the Environment and Energy, Fedral Government of Australia*, viewed 05/03/2018, <http://www.environment.gov.au/protection/waste-resource-recovery/food-waste>.

Espinoza-Acosta, JL, Torres-Chavez, PI, Carvajal-Millan, E, Ramirez-Wong, B, Bello-Perez, LA & Montano-Leyva, B 2014, 'Ionic Liquids and Organic Solvents for Recovering Lignin from Lignocellulosic Biomass', *Bioresources*, vol. 9, no. 2, pp. 3660-87.

Girotto, F, Alibardi, L & Cossu, R 2015, 'Food waste generation and industrial uses: A review', *Waste Management*, vol. 45, pp. 32-41.

Hegde, S, Lodge, JS & Trabold, TA 2018, 'Characteristics of food processing wastes and their use in sustainable alcohol production', *Renewable & Sustainable Energy Reviews*, vol. 81, pp. 510-23.

Kim, JS, Lee, YY & Kim, TH 2016, 'A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass', *Bioresource Technology*, vol. 199, pp. 42-8.

Kiran, EU, Trzcinski, AP & Liu, Y 2015, 'Platform chemical production from food wastes using a biorefinery concept', *Journal of Chemical Technology and Biotechnology*, vol. 90, no. 8, pp. 1364-79.

Kiran, EU, Trzcinski, AP, Ng, WJ & Liu, Y 2014, 'Bioconversion of food waste to energy: A review', *Fuel*, vol. 134, pp. 389-99.

Li, HQ, Qu, YS, Yang, YQ, Chang, SL & Xu, J 2016, 'Microwave irradiation - A green and efficient way to pretreat biomass', *Bioresource Technology*, vol. 199, pp. 34-41.

Li, L, Peng, XY, Wang, XM & Di Wu, D 2018, 'Anaerobic digestion of food waste: A review focusing on process stability', *Bioresource Technology*, vol. 248, pp. 20-8.

M.Galanakis, C 2012, 'Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications', *Trends in Food Science & Technology*, vol. 26, no. 2, pp. 68-87.

Ma, HZ, Yue, SY, Li, HG, Wang, QH & Tu, MB 2019, 'Recovery of lactic acid and other organic acids from food waste ethanol fermentation stillage: Feasibility and effects of substrates', *Separation and Purification Technology*, vol. 209, pp. 223-8.

Mathew, AK, Parameshwaran, B, Sukumaran, RK & Pandey, A 2016, 'An evaluation of dilute acid and ammonia fiber explosion pretreatment for cellulosic ethanol production', *Bioresource Technology*, vol. 199, pp. 13-20.

Mckenzie, S 2018, *Australia Bioenery policies and status of implementation 2018 updates* IEA Bioenergy IEA Bioenergy, https://www.ieabioenergy.com/wp-content/uploads/2018/10/CountryReport2018_Australia_final.pdf>.

Mirabella, N, Castellani, V & Sala, S 2014, 'Current options for the valorization of food manufacturing waste: a review', *Journal of Cleaner Production*, vol. 65, pp. 28-41.

Muhammad Waqasa, MR, Muhammad Daud Khanb, Abdul-Sattar Nizamia 2019, 'Conversion of Food Waste to Fermentation Products', *Encyclopedia of Food Security and Sustainability*, vol. 1, pp. 501-9.

Nazzaro, F, Fratianni, F, Ombra, MN, d'Acierno, A & Coppola, R 2018, 'Recovery of biomolecules of high benefit from food waste', *Current Opinion in Food Science*, vol. 22, pp. 43-54.

O’Connell, DD 2007, *Biofuels in Australia — issues and prospects*, CSW – 44A, RIRaD Corporation, http://biomassproducer.com.au/wp-content/uploads/2013/11/BiofuelsinAustralia_CSIRO.pdf>.

Paritosh, K, Kushwaha, SK, Yadav, M, Pareek, N, Chawade, A & Vivekanand, V 2017, 'Food Waste to Energy: An Overview of Sustainable Approaches for Food Waste Management and Nutrient Recycling', *Biomed Research International*.

Park, J, Jones, B, Koo, B, Chen, X, Tucker, M, Yu, JH, Pschorn, T, Venditti, R & Park, S 2016, 'Use of mechanical refining to improve the production of low-cost sugars from lignocellulosic biomass', *Bioresource Technology*, vol. 199, pp. 59-67.

Pham, TPT, Kaushik, R, Parshetti, GK, Mahmood, R & Balasubramanian, R 2015, 'Food waste-to-energy conversion technologies: Current status and future directions', *Waste Management*, vol. 38, pp. 399-408.

Ravindran, R & Jaiswal, AK 2016, 'A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities', *Bioresource Technology*, vol. 199, pp. 92-102.

Ren, YY, Yu, M, Wu, CF, Wang, QH, Gao, M, Huang, QQ & Liu, Y 2018, 'A comprehensive review on food waste anaerobic digestion: Research updates and tendencies', *Bioresource Technology*, vol. 247, pp. 1069-76.

Roger Farrell 2017, *Australia Biofuels Annual 2017*, ASI1712, USDA Foreign Agricultural Service Canberra, https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Biofuels%20Annual_Canberra_Australia_8-15-2017.pdf>.

Schieber, A, Stintzing, FC & Carle, R 2001, 'By-products of plant food processing as a source of functional compounds - recent developments', *Trends in Food Science & Technology*, vol. 12, no. 11, pp. 401-+.

Serna, LVD, Alzate, CEO & Alzate, CAC 2016, 'Supercritical fluids as a green technology for the pretreatment of lignocellulosic biomass', *Bioresource Technology*, vol. 199, pp. 113-20.

Sharma, HK, Xu, CB & Qin, WS 2019, 'Biological Pretreatment of Lignocellulosic Biomass for Biofuels and Bioproducts: An Overview', *Waste and Biomass Valorization*, vol. 10, no. 2, pp. 235-51.

Sindhu, R, Binod, P & Pandey, A 2016, 'Biological pretreatment of lignocellulosic biomass - An overview', *Bioresource Technology*, vol. 199, pp. 76-82.

Sonja Djtlas Jasna. Čanadanovic-Brunet, GC 2009, 'By-products of ruits processing as a source of phytochemicals', *Chemical Industry & Chemical Engineering Quarterly*, vol. 15, no. 4, pp. 191-202.

Sundaram, V & Muthukumarappan, K 2016, 'Influence of AFEX (TM) pretreated corn stover and switchgrass blending on the compaction characteristics and sugar yields of the pellets', *Industrial Crops and Products*, vol. 83, pp. 537-44.

team, AESp 2017, *Australian Energy Update 2017*, DotEa Energy, Department of the Environment and Energy, Government of Australia CANBERRA, viewed 8/1/2019, <https://www.energy.gov.au/sites/default/files/energy-update-report-2017.pdf>.

Vigano, J, Machado, APD & Martinez, J 2015, 'Sub- and supercritical fluid technology applied to food waste processing', *Journal of Supercritical Fluids*, vol. 96, pp. 272-86.

W.Babbitt, TATaC 2018, ' Introduction to Sustainable Food Waste-To-energy Systems', in TATaC W.Babbitt (ed.), *Sustainable Food Waste-to-Energy Systems*, Academic Press, vol. 1, pp. 1-10.

Zhang, K, Pei, ZJ & Wang, DH 2016, 'Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review', *Bioresource Technology*, vol. 199, pp. 21-33.

CHAPTER 2: Converting Pineapple *(Ananas comosus)* wastes into value-added products: environmentally friendly approaches

Kiran R. Mahale [a*], Mark Lynch [b], Antoine P. Trzcinski [a], and Les Bowtell [c].

Introduction Chapter 2

The present articles provide information about the different and innovative approaches to develop value-added products from pineapple waste. The present article aimed to review the latest advances in various popular methods of extraction for obtaining value-added products from pineapple waste/byproducts and their potential utility as a source of various functional compounds.

Review:

Converting Pineapple *(Ananas comosus)* wastes into value-added products: environmentally friendly approaches

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Abstract:

Pineapple *(Ananas comosus)* is one of the most popular fruit in the world due to its tropical aroma and exotic taste. Over 25 million tonnes of pineapples are produced globally every year. The pineapple processing industry generates huge amounts of wastes every year and it's almost 50% of the wet fruit mass. Pineapple waste has vast economic value as it contains an abundance of various polyphenols, very important enzymes, fibers, sugars, and other organic acids. Pineapple waste also contains high levels of sugars that can be further used in the fermentation of bioethanol and other energy-driven products. The present article aims to review the latest advances in various popular methods of extraction of value-added products from pineapple processing waste and their potential use as a source of various functional compounds.

Keywords: Pineapple, value-addition, by-products, ethanol, waste management,

2.1 Introduction:

Pineapple, *Ananas comosus* is the third most popular and growing tropical fruit after citrus and banana in the world and is the third most popular juice after orange and apple [35]. The origin of pineapple is thought to be in South America, particularly in the region between Brazil and Paraguay [36]. The tropical aroma and exotic taste make pineapple one of the vital fruit. The processing of pineapple has made the fruit well known even in the temperate parts of the world [37]. The annual global production of approximately 25 million tonnes, with Costa Rica being the largest producer with approximately 2.93 million tonnes closely followed by Brazil with 2.69 million tonnes while Philipines, India, Thailand, and Indonesia are the other leading producers of pineapple in the world [38]. The current and increasing consumption of fruit juice and products has resulted in the growth of the fruit processing industry, which translated into the generation of a large amount of waste [39]. The processing industry process the pineapple for the production of canned juice or slices, jam, glaze so the fruit can be available throughout the year [40, 41].

Based on the location of the production, Pineapple waste can be classified as Pineapple-On-Farm-Waste (POFW) and Pineapple-Processing-Waste (PPW). POFW mostly contains leaves, stems, and roots that are left on the farm after the harvesting. A survey conducted in India estimated that every pineapple plant generates around 6-8kg of fresh POWF [42]. The processing industry generates 40-55% of waste in the form of a crown, outer peel, core, and bottom slice [43, 44]. Pineapple is processed in different ways depending on the use of end products like slice, pulp, and juice. The juicing industry generates liquid waste along with residues like peel, crown, and core as shown in figure 1 while Figure 2 shows the general anatomy of the fruit.

The waste produced during the processing of the fruits poses significant environmental problems. The debris from the fruit processing industry contains large quantities of nitrogen and phosphates along with high content of carbohydrates and moisture which makes it an ideal substrate for microbes to grow and create an environmental hazard [45]. Until the last decade, processing waste was considered a liability and mainly sent to animal feed processing plants, composting, or landfills. In recent years, strict government policies on landfills mean that sending processing waste to landfills is becoming a non-economical option. To reduce the quantity of waste, processors are forced to fo develop alternative technologies that can create new products from waste or reduce the environmental impacts of their operations [46].

Destruction of PPW is an issue for the processing industry as it requires enormous investment and logistics. The changing rules of landfills, gate fees, and strict government policies are making it difficult for the processing industry to dispose of waste in landfills. Landfilling can contaminate the soil and waterways as well as releasing greenhouse gases [47]. A high amount of carbohydrates in PPW attracts fungi and bacteria very quickly which can generate food contamination [48]. Burning solid residues is also not a good option due to the high moisture content and it also releases pollutants such as dioxins, furans, acid gases, and particulate matter [49]. Table 2.1 shows the contains valuable constituents present in pineapple waste like sucrose, glucose, fructose, cellulose, fibers, bromelain, and other nutrients [50].

Table 2.1. Chemical Composition of pineapple waste generated during the pineapple canning process

Composition	Parameters	Liquid waste (g/L)	Solid waste (%)
Sugars	Reducing sugars	40.40 g/L	20.93 %
	Sucrose	16.75 g/L	0.00%
	Glucose	19.72 g/L	8.24%
	Fructose	20.62 g/L	12.71%
	Total Sugar	73.76 g/L	---
Proteins	Soluble Proteins	1.13 g/L	---
	Kjeldahl nitrogen	0.64 g/L	---
Organic acids	Acidity as citric acid	2.95 g/L	---
	Malic acid	0.29 g/L	---

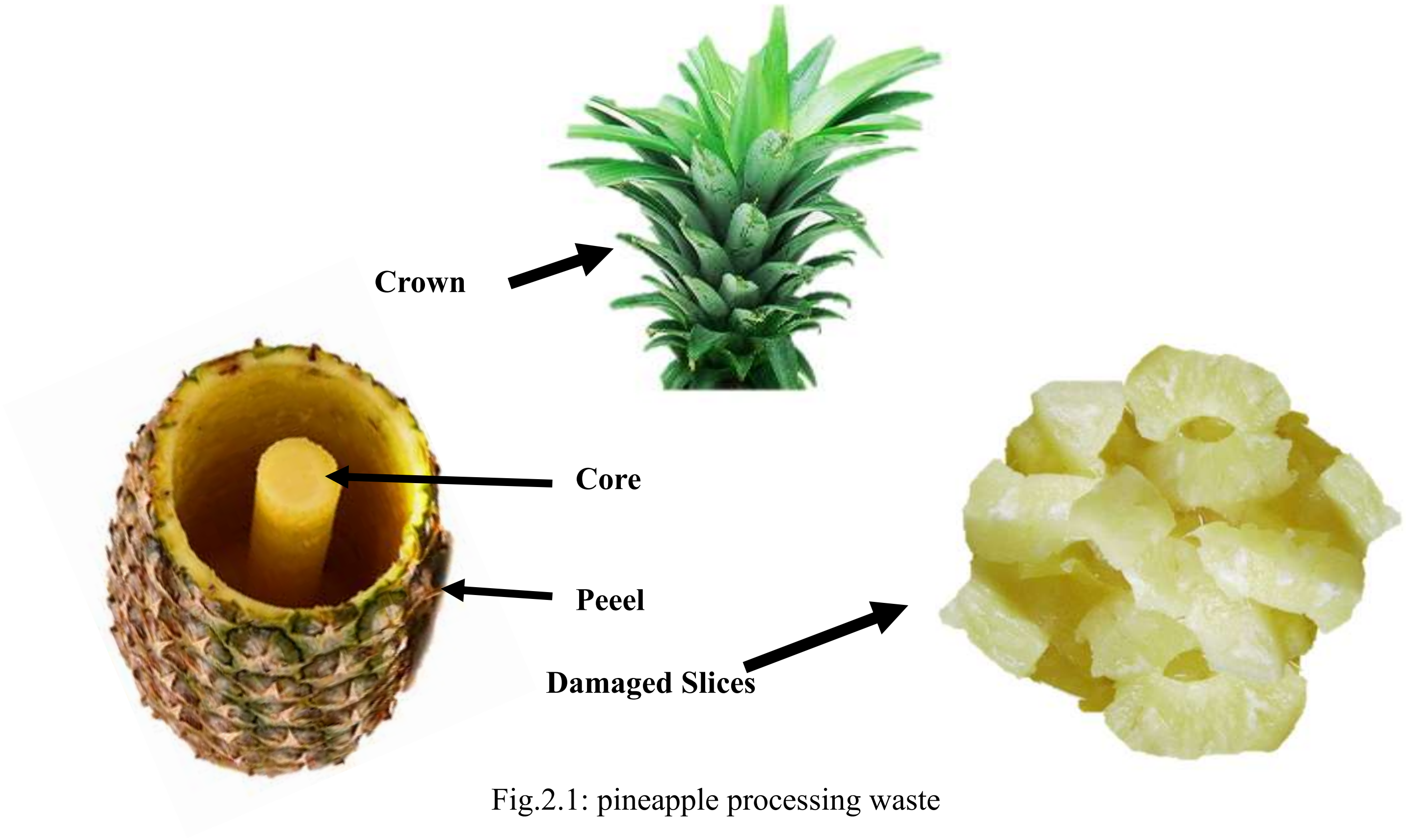


Fig.2.1: pineapple processing waste

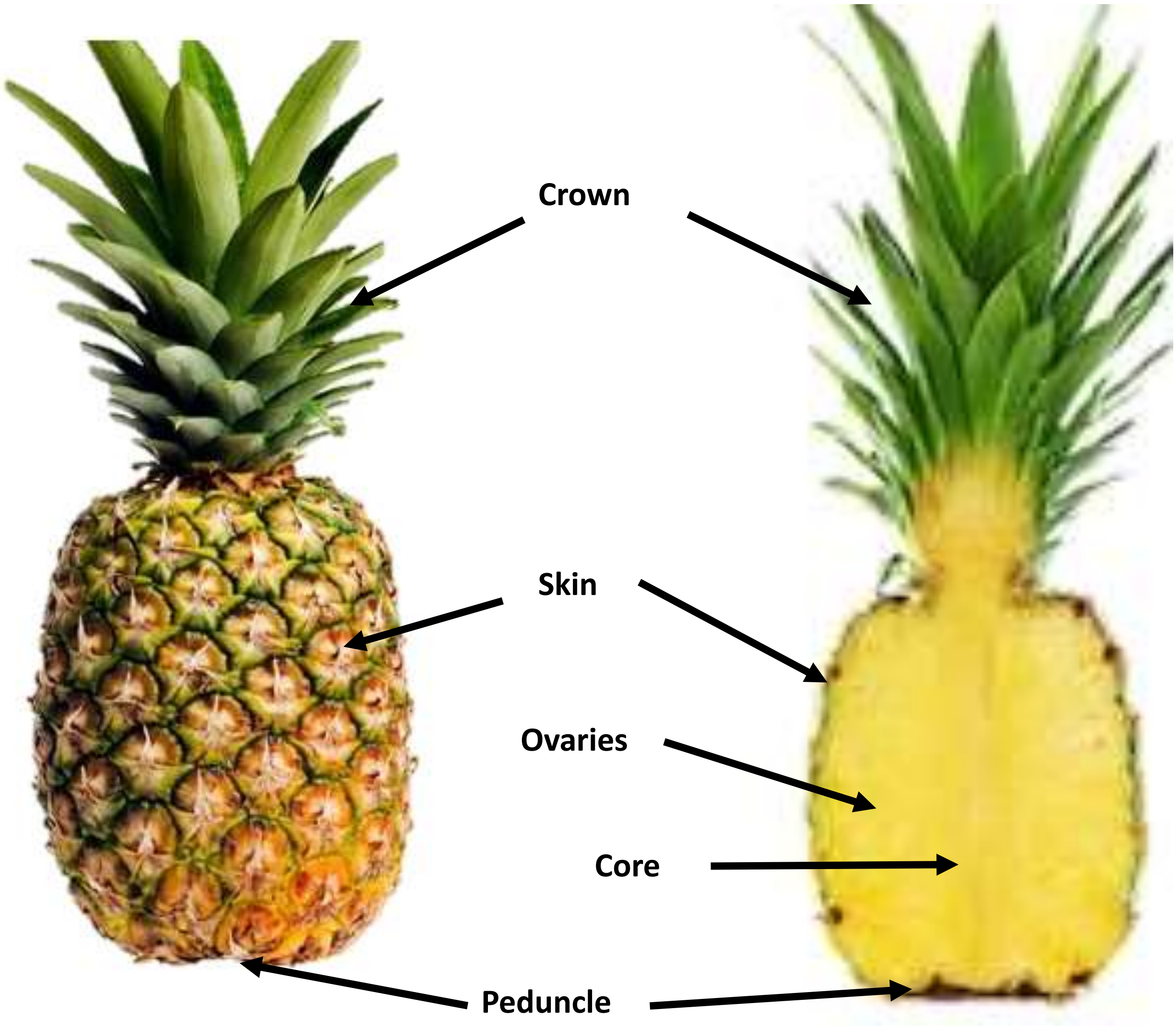


Fig. 2.2. Showing General anatomy of pineapple fruit

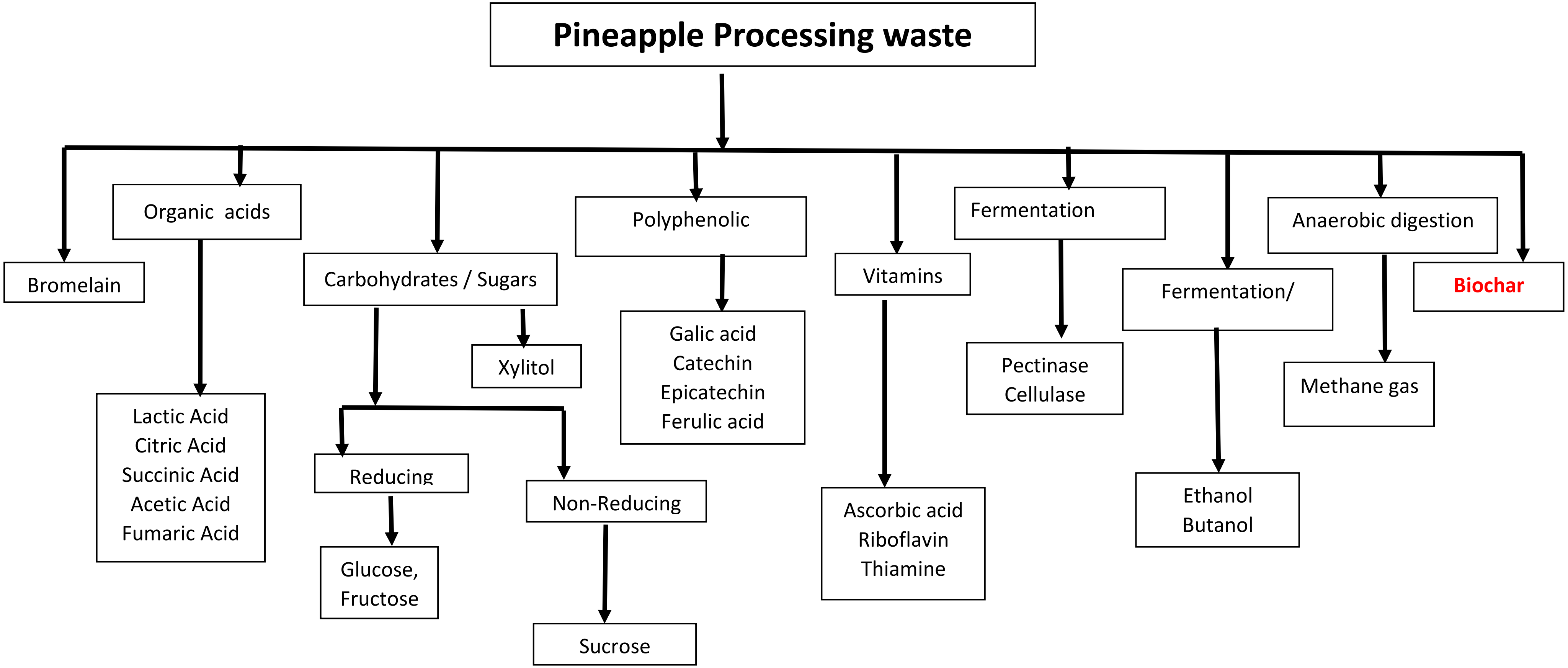


Figure 2.3. Different value-added products can be made from pineapple waste.

2.2 Australian Pineapple Industry

Pineapple is exotic to Australia and is grown as an agricultural crop almost exclusively in Queensland. The pineapple was first introduced to Australia from India by Lutheran missionaries in the southern coastal district of Queensland in about 1838 [51]. The major producing areas include southeast Queensland from Beerwah to Wamuran through to Mareeba, Rollingstone, Yeppoon, and Wide Bay in Queensland and Darwin in the Northern Territory as shown in (figure 2.4).

Figure 2.4. Major Pineapple production areas in Australia. *(Source: Australian Horticulture Statistics Handbook Fruit 2017/18 page183)*

In the financial year ending June 2018, the total production of pineapple in Australia was over 76,002 tonnes valued at $53.4 million. 38% of the total produced, i.e. over 28,000 tonnes of pineapple were sent to the processing industry to be either tinned or juiced. 62% of the total production was supplied as fresh supply with an estimated total value of $48.3 million [52]. Table (2) shows the changes in production and value of the pineapple industry over the last three years. There has been a notable decrease in production from the year 2016-2018.

Table 2.2. Comparison of Australian Pineapple industry year 2016-2018) *(Source: Australian Horticulture Statistics Handbook Fruit 2017/18 page183)*

Year Ending June	2016	2017		2018	
	Value	Value	%YoY	Value	%YoY
Production (t)	75,376	77,482	+3%	76,002	-2%
Production value ($m)	$55.0	$54.2	-1	$53.4	-1
Fresh supply (t)	47,486	45,897	-3	47,206	+3
Fresh supply Wholesale Value ($m)	$50.7	$47.7	-6	$48.3	+1

2.2.1 Australian pineapple problems and International trade

The Australian pineapple processing industry has been struggling to compete with the global market due to heavy competition from cheaper imported products from Thailand, Malaysia, and Asian countries. A steady decrease in processing industry turnover has been recorded over the past few years [53]. Australia imported over 19,000 tonnes of preserved pineapples along with over 4,000-kiloliters of pineapple juice in the year 2018. On the other hand, 126 tonnes of preserved pineapple and over 16 kiloliters of pineapple juice were exported from Australia, generating over \$2.3 million [52]. Australian pineapple industry has been going through a tough time due to many reasons like severe climatic conditions, unavailability of processing industry, expensive labor costs, and one of the crucial reasons is heavy competition from cheaper imported products.

2.3 Pineapple waste and utilization trend

POFW and PPW are conventionally used for compost or as animal feed [54]. Pineapple waste is high in carbohydrate content which makes pineapple waste a good source for biogas production [54]. The pineapple processing industry generally produces a large amount of processing waste in the form of pineapple core and peel, which accounts for approximately 29-40% of the total pineapple weight [55]. Figure 2.5 shows the trend of utilization of pineapple peel waste and different value-added products made from pineapple peel waste

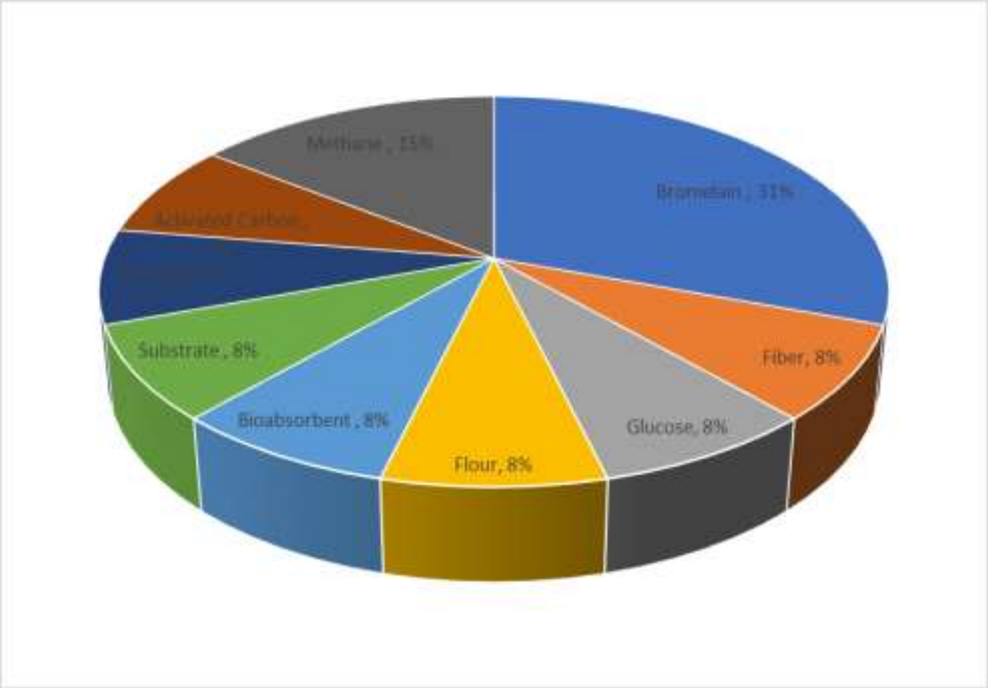


Figure 2.5. Pineapple peel waste utilization trend. (Source: Current trends of tropical fruit waste utilization)

2.4 Recovery of value-added components

The waste generated by the pineapple processing industry is rich in sugars, pectin's, fibers, proteins, flavor compounds, and phytochemicals, followed by the right level of micronutrients such as vitamins, minerals, and phenolic compounds [56]. Such value-added products if extracted and purified have immense value as a food additive, nutraceutical, therapeutics, and cosmetics [57].

2.4.1 Organic acids from pineapple waste

Pineapple waste serves as an excellent low-cost substrate for the production of organic acids. The high carbohydrate content of pineapple waste makes it a suitable substrate for the production of many organic acids like lactic, citric, succinic, and acetic acids along with ferulic acid [42, 58, 59]. These organic acids have a wide range of applications in many industries, including food, pharmaceuticals, polymers, cosmetics, and other industries. The production of lactic acid, citric acid, succinic acid, acetic acid, and Ferulic acid (esterified) from pineapple waste has been reported in the literature and summarized in table 3.

2.4.1.1 Lactic acid

Lactic acid is an essential acid from carboxylic acids as it has widespread applications in the food, cosmetics, biopolymer, and pharmaceutical industries. Lactic acid is used as a preservative and acidulant in the food, cosmetic and pharmaceutical industries [42]. Lactic acid is also known for its disinfectant properties. Production of lactic acid using fermentation technology has been reported where simple carbohydrates like glucose, sucrose, or galactose are converted to lactic acid using *lactobacillus* [42, 58, 60]. Jin Bo et al. (2005) reported that pineapple waste was the best substrate among other substrates such as potato, corn, and wheat. The bacterial strain used in the process was *Rhizopus arrhizus 2062,* with the highest yield of 0.65-0.76 g/g of lactic acid [61]. In 2003, Ueno reported that canned pineapple syrup was used as a substrate for the production of lactic acid using *Lactococcus lactis.* To improve the sucrose utilization, the enzyme invertase was used for sucrose hydrolysis. The highest yield of 20 and 92 g L^{-1} was obtained from 20 and 100 g total sugars l^{-1}, respectively [60]. In 2006, Idris et al. (2006) used pineapple waste for the production of lactic acid using *Lactobacillus delbrueckii (*ATCC 9646) under anaerobic conditions for 72 h. The highest yield of 29.02 g/L of lactic acid was observed at pH 6.5 after 56 h [62]. Nakanishi et al. (2010) used canned pineapple syrup as a substrate for the production of L-Lactic acid using *Lactobacillus paracasei NRIC 0765* and achieve titers of 19 and 93 g l^{-1} from 20 and 100 g l^{-1} total sugar [63]. Aziman et al. (2015) used solid pineapple waste for the production of lactic acid using *Rhizopus oryzae*, resulting in a final yield of 23 g/kg of the substrate [64].

2.4.1.2 Citric acid

Citric acid is a weak organic acid with massive demand in the world as it has widespread applications in many industries including food, cosmetics, pharmaceutical. It improves the flavor of food and is used as an acidulant in the pharmaceutical industry [65, 66]. Tran et al. (1998) investigated various *Aspergillus* strains for the production of citric acid utilizing pineapple waste and found a maximum yield of 19.4g/100 g of pineapple waste using *Aspergillus niger* ACM 4992 with the following optimum conditions: 65% moisture (w/w) with 3% v/w methanol, 30 °C, and pH 3.4 [67]. Kareem et al. (2010) used *A. Niger KS-7* in solid-state fermentation and achieved a yield of 60.61 g/kg when 15% sucrose, 0.25% of ammonium nitrate (w/v), and 0.2% v/v of methanol was supplemented to the substrate [68]. Kumar et al. (2003) used *A. niger* under solid-state fermentation and investigated the effect of methanol on the fermentation process, which was found to increase the yield from 37.8% to 54.2% [69]. Imandi et al. (2008) reported a citric acid yield of 202.35g/kg of pineapple using *Yarrowia lipolytica* NCIM *3589* in solid-state fermentation were the optimum conditions were: moisture content of 70.71%, 0.34% w/w of yeast extract, 0.61%w/w of KH_2PO_4 and0.69%w/w of Na_2HPO_4 used [70].

2.4.1.3 Succinic acid

Succinic acid is an essential organic acid with applications in the food and beverage industry as an acidity regulator. Succinic acid is in substantial demand in the pharmaceutical sector as it is used as a prime ingredient in drugs such as metoprolol succinate which is prescribed for cardiac diseases [71]. Jusoh et al. (2014) achieved produced a maximum of 6.26 g/L of succinic acid using pineapple waste as the sole substrate [72]. Ferone et al. (2019) used different high sugar-content beverages for the production of succinic acid using *Actinobacillus succinogenes*. fruit juices (pineapple and ace), syrups (almond), and soft drinks (cola and lemon) were investigated and the highest yield of 30-40 g/L of succinic acid was reported while the rate of production was improved by 6-24% [73].

2.4.1.4 Acetic acid

Acetic acid is an organic acid with vast applications in the food and pharmaceutical industry as a flavor enhancer and food preservative. There is an immense demand in the pharmaceutical industry due to its use as antiseptic and in cervical cancer screening tests, [74]. Roda et al. (2017) reported 5% yield of acetic acid using *Acetobacter aceti* growing in pineapple waste. The pineapple waste was first saccharified by physical and enzymatic pretreatments and then fermented with *S. cerevisiae* for 7–10 days under aerobic conditions at 25 °C resulted in 7% of alcohol production[75]. Roda et al investigated the effect of three strains of S.cerevisiae on the saccharification of pineapple waste for the production of pineapple wine and acetic acid [76].

Table 2.3: Organic acids produced by fermentation methods using pineapple waste as substrate

Organic acid	Method	Micro-Organism [name in italics]	References
Lactic acid	Fermentation	*Rhizopusarrhizus* *R. oryzae* *Lactobacillus* *Delbrueckii* *Lactococcuslactis* *Lactobacillus paracasei*	[61] [60] [62] [63, 64]
Citric acid	Fermentation	*Yarrowia lipolytica* *A. Foetidus* *A. Niger*	[77] [67-69, 78]
Succinic acid	Fermentation	*E. Coli AFP 184*	[79]
Acetic acid	Fermentation	*Saccharomyces cerevisiae* *Clostridium acetobutylicum B 527*	[75] [80]
Ferulic acid (esterified)	Alkali exchange chromatography	--	[81]

2.4.1.5 Ferulic acid

Ferulic acid and its derivatives mainly vanillin, 4-vinyl guaiacol and sodium ferulate [82, 83] have great potential as food additives and drug development. Vanillin is commonly used as a flavoring agent in many sweets, confectionery, and baking products [82], while sodium ferulate is one of the critical ingredients in drugs for cardiovascular diseases [84]. On the other hand, 4-vinyl guaiacol is used in the food industry as a flavoring agent. Ong et al. in 2014 reported the utilization of pineapple waste for the production of ferulic acid and showed that autoclaved and non-autoclaved pineapple waste resulted in a different yield of 3.25 ± 0.12mg/g and 0.64 ± 0.03mg/g of substrate, respectively [82].

2.4.2 Enzyme Production

The residue from the pineapple processing industry has polysaccharides composed of carbohydrate polymers (cellulose and hemicelluloses), lignin, proteins, and organic acids. these can serve as nutrients for the microbial growth and production of enzymes [85]. Enzymes are commonly used in many industrial applications. Production of enzymes by solid-state fermentation is a low-cost technique and several researchers demonstrated the feasibility of producing enzymes from industrial food waste [85]. The following paragraphs explore the use of pineapple waste for the production of common enzymes used in the industry.

2.4.2.1 Pectinase Enzyme

Pectinase is an enzyme that breaks down pectin that is found in many fruits (polysaccharide composed of α-1,4-linked d-galacturonic acid). Not many studies have reported the utilization of pineapple waste for the production of pectinase enzymes. Kavuthodi et al. in 2018 used pineapple stem extract as a substrate for the production of pectinase using *Bacillus subtilis BKDS1*. The highest activity of the crude enzyme was 502.61 U/mlL [86].

2.4.2.2 Cellulase enzyme

Cellulases a lignocellulolytic enzyme that has many applications in various industries including food, animal feed, brewing, winemaking, agriculture, pulp and paper, textile, biomass refining, and laundry [87]. Conesa et al.in 2018 reported the use of *Aspergillus niger* and *Trichoderma* *reesei* for the production of cellulase enzyme utilizing pineapple processing waste. It was found that the cellulose from *Trichoderma reesei* exhibited a significantly faster initial hydrolyzing rate than that from *A. niger*. 0.258 ± 0.004 Vs 0.15 ± 0.07.

2.4.2.3 Bacterial cellulase

Bacterial cellulose (BC) is a natural biomaterial that is produced by bacteria and has unique physical, and chemical properties with potential applications in many industries including biomedical, environmental, agricultural, electronic, food [88, 89]. However, the high cost of raw material and the lower yield makes the process expensive [88]. Anwar et al. in 2015 reported the isolation of cellulose nanocrystals from pineapple peel waste juice using *Gluconacetobacter xylinus* [90]. The pineapple peel was acid-treated using sulfuric acid (concentration?) for 25-40 min at 50 °C [90]. Algar et al. in 2014 published the utilization of pineapple residue for the production of bacterial cellulose using *Gluconacetobacter medellinensis* using pineapple and sugar cane juice as a source of carbon and other nutrients [91].

2.4.2.4 Bromelain Enzyme

Bromelain, a proteolytic enzyme, is found in the tissues of the (*Ananas comosus*) pineapple. Bromelain is present in all parts of pineapple fruit including pulp, core, stem, and peel in a small amount [92]. Pineapple contains a high amount of protease at the mature stage and is not present during the pre-mature stage. The percentage of bromelain increases as the fruit matures and it remains high until ripening, then its content diminishes slightly [93]. Bromelain is found abundantly in the stem and fruit of pineapple plant and it can also be isolated in small amounts from pineapple waste such as core, leaves, peel, etc [94]. Figure 2.6 shows the structure of the enzyme bromelain.

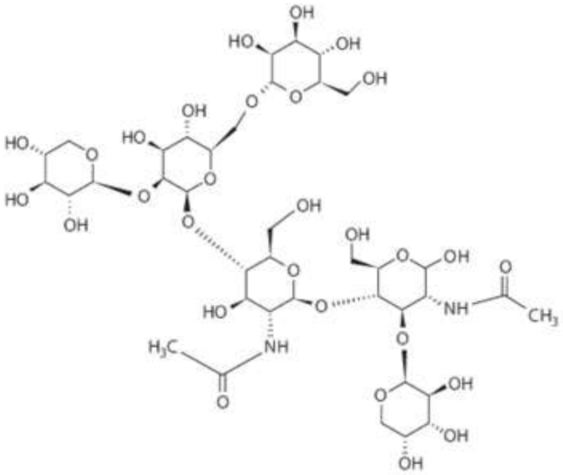


Figure 2.6. Showing structure of Bromelain enzyme

2.4.2.4.1 Method of extraction and purification

Bromelain has been attracting the interest of researchers due to its diverse applications in many industries. Different methods have been studied for the extraction and purification of the enzyme using pineapple waste are summarised in table 2.4.

Table 2.4. : Methods of extraction and purification of Bromelain

Purification method	**Methos**	**Yield (%)**	**Ref.**
Aqueous two-phase System (ATPS)	PEG/K2So4 aqueous two-phase system	228	[95]
	PEG/poly(acrylic acid) aqueous two-phase system	335.27	[96]
	PEG/MgSo4 aqueous two-phase system	113.54	[97]
	PEG/MgSo4 aqueous two-phase system	206	[98]
Filtration	Microfiltration & ultrafiltration	85-100	[99]
	Microfiltration, ammonium sulfate precipitation, ultracentrifugation	50	[100]
Reverse micellar systems	Reverse micelle systems	85	[101]
	Reverse micelle systems	102	[102]
	Affinity-based reverse micelle system	185.6	[103]
Chromatography	Immobilized metal affinity membrane	94.6	[104]
	Cation exchange chromatography	-	[105]

2.4.2.4.2 Application of Bromelain in different industries

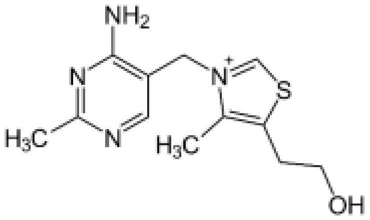
Bromelain is attracting the attention of researchers due to its enormous benefits and applications in different industries such as baking, alcohol, and meat. It is also used as an anti-browning agent in the horticulture industry to reduce phenol oxidation (79). The major and important applications of bromelain have been summarised in Table 2.5.

Table 2.5: Industrial applications of Bromelain

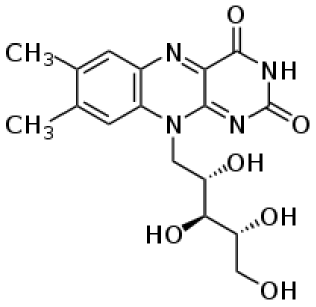
Application	Reasons	References
Baking industry	• Improve dough relaxation and allow the dough to rise evenly • Produce hypoallergenic flour	[106] [107]
Tenderization of meat	• Hydrolyzing agent for meat, oyster, chicken, and squid	[108] [109]
Alcohol Production	• Enhances the protein stability to beers • Prevent haze formation	[110]
Antibrowning agent	• Inhibit the browning of fruits and phenol oxidation	[111]

2.4.3 Polyphenols

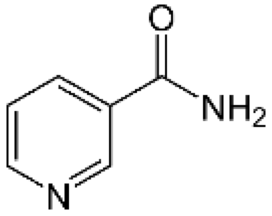
Fresh pineapple is low in calories (50 Kcal/100 g) and unique for its health-promoting compounds, minerals, and vitamins that are essential for optimum health (Table 3.2). The fruit is a good source of carbohydrates, fiber and minerals especially Ca, P, Fe, Na, and K. It also contains some vitamins including A, B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B9 (folate) and C (ascorbic acid). The nutritional content is influenced by several factors including variety, soil, climatic conditions, irrigation water, fertilizers, maturity stage at harvest, postharvest handling, and processing (Flath, 1980; Tee *et al.* ,1988; Hassan *et al.*, 2011). The flesh contains 13.52 g/100 g of carbohydrates, is low in proteins (0.54 g/100 g) and total fats (0.12 g/100 g) with no saturated fats or cholesterol, and it is a rich source of soluble and insoluble dietary fibers like pectins (USDA, 2015). Figure 2.7 shows the structure of the polyphenols present in the fresh pineapple and pineapple peel waste.



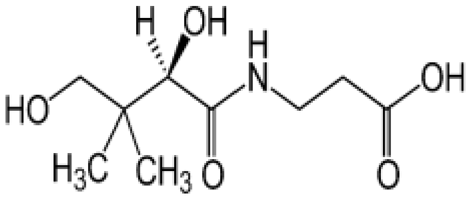
Thiamine



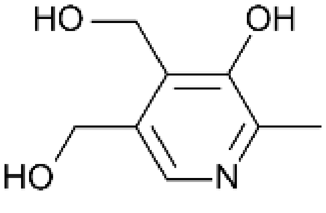
Riboflavin



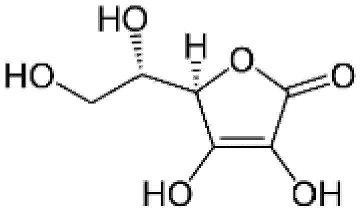
Niacin



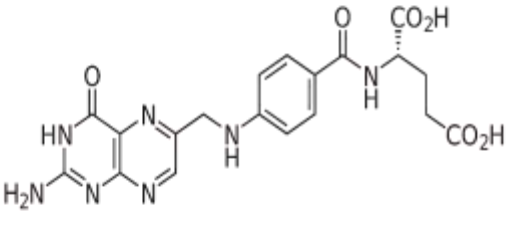
Pantothenic acid.



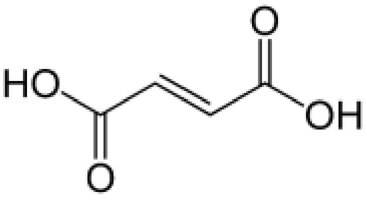
Pyridoxine



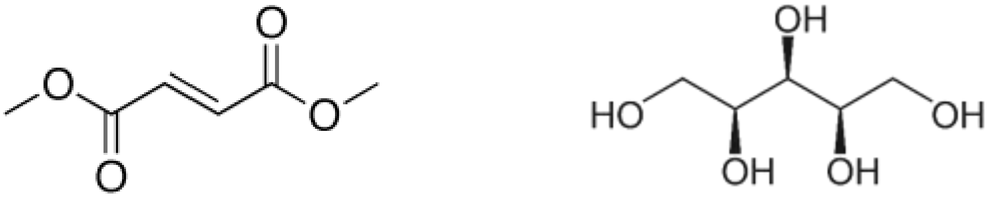
Ascorbic acid



Folate



Fumaric acid



Dimethyl fumarate Xylitol

Xylooligosaccharides (XOS) Sodium Ferulate

Figure. 2.7. Structure of different polyphenols present in pineapple and pineapple peel waste.

2.4.4 Single-cell protein

Single-cell protein (SCP) is used as a protein supplement in animal feed and protein-rich foods for humans. The increasing demand for protein-rich food has led to the development of single-cell protein from different sources using fermentation technology. Single-cell protein is in the form of dry microbial cells or total protein extract from pure microbial cell culture using different microorganisms [112]. The substrate of agricultural origin is commonly used for the production of SCP including whey[reference], orange processing waste [113] sugarcane waste[114], rice husk, wheat straw [115], grape waste [116]mango [117], and, pineapple processing waste [118]. Nigam (1998) reported the production of SCP using *Candida utilis Y900* utilizing pineapple cannery effluent. A maximum specific growth rate of μ_m =0.46h^{-1} and cell yield coefficient of$Y_{c/s}$ = 0.30 were obtained with 23.2 g carbohydrate/L in the growth medium [118]. Correia et al. (2007) reported the fermentation of pineapple waste using *Saccharomyces cerevisiae* which resulted in 3.5 times higher protein content compared to unfermented residues. Yousufi et al.(2012) combined pineapple waste and pomegranate rind for the production of SCP using *Rhizopus oligosporus* and obtained 61.2 mg protein content per 100g substrate

2.4.5 Production of bio Energy

2.4.5.1 Biomethane production

Anaerobic digestion of food waste for the production of biogas in the form of methane is another alternative option for the utilization of biomass due to its low operating cost and utilization of biomass as a renewable energy source. The solid residue or digest is rich in nutrients and can be used as fertilizer or soil conditioner resulting in an added avenue of revenue from the same waste biomass [119, 120]. As pineapple waste is rich in cellulose, hemicellulose, and carbohydrates, it is a promising substrate for methane generation by anaerobic digestion [121]. Rani et al.in 2004 reported that the ensilage of pineapple processing wastes reduced the biological oxygen demand by 91%. Biogas digester fed with ensilaged pineapple peel resulted in the biogas yield of 0.67 m^3/kg volatile solids (VS_{added}) with a methane content of 65%, whereas fresh and dried pineapple peels gave biogas yields of 0.55 and 0.41 m^3/kg VS_{added} and methane content of 51% and 41%, respectively [121] Namsree et al.(2012) digested pineapple pulp and peel of 7 days HRT and obtained a biogas production rate, biogas yield and methane yield of 0.12 m3/m3.d, 0.26 m3/ kg COD removed and 0.11 m3/kg COD removed, respectively, while the COD removal was 64.1%. The performance improved to 0.25 m3/m3.d, 0.43 m3/kg COD removed and 0.14 m3/kg COD removed, but the COD removal was only 60.41% when the organic loading rate was doubled [122]. Rosli et al.(2019) reported that the co-digestion of rice straw wash water and pineapple waste extract[need to explain what extract] at a ratio of 50:50 improved the COD removal and production of methane compared to the mono-digestion of rice straw wash water from 81% to 86% and 0.093 to 0.13 L CH_4/g COD_{rem} [123].

2.4.5.2 Biohydrogen Production

Researchers are now investigating new ways of producing bio-hydrogen as a new source of energy from food waste; as it has high energy density (142 kJ/gram) along with other qualities such as lower greenhouse gas emissions and high energy conversion using fuel cell [124]. Pineapple waste can be a good substrate as it has enough nitrogen required for the production of hydrogen [125]. Cahyari et al.(2018 reported mesophilic dark fermentation (30 °C, 1 atm, and 5.0 pH) of pineapple waste including peel, core, and leaves for the production of bio-hydrogen. The highest yield of hydrogen of 2.4 ml STP/gram VS was reported at a substrate concentration of 26.4 gram VS/L [124]. Cahyari et al. reported that the acidic pretreatment of a substrate with 0.3 N dilute H_2SO_4 might improve the fermentation process with a higher yield of hydrogen at 0.8 ml/grams VS [124]. Reungsang el al. (2013) reported that the substrate concentration, initial pH, and $FeSO_4$ had a significant influence on the specific hydrogen production rate. The optimum yield of 1.83 mol H_2/mol glucose was observed at a substrate concentration of 25.76 g-total sugar/L, initial pH of 5.56, and 0.81g/L of $FeSO_4$ [126]. Wang et al. (2006) studied the combination of sucrose, municipal sewage, and pineapple waste as carbon sources and for the production of hydrogen. A maximum of 745 mL/L.h and 2.46 mol H_2/mol sucrose was produced at an optimum pH of 7.5. Wang et al.also reported similar results were observed while using pineapple waste as substrate [127].

2.4.5.3 Bioethanol from Pineapple waste

The literature review has shown that much attention has been given to the utilization of fruit and vegetable waste in the production of ethanol. Pineapple processing waste is a potential source of sugars and growth factors, can be used as a substrate for ethanol production. Pineapple is a lignocellulosic material that is composed of cellulose, hemicellulose, lignin, extractives, and several inorganic materials that are hard to hydrolyze and need some pre-treatment to produce fermentable sugars. [128]. Literature has shown a limited amount of work has been done with pineapple and processing waste. According to the study of Italian et al. [129], the highest optimal ethanol yield of 8.34% (v/v) using pineapple peel was recorded using simultaneous saccharification and fermentation which was compared with banana and plantain. Another study of bioethanol production from pineapple peel was done by Choonut et al. [130], in which 1.23% v/v (which is also equal to 9.69 g/L) of ethanol yield was produced after 72 h of fermentation of hot water bath pretreated pineapple peel. The latest study was conducted by Gil and Maupoey [131] regarding pineapple waste valorization in which it was found out that 5.4 ± 0.1% v/v ethanol production was observed under simultaneous saccharification and fermentation. Various thermotolerant/thermophilic fermenting strains such as *Clostridium accetobutylicum, P. stipites* NCIM 3498, *Issatchenkia orientalis, thermoanaerobacter ethanolicus,* and *K. marxianus* have been reported for the ethanol fermentation using different feedstock, where as *S. cervisiae* being the most popular fungi used for the ethanol production [132].

During the alcoholic fermentation of glucose ($C_6H_{12}O_6$) into ethanol ($2C_2H_5OH$) and carbon dioxide during the process ATP is also produce is show in reaction (i).

$$C_6H_{12}O_6\ (aq) \rightarrow 2C_2H_5OH\ (aq) + 2CO_2\ (gas) \uparrow \ldots\ldots\ldots (i)$$

During alcoholic fermentation of one mole of glucose using *S. cervisiae* commonly known as baker's yeast which contains enzyme zymase which acts a as catalyst and produces two moles of ethanol and two moles of carbon dioxide, producing two moles of ATP in the process. For example 250g of glucose will produce 127.9g of ethanol the theoretical yield will be 130 g which is 160mL of ethanol.

2.4.5.4 Biochar from pineapple waste

The solid residue from processing waste can be used in the adsorption of heavy metals using biochar as a sorptive material is a promising approach for the remediation of ground and or surface water [133]. Biochar is a C-rich solid material synthesized from lignocellulosic biomass pyrolysis under a low/no oxygen environment [134]. There has been no evidence of utilization of pineapple peel-derived biochar for arsenic removal from ground or surface water. Pineapple biochar has been used for Cr (VI) sorption, the greatest sorption capacity, 7.44 mg g_1, occurred with PABC pyrolyzed at 750 _C for 2 h [135]. This thesis will address the gaps in the literature review and investigate the mechanisms of sorption of Ar (IV) using biochar derived from pineapple peel waste.

2.5 Conclusion

Due to its tropical aroma and exotic flavors, pineapple is one of the popular fruits among all age groups. More than 50 % of the fruit is used for manufacturing processed items such as juice, slices, hames, jellies, and so on. The waste generated by the processing industry is almost 50% of wet fruit weight. The present review focuses on the extraction of various beneficial value-added compounds from these wastes, which are otherwise very expensive and time-consuming to synthesize from conventional chemical routes. Enzymes, organic acids, and sugars are actively used in the food and pharmaceutical industry as additives or as functional foods. The present reviews provide brief information on each aspect of the value-added products from pineapple waste.

Declaration of competing interest:

The authors declare no conflict of interest.

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2.6 References

1. Tran AV (2006) Chemical analysis and pulping study of pineapple crown leaves. Ind Crop Prod 24 (1):66-74. doi:10.1016/j.indcrop.2006.03.003

2. Loudon JC (1822) The Different Modes of Cultivating the Pineapple, from its First Introduction to Europe to the Late Improvements of T.A. Knight Esq. London: Houlgman Hurst Resorme Brown,

3. María Gloria Lobo MS (2016) Overview of pineapple production, postharvest physiology, processing and nutrition. In: Paull MGLaRE (ed) Handbook of pineapple technology. Wileys, Chichester, West Sussex, pp 1-15

4. siddiq MGLaM (2017) Overview of pineapple production, postharvest physiology In: Paull MGLaRE (ed) Handbook of pineapple technology John wiley and Sones pp 1-15

5. Wadhwa M, & Bakshi, S. (2013) Utilization of fruit and vegetable wastes as livestock feed and as substrate for generation of other value-added products (trans: FAO).

6. Gorinstein S, Zemser M, Haruenkit R, Chuthakorn R, Grauer F, Martin-Belloso O, Trakhtenberg S (1999) Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. J Nutr Biochem 10 (6):367-371

7. Barretto LCD, Moreira JDD, dos Santos JAB, Narain N, dos Santos RAR (2013) Characterization and extraction of volatile compounds from pineapple (Ananas comosus L. Merril) processing residues. Food Sci Tech-Brazil 33 (4):638-645

8. Banerjee S, Ranganathan V, Patti A, Arora A (2018) Valorisation of pineapple wastes for food and therapeutic applications. Trends in Food Science & Technology 82:60-70. doi:10.1016/j.tifs.2018.09.024

9. Nor MZM, Ramchandran L, Duke M, Vasiljevic T (2015) Characteristic properties of crude pineapple waste extract for bromelain purification by membrane processing. J Food Sci Tech Mys 52 (11):7103-7112. doi:10.1007/s13197-015-1812-5

10. Parfitt J, Barthel M, Macnaughton S (2010) Food waste within food supply chains: quantification and potential for change to 2050. Philos T R Soc B 365 (1554):3065-3081. doi:10.1098/rstb.2010.0126

11. Miranda RC, Schaffner DW (2019) Virus risk in the food supply chain. Curr Opin Food Sci 30:43-48. doi:10.1016/j.cofs.2018.12.002

12. Sogi EDaDS (2017) Value added processing and utilization of pineapple by-products. In: Paull DMGLaDRE (ed) Handbook of pineapple technology. Wiley pp 196-220

13. Food waste footprint impacts on natural resources (2013). Food And Agriculture Organization of United Nations,

14. María Elena Sánchez Pardo1* MERC, Rosalva Mora Escobedo1, Epifanio Jiménez García1 (2014) Chemical Characterisation of the Industrial Residues of the Pineapple (Ananas comosus). Journal of Agricultural Chemistry and Environment 3:53-56. doi:http://dx.doi.org/10.4236/jacen.2014.32B009

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

16. Hemalatha RA, S (2013) Physicochemical constituents of pineapple pulp and waste. . Journal of Chemical and Pharmaceutical Research 5 (2):240-242

17. Collins J (1960) The Pineapple: Botany, Cultivation and Utilisation. Interscience Publishers, New York.,

18. Australian Horticulture Statistics Handbook 2017/18 (2019).

19. Pineapples. (2018) The State of Queensland (Department of Agriculture and Fisheries) 2010–2019. https://www.daf.qld.gov.au/business-priorities/agriculture/plants/fruit-vegetable/fruit-vegetable-crops/pineapples. Accessed 15/05/2019 2019

20. Castillo-Gonzalez E, Giraldi-Diaz MR, De Medina-Salas L, Sanchez-Castillo MP (2019) Pre-Composting and Vermicomposting of Pineapple (Ananas Comosus) and Vegetable Waste. Appl Sci-Basel 9 (17). doi:ARTN 3564

10.3390/app9173564

21. AophatChoonutMakornSaejongKanokphornSangkharak (2014) The Production of Ethanol and Hydrogen from Pineapple Peel by Saccharomyces Cerevisiae and Enterobacter Aerogenes. Energy Procedia 52:242-249. doi:https://doi.org/10.1016/j.egypro.2014.07.075

22. M AAaH (2008) Characterisation of solid and liquid pineapple waste. Reaktor 12:48-52

23. Shalini Gaur Rudra* JN, Neetu Jakhar and Charanjit Kaur (2015) FOOD INDUSTRY WASTE: MINE OF NUTRACEUTICALS. International Journal of Science, Environment and Technology 4 (1):205-229

24. ATUL UPADHYAY, JEEWAN PRAVA LAMA and SHINKICHI TAWATA (2010) Utilization of Pineapple Waste: A Review. Journal of Food Science and Technology-Nepal 6:10-18

25. Roda A, Lambri M (2019) Food uses of pineapple waste and by-products: a review. Int J Food Sci Tech 54 (4):1009-1017. doi:10.1111/ijfs.14128

26. Jin B, Yin PH, Ma YH, Zhao L (2005) Production of lactic acid and fungal biomass by Rhizopus fungi from food processing waste streams. Journal of Industrial Microbiology & Biotechnology 32 (11-12):678-686

27. Ueno T, Ozawa Y, Ishikawa M, Nakanishi K, Kimura T (2003) Lactic acid production using two food processing wastes, canned pineapple syrup and grape invertase, as substrate and enzyme. Biotechnology Letters 25 (7):573-577

28. Idris A, Suzana W (2006) Effect of sodium alginate concentration, bead diameter, initial pH and temperature on lactic acid production from pineapple waste using immobilized Lactobacillus delbrueckii. Process Biochem 41 (5):1117-1123

29. Nakanishi K, Ueno T, Sato S, Yoshi S (2010) L-Lactic Acid Production from Canned Pineapple Syrup by Rapid Sucrose Catabolizing Lactobacillus paracasei NRIC 0765. Food Science and Technology Research 16 (3):239-246

30. Aziman SN, Tumari HH, Zain NAM (2015) Determination of Lactic Acid Production by Rhizopus Oryzae in Solid State Fermentation of Pineapple Waste. J Teknol 77 (31):95-102

31. deLima VLAG, Stamford TLM, Salgueiro AA (1995) Citric acid production from pineapple waste by solid state fermentation using Aspergillus niger. Arquivos De Biologia E Tecnologia 38 (3):773-783

32. Tran CT, Sly LI, Mitchell DA (1998) Selection of a strain of Aspergillus for the production of citric acid from pineapple waste in solid-state fermentation. World J Microb Biot 14 (3):399-404

33. Patagundi BI, Kaliwal BB (2008) Biotechnological Production of Citric Acid Using Different Substrates by Aspergillus Niger ATCC 26550. Research Journal of Biotechnology:196-206

34. Kareem SO, Akpan, I. and Alebiowu, O. O. (2010) Production of citric acid by Aspergillus niger using pineapple waste. Malaysian Journal of Microbiology 6 (2):161-165. doi:DOI: 10.21161/mjm.19009

35. Kumar D, Jain VK, Shanker G, Srivastava A (2003) Utilisation of fruits waste for citric acid production by solid state fermentation. Process Biochem 38 (12):1725-1729. doi:Pii S0032-9592(02)00253-4

Doi 10.1016/S0032-9592(02)00253-4

36. Aruna TE (2019) Production of value-added product from pineapple peels using solid state fermentation. Innov Food Sci Emerg 57

37. Roda A, Lucini L, Torchio F, Dordoni R, De Faveri DM, Lambri M (2017) Metabolite profiling and volatiles of pineapple wine and vinegar obtained from pineapple waste. Food Chem 229:734-742

38. Khedkar MA, Nimbalkar PR, Gaikwad SG, Chavan PV, Bankar SB (2017) Sustainable biobutanol production from pineapple waste by using Clostridium acetobutylicum B 527: Drying kinetics study. Bioresource Technol 225:359-366

39. Tilay A, Bule M, Kishenkumar J, Annapure U (2008) Preparation of ferulic acid from agricultural wastes: Its improved extraction and purification. J Agr Food Chem 56 (17):7644-7648. doi:10.1021/jf801536t

40. Sawant O, Mahale S, Ramchandran V, Nagaraj G, Bankar A (2018) Fungal Citric Acid Production Using Waste Materials: A Mini-Review. J Microb Biotec Food 8 (2):821-828. doi:10.15414/jmbfs.2018.8.2.821-828

41. Dhillon GS, Brar SK, Verma M, Tyagi RD (2011) Recent Advances in Citric Acid Bio-production and Recovery. Food Bioprocess Tech 4 (4):505-529. doi:10.1007/s11947-010-0399-0

42. Imandi SB, Bandaru VVR, Somalanka SR, Bandaru SR, Garapati HR (2008) Application of statistical experimental designs for the optimization of medium constituents for the production of citric acid from pineapple waste. Bioresource Technol 99 (10):4445-4450. doi:10.1016/j.biortech.2007.08.071

43. R D (2008) Overview of pharmaceutical excipients used in tablets and capsules.

44. Norela Jusoh NO, Ani Idris, Alina Nasruddin (2014) Characterization of Liquid Pineapple Waste as Carbon Source for Production of Succinic Acid. Jurnal Teknologi 69 (4):11-13

45. Ferone M, Ercole A, Raganati F, Olivieri G, Salatino P, Marzocchella A (2019) Efficient succinic acid production from high-sugar-content beverages by Actinobacillus succinogenes. Biotechnol Progr 35 (5). doi:UNSP e2863

10.1002/btpr.2863

46. Fokom-Domgue J, Combescure C, Fokom-Defo V, Tebeu PM, Vassilakos P, Kengne AP, Petignat P (2015) Performance of alternative strategies for primary cervical cancer screening in sub-Saharan Africa: systematic review and meta-analysis of diagnostic test accuracy studies. Bmj-Brit Med J 351. doi:ARTN h3084

10.1136/bmj.h3084

47. Roda A, De Faveri DM, Dordoni R, Cases EV, Jauregui NN, Barrachina AAC, Fernandez MJF, Lambri M (2017) Pineapple Wines Obtained from Saccharification of Its Waste with Three Strains of Saccharomyces cerevisiae. J Food Process Pres 41 (5)

48. Ong Khai Lun, and TBW, Ling LS (2014) Pineapple cannery waste as a potential substrate for microbial biotranformation to produce vanillic acid and vanillin. International Food Research Journal 21 (3):953-958

49. Ou SY, Kwok KC (2004) Ferulic acid: pharmaceutical functions, preparation and applications in foods. J Sci Food Agr 84 (11):1261-1269. doi:10.1002/jsfa.1873

50. Wang BH, Ou-Yang JP (2005) Pharmacological actions of sodium ferulate in cardiovascular system. Cardiovasc Drug Rev 23 (2):161-172

51. Trzcinski AP (2018) Enzyme production from food waste In: Trzcinski AP (ed) Biofules from Food Waste vol 1. CRC Place New York pp 49-68

52. Kavuthodi B, Sebastian D (2018) Biotechnological valorization of pineapple stem for pectinase production by Bacillus subtilis BKDS1: Media formulation and statistical optimization for submerged fermentation. Biocatal Agric Biote 16:715-722. doi:10.1016/j.bcab.2018.05.003

53. Ramesh Chander Kuhad RG, and Ajay Singh (2011) Microbial Cellulases and Their Industrial Applications. Enzyme Reserach 1-11. doi:doi:10.4061/2011/280696

54. Mohite BV, Patil SV (2013) A novel biomaterial: bacterial cellulose and its new era applications. Biotechnology and Applied Biochemistry 61 (2):101-110. doi: https://doi.org/10.1002/bab.1148

55. Cacicedo ML, Castro MC, Servetas I, Bosnea L, Boura K, Tsafrakidou P, Dima A, Terpou A, Koutinas A, Castro GR (2016) Progress in bacterial cellulose matrices for biotechnological applications. Bioresour Technol 213:172-180. doi:10.1016/j.biortech.2016.02.071

56. Budiman Anwar BB, I. Made Arcana, (2015) Isolation of Cellulose Nanocrystals from Bacterial Cellulose Produced from Pineapple Peel Waste Juice as Culture Medium, . Procedia Chemistry 16:279-284. doi:https://doi.org/10.1016/j.proche.2015.12.051.

57. Itxaso Algar SCMF, Gurutz Mondragon, Cristina Castro,, Clara Garcia-Astrain NG, Alona Retegi, Arantxa Eceiza (2015) Pineapple Agroindustrial Residues for the Production of High Value Bacterial Cellulose with Different Morphologies. Journal of Applied Polymer science 132:41237(41231-41238). doi:10.1002/APP.41237

58. Zoya Manzoor AN, Hamid Mukhtar, Ikram Haq (2016) Bromelain: Methods of Extraction, Purification and Therapeutic Applications. BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY 59:1-16

59. Marialice Pinto Coelho Silvestre, Raquel Linhares Carreira, Mauro Ramalho Silva, Flávia Campos Corgosinho, Márcia Regina Pereira Monteiro a, Morais HA (2012) Effect of pH and Temperature on the Activity of Enzymatic Extracts from Pineapple Peel. Food Bioprocess Technol 5:1824-1831. doi:10.1007/s11947-011-0616-5

60. Hossain MM LS, Kim IH. (2015) Effects of bromelain supplementation on growth performance, nutrient digestibility, blood profiles, faecal microbial shedding, faecal score and faecal noxious gas emission in weanling pigs. Veterinarni Medicina 60 (10):544-552

61. Babu BR, Rastogi NK, Raghavarao KSMS (2008) Liquid-liquid extraction of bromelain and polyphenol oxidase using aqueous two-phase system. Chemical Engineering and Processing 47 (1):83-89. doi:10.1016/j.cep.2007.08.006

62. Novaes LC, Ebinuma Vde C, Mazzola PG, Pessoa A, Jr. (2013) Polymer-based alternative method to extract bromelain from pineapple peel waste. Biotechnol Appl Biochem 60 (5):527-535. doi:10.1002/bab.1121

63. Ketnawa S RS, Chaiwut P. (2010) Two phase partitioning and collagen hydrolysis of bromelain from pineapple peel Nang Laecultivar. J Biochem Eng 52 (3):205-211

64. Ketnawa S CP, Rawdkuen S. (2011) Aqueous Two-phase Extraction of Bromelain from Pineapple Peels (‘PhuLae’ cultv.) and Its Biochemical Properties. Food Sci Biotechnol 20 (5):1219-1226

65. Lopes FLG, Severo JB, de Souza RR, Ehrhardt DD, Santana JCC, Tambourgi EB (2009) Concentration by Membrane Separation Processes of a Medicinal Product Obtained from Pineapple Pulp. Brazilian Archives of Biology and Technology 52 (2):457-464

66. M.B.Doko VB, J.Casadebaig, L. Cavailles, M.Jacob. (1991) Preparation of proteolytic enzyme extracts from Ananas comosus L., Merr. fruit juice using semipermeable membrane, ammonium sulfate extraction, centrifugation and freeze-drying processes. International Journal of Pharmaceutics 76 (3):199-206. doi:https://doi.org/10.1016/0378-5173(91)90272-P

67. Chaurasiya RS, Sakhare PZ, Bhaskar N, Hebbar HU (2015) Efficacy of reverse micellar extracted fruit bromelain in meat tenderization. J Food Sci Tech Mys 52 (6):3870-3880

68. Hebbar HU HA, Sumana B, Raghavarao KSMS (2011) Reverse micellar extraction of bromelain from pineapple (ananascomosus l. merryl) waste: scale-up, reverse micelles characterization and mass transfer studies. Sep Sci Technol 46 (10):1656-1664

69. Kumar S, Hemavathi AB, Hebbar HU (2011) Affinity based reverse micellar extraction and purification of bromelain from pineapple (Ananas comosus L. Merryl) waste. Process Biochem 46 (5):1216-1220

70. Nie HL, Li SB, Zhou YT, Chen TX, He ZY, Su SN, Zhang HT, Xue Y, Zhu LM (2008) Purification of bromelain using immobilized metal affinity membranes. Journal of Biotechnology 136:S416-S416

71. Nadzirah KZ, Zainal S, Noriham A, Normah I (2013) Efficacy of selected purification techniques for bromelain. International Food Research Journal 20 (1):43-46

72. Kong XZ, Zhou HM, Qian HF (2007) Enzymatic hydrolysis of wheat gluten by proteases and properties of the resulting hydrolysates. Food Chem 102 (3):759-763

73. Watanabe M, Watanabe J, Sonoyama K, Tanabe S (2000) Novel method for producing hypoallergenic wheat flour by enzymatic fragmentation of the constituent allergens and its application to food processing. Bioscience Biotechnology and Biochemistry 64 (12):2663-2667

74. Hage DS, Anguizola JA, Bi C, Li R, Matsuda R, Papastavros E, Pfaunmiller E, Vargas J, Zheng XW (2012) Pharmaceutical and biomedical applications of affinity chromatography:

Recent trends and developments. Journal of Pharmaceutical and Biomedical Analysis 69:93-105

75. Sunantha Ketnawa SR (2011) Application of Bromelain Extract for Muscle Foods Tenderization. Food and Nutrition Sciences 2:393-401

76. Benucci I, Liburdi K, Garzillo AMV, Esti M (2011) Bromelain from pineapple stem in alcoholic-acidic buffers for wine application. Food Chem 124 (4):1349-1353

77. C.Ramalingam RSaNNI (2012) Isolation and characterization of Bromelain from pineapple (Ananas Comosus) and comparing its anti-browning activity on apple juice with commercial antibrowning agents. Elixir Food Science 45:7822-7826

78. Anupama, Ravindra P (2000) Value-added food: Single cell protein. Biotechnol Adv 18 (6):459-479. doi:Doi 10.1016/S0734-9750(00)00045-8

79. Zhou Y-M, Chen Y-P, Guo J-S, Shen Y, Yan P, Yang J-X (2019) Recycling of orange waste for single cell protein production and the synergistic and antagonistic effects on production quality. J Clean Prod 213:384-392. doi:https://doi.org/10.1016/j.jclepro.2018.12.168

80. Shahla Samadi MM, Ghasem Najafpour (2016) Production of Single Cell Protein from Sugarcane Bagasse by Saccharomyces cerevisiae in Tray Bioreactor P A P E R I N F O Paper history. Enzyme Technology and Commercial Enzyme Production 29 (8):1029-1036. doi:DOI: 10.5829/idosi.ije.2016.29.08b.01

81. Gabriel AY, Mahmoud RM, Goma M, Abou-Zeid M (1981) Production of single cell protein from cereal by-products. Agricultural Wastes 3 (3):229-240. doi:https://doi.org/10.1016/0141-4607(81)90030-5

82. Zacharof M-P (2017) Grape Winery Waste as Feedstock for Bioconversions: Applying the Biorefinery Concept. Waste Biomass Valori 8 (4):1011-1025. doi:10.1007/s12649-016-9674-2

83. Rashad MM, Moharib SA, Jwanny EW (1990) Yeast conversion of mango waste or methanol to single cell protein and other metabolites. Biological Wastes 32 (4):277-284. doi:https://doi.org/10.1016/0269-7483(90)90059-2

84. Nigam JN (1998) Single cell protein from pineapple cannery effluent. World J Microb Biot 14 (5):693-696

85. Nasir IM, Ghazi TIM, Omar R (2012) Production of biogas from solid organic wastes through anaerobic digestion: a review. Appl Microbiol Biot 95 (2):321-329. doi:10.1007/s00253-012-4152-7

86. Barz M, Delivand MK, Dinkier K (2019) Agricultural Wastes - A Promising Source for Biogas Production in Developing Countries of the Tropical and Subtropical Regions. Rev for Mesoam Kuru- 16 (38):2-12. doi:10.18845/rfmk.v16i38.3991

87. Rani DS, Nand K (2004) Ensilage of pineapple processing waste for methane generation. Waste Manage 24 (5):523-528. doi:10.1016/j.wasman.2003.10.010

88. Namsree P, Suvajittanont W, Puttanlek C, Uttapap D, Rungsardthong V (2012) Anaerobic digestion of pineapple pulp and peel in a plug-flow reactor. J Environ Manage 110:40-47. doi:10.1016/j.jenvman.2012.05.017

89. Nurul Shafiqah Rosli SI, Azmir Md Dom and Nik Norsyahariati Nik Daud (2019) Potential of pineapple waste extract (PWE) as co-substrate in anaerobic digestion of rice straw washwater (RSWW): enhancement of biogas production. In: Lecture Notes in Civil Engineering, vol 9. Springer pp 1479-1493

90. Cahyari K, Putri AM, Oktaviani ED, Hidayat MA, Norajsha JD (2018) Biohydrogen Production from Pineapple Waste: Effect of Substrate Concentration and Acid Pretreatment. Iop Conf Ser-Mat Sci 358. doi:Unsp 01200110.1088/1757-899x/358/1/012001

91. Han SK, Shin HS (2004) Biohydrogen production by anaerobic fermentation of food waste. Int J Hydrogen Energ 29 (6):569-577. doi:10.1016/j.ijhydene.2003.09.001

92. Reungsang A, Sreela-or C (2013) Bio-Hydrogen Production from Pineapple Waste Extract by Anaerobic Mixed Cultures. Energies 6 (4):2175-2190. doi:10.3390/en6042175

93. Wang CH, Lin PJ, Chang JS (2006) Fermentative conversion of sucrose and pineapple waste into hydrogen gas in phosphate-buffered culture seeded with municipal sewage sludge. Process Biochem 41 (6):1353-1358. doi:10.1016/j.procbio.2006.01.016

CHAPTER 3: Effects of alkali, acidic and enzymatic pre-treatments on the ethanol fermentation of pineapple (Ananas comosus) processing waste

Kiran R. Mahale[a*] and Antoine P. Trzcinski[a]

Research Paper-I

Introduction Chapter 3

The manuscript presents the effects of different pre-treatments on pineapple processing waste for the production of reducing sugars and subsequently bioethanol. A range of different concentrations of acids, alkalies, and enzyme was examined on pineapple *(Ananas comosus)* waste for 7 days in a controlled environment. A range of analytical tests was conducted including total reducing sugars estimation, HPLC sugars profiling, determination of total phenolic content, and fermentation inhibitors including furfural and hydroxymethylfurfural to estimate the sugars produced and ethanol fermentation. Along with Scanning electron microscopy to examine the physical changes in biomass due to different chemical pre-treatments. The mechanism of production of reducing sugars and ethanol production was predicted based on the results of the experiments conducted.

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Processing of Biogenic Material for Energy and Chemistry

Effects of alkali, acidic and enzymatic pretreatments on the ethanol fermentation of pineapple (Ananas comosus) processing waste.

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Abstract

The pineapple processing industry produces large amounts of waste which is classified as lignocellulosic biomass. In the current study, alkaline, acidic, and enzymatic pre-treatment were investigated to improve the saccharification of pineapple waste before ethanol fermentation. The sample treated with 1% NaOH gave the highest amount of reducing sugars (265.8 ± 4.1 g/L) after 7 days followed by 229.6 ± 5.2 g/L in the untreated raw pineapple waste samples and 0.5% Sulphuric acid with 191 ± 5.2 g/L. Enzymatic hydrolysis using a dosage of 320 µL of commercial cellulase was investigated and resulted in a 79% increase in reducing sugar concentration after 24 hours. Eventually, the sequence of alkali pre-treatment followed by enzymatic hydrolysis resulted in a maximum reducing sugars concentration of 309.3 g/L. Unfortunately, due to the presence of fermentation inhibitors, a maximum bioethanol yield of only 9.45 ±0.94 g/L was obtained after 48 h of fermentation.

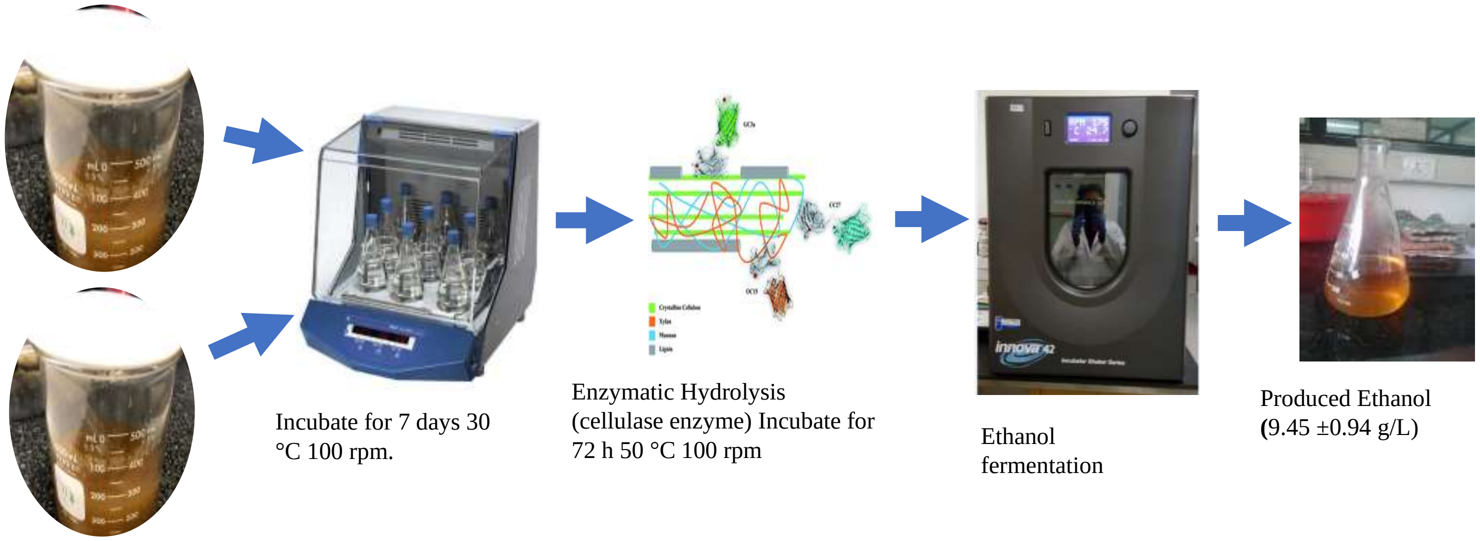
Keywords: bioethanol, pineapple waste, pre-treatments, fermentation, bioprocess, enzymatic hydrolysis.

Highlights:

- 1% NaOH produced the highest amount of reducing sugars among all the Alkali and acidic pre-treatments.
- SEM analysis showed that the pineapple peel structure deteriorated after the pre-treatments.
- A maximum bioethanol yield of 9.45 ±0.94 g/L was recorded

Graphical Abstract:

Acidic Pre-treatment (0-7 days) (0.5-5%) 20% (w/v) PW loading.



Incubate for 7 days 30 °C 100 rpm.

Enzymatic Hydrolysis (cellulase enzyme) Incubate for 72 h 50 °C 100 rpm

Ethanol fermentation

Produced Ethanol (9.45 ±0.94 g/L)

Alkaline Pre-treatment (0-7 days) (0.5-5%) 20% (w/v) PW loading

3.1 Introduction

Waste disposal is one of the major problems for food processing companies throughout the globe [136]. The fruits and vegetable processing industries generate large amounts of industrial residues that are disposed of in landfills and become a potential hazard to the environment. In particular, the pineapple processing industry generates a significant amount of waste residues as high as 70-80% [137]. Pineapple (*Ananas comosus*) is the third most popular and growing tropical fruit after citrus and banana in the world and is the third most popular juice after orange and apple [35]. The annual global production of pineapple is approximately 25 million tonnes, Australia contributing over 76,000 tonnes [52]. The canning industry uses the fruit for the production of juice, jam, and slices as process fruits. This results in the production of processing waste in the form of core, peel, crown, and damaged slices. Pineapple waste is composed of cellulose, hemicellulose, lignin, extractives, and several inorganic materials. It is a rich source of carbohydrates and can be used in the production of reducing sugars which can be fermented to biofuels [138].

Bioethanol is one of the promising biofuels and pineapple waste is a prominent feedstock for its production [42]. Direct conversion of lignocellulosic material to biofuels without pre-treatment always results in a low-ethanol yield [138]. To improve the ethanol yield, different pre-treatments are employed. The pre-treatment process is important to break down the lignin structure, remove hemicellulose and increase the accessible surface area for enzymatic digestion [139]. The literature review showed only a few studies are reporting alkaline pre-treatment of pineapple waste, so the objective of this study was to investigate different concentrations of common alkali such as NaOH and $Ca(OH)_2$ and acids sulfuric acid (H_2SO_4) and phosphoric acid (H_3PO_4) to produce reducing sugars. Recently, acid- and alkali based pre-treatments have been widely used on lignocellulosic biomass. The acidic pre-treatment hydrolyzes hemicellulose and makes cellulose structure accessible for enzymatic digestion [139]. Sulfuric acid (H_2SO_4) and phosphoric acid (H_3PO_4) are commonly used as cost-effective and environmentally friendly treatment [139, 140]. While the alkali-based treatment of biomass causes the breakdown of ester bonds cross-linking lignin and xylan and partial decrystallization of cellulose [139, 141]. In 2012, Kaur et al. reported the enzymatic hydrolysis of 4% alkali-treated corn stalk (121 °C, for 60 min) resulted in 65% of the theoretical glucose yield from cellulose after 48 hours [142]. Liberating sugars from lignocellulosic biomass at low cost to develop economically competitive biofuel is a challenge. The lignocellulosic biomass contains cellulose as a major constituent and is resistant to biodegradation in nature [143, 144]. Therefore, the cellulase enzyme is required for the hydrolysis of cellulose. A study conducted by Merino and Cherry et al in 2007 suggested that a concentration of 40-fold to 100-fold of higher enzymes is required in the degeneration of cellulose as compared to starch hydrolysis [145]. Roda *et al* in 2014 used a combination of cellulase (6 g/kg), hemicellulase (1 g/kg) and pectinase (0.05 g/kg)which showed a significant increase in reducing sugar of 14.3g/L in reducing sugars from pineapple peel waste, while the same study reported an increase of 9.1g/L using pineapple core a feedstock [146].

In the current experiment, the yeast *Saccharomyces Cerevisiae* (Thermosac Dry) provided by was used for anaerobic fermentation of extracted sugar from pineapple waste to produce ethanol and the details of experimental parameters are discussed in methodology. The objective of this study was to investigate the efficacy of different chemical and enzymatic pre-treatments on pineapple waste, as well as their combination.

3.2 Materials and methods

3.2.1 Raw pineapple and feedstock characterization

The feedstock used in this experiment is pineapple waste (PW), which was collected from a local pineapple processing company near Brisbane, Queensland Australia. The samples were collected at different times, from different batches of pineapple from different points of waste generation within the processing plant. All the samples were packed in bags and stored in a freezer at -20 °C until use. Frozen PW was then used for feedstock characterization according to the acid detergent fiber, neutral detergent fiber, and acid detergent lignin (ADF-NDF-ADL) method by Goering and van Soest (1970) [147]. Hemicellulose and cellulose contents were calculated using the formulas as below.

Hemicellulose (%) = NDF (%) – ADF (%)

Cellulose (%) = NDF (%) – Hemicellulose (%) – Lignin (%)

A pH of 3.7 ± 0.2 was measured using a benchtop pH meter (TPS, Australia). These thawed samples were then homogenized in a kitchen blender and passed through a mesh sieve (<0.5 mm) before being used in the pre-treatment experiments. . PW samples were prepared freshly before each experiment. Moisture in the samples was measured using an OHAUS moisture balance (model MB120).

3.2.2 Alkaline pre-treatment

The analytical grade of Sodium Hydroxide (NaOH) and calcium hydroxide $Ca(OH)_2$ were purchased from Sigma Aldrich. PW was treated with sodium hydroxide and calcium hydroxide separately at concentrations of 0.5%, 1%, 3%, 5%, (w/v) resulting in a pH of NaOH 0.5% and 1% was 4.1 and 5.6 respectively whereas $Ca(OH)_2$ was measured 4.8 and 6.4 respectively as mentioned in table 3.1. The alkaline pre-treatment was carried out with 20% (w/v) PW loading.

A control was run in parallel with no alkali. Each condition was tested in triplicate. The treatment was carried out in a 250-mL Erlenmeyer flask placed in a shaker incubator at 30°C and 100 rpm for 7 days. After the alkali pre-treatment, the pH was adjusted with 1 N HCl until it reached the desired pH of (5.5) for enzymatic hydrolysis. All samples were done in triplicate.

3.2.3 Acidic pre-treatment

Analytical grade of sulfuric acid (H_2SO_4) and phosphoric acid (H_3PO_4) were used for the acidic pre-treatment and were purchased from Sigma Aldrich. PW was treated with sulphuric acid and phosphoric acid separately at a concentration of 0.5%, 1%, 3%, 5%, (w/v) resulting in a pH of sulfuric acid 0.5% and 1% was recorded as 2.9 and 2.4 respectively as recorded in table 3.1 respectively. The acidic pre-treatment was carried out with 20% (w/v) PW loading. A control was run in parallel with no acid. Each condition was tested in triplicate. The treatment was carried out in a 250-mL Erlenmeyer flask placed in a shaker incubator at 30 °C at 100 rpm for 7 days. After the acidic pre-treatment, the pH was adjusted to 5.5 with 1 N NaOH

3.2.4 Enzymatic pre-treatment

For the enzymatic pre-treatment of PW, commercial *cellulase* enzyme from *Trichoderma reesei* (Sigma Aldrich C2730 aqueous solution, ≥700 units/g) was used. The PW samples used for this pre-treatment were from a different batch of pineapple waste used in acidic and alkaline pre-treatments. PW was hydrolyzed in 250-mL Erlenmeyer flasks filled with 100 mL of PW samples adjusted to pH 5.5 and subjected to different amounts of cellulase enzyme. The samples were incubated at 50 °C for 72 h and 2mL of samples was then taken after 0, 3, 6, 9, 12 and 24 hours. All samples were centrifuged for 10 min at 10,000 rpm and filtered through a 0.45µm membrane filter. The filtered supernatant was then collected and analysed for reducing sugar and fermentation inhibitors. All the samples were done in triplicate.

3.2.5 Ethanol fermentation

The yeast *Saccharomyces Cerevisiae* in powder form was provided by Lallemand Biofuels and Distilled Spirits (USA) under the name Thermosacc Dry. 5g of yeast was activated in a 100 mL growth medium containing 0.4 g yeast extract with 3g of glucose as suggested in the literature. The culture was then incubated at 35°C and 120 rpm for 12 hrs. The hydrolysate obtained after the pre-treatment was filtered and centrifuged (10,000 rpm, 10 min) to remove the unhydrolyzed residue. The fermentation was carried out in a 250-mL Erlenmeyer flask with 75 mL hydrolysate at a pH of 5.6. This was inoculated with 2% of *S. cerevisiae* (Thermosacc Dry) culture and was incubated at 36 °C for 72 h. The ethanol concentration monitoring was done every 24 h of fermentation using a brewing hydrometer brought from the local market. After completion of the fermentation, the fermented liquor was filtered through a 0.45µm membrane filter and analyzed using A Shimadzu QP2010 Gas Chromatograph Mass Spectrophotometer (GC-MS) for ethanol concentration using a Restek Stabilwax-DA column and the following program settings: oven temperature 100 °C, hold time 10 min, increasing to 250 °C at a rate of 5°C min^{-1}, hold time 0 min. The injection temperature was 150 °C, the ion source temperature 250 °C; total flow 14.2 ml/min; column flow 1.87 ml/min; linear velocity 49.9 cm/sec; purge flow 3 ml/min.

3.2.6 Analytical methods

3.2.6.1 Total reducing sugars

The reducing sugar concentration was determined using the di-nitro salicylic acid (DNS) assay method as described by Miller et al. 1959 [148]. This is based on the color reaction of 3,5-dinitro salicylic acid and reducing sugar in the alkaline solution and boiled water. The calibration curve was prepared using glucose as a standard with R^2= 0.9959 within the test ranges. About 1 mL of sample was diluted with DI water and added with 1 mL of DNS solution in the test tube before boiling at 90 °C for 5 min. The absorbance of the sample was detected using a UV–vis spectrophotometer HACH DR 6000 series) at a wavelength of 540 nm for the determination of total reducing sugar concentration. All the samples were done in triplicate and the standard deviation was reported as error bars on the graphs.

3.2.6.2 HPLC Profiling of sugars

The PW hydrolysates were analysed for sugar profiling using High-Performance Liquid Chromatography (HPLC) (Model 1600, Waters, USA) with a Refractive Index detector (Model 2414). The hydrolysates were filtered through a 0.45µm membrane filter before it was run through a Carbohydrate High-Performance 4µm (4.6 mm x 250 mm cartridge) column at a flow rate of 1.3 mL/min using water and acetonitrile (10:90) as mobile phase run for 15 mins.

3.2.6.3 Scanning Electron Microscopy (SEM) analysis

Scanning electron microscopy analysis was conducted to determine the changes in the morphological structure of PW before and after the pre-treatments. All samples were fixed on tape and coated with gold-palladium particles to obtain clear images. The SEM images were visualized at 2000× magnification, 50-µm diameter, and 5 kV using an Apera S SEM (model JSM-6000 plus, JEOL, Japan).

3.2.6.4 Detection of fermentation inhibitors

The presence of fermentation inhibitors including furfural (F) and hydroxymethylfurfural (HMF) and other organic acids in the liquid phase of the samples after pre-treatment were evaluated as described below along with phenolic contents.

3.2.6.5 Determination of furfural

The presence of furfural (F) and other acids in the liquid phase of the samples before and after pre-treatment were evaluated using A Shimadzu QP2010 Gas Chromatograph Mass spectrophotometer (GC-MS) (Shimadzu, Duisburg, Germany) using a Restek stabilwaxDA Column with the following program: oven 60 °C, hold time 0 min, increasing to 250 °C at a rate of 5°C min^{-1}, hold time 0 min. The injection temperature was 200 °C, the ion source temperature 250 °C; interface temperature 250 °C; solvent cut time 2 min; split ratio 5; flow control mode: linear velocity; pressure 115.9 kPa; total flow 14.2 mL/min; column flow 1.87 mL/min; linear velocity 49.9 cm/sec; purge flow 3 mL/min.

3.3 Results and discussion

3.3.1 Feedstock characterization

The pH of the raw pineapple samples was 3.7 (± 0.2) and the moisture content 42.2% ±0.34 (w/w). Untreated pineapple samples contained 23.9% ± 0.29 (w/w) hemicellulose, 21.1%±0.34 (w/w) cellulose and 5.5% ± 0.33 (w/w) lignin (Table 3.1).

3.3.2 Effect of Sodium hydroxide (NaOH) and Calcium hydroxide $Ca(OH)_2$

In alkaline pre-treatment, PW was treated with different concentrations of sodium hydroxide (NaOH) or calcium hydroxide $Ca(OH)_2$ for 7 days at 30°C in a shaker incubator at 100 rpm. The alkaline pre-treatment specifically degrades the ester and glycosidic side chains of the biomass and disrupts the lignin content of the biomass [149, 150], and makes hemicellulose and cellulose available for enzymatic degradation. This was confirmed in Table 1 where the lignin content of PW decreased from 5.5% to 4.8% and 4.1 %, respectively, in 0.5% and 1% NaOH pretreatment. However, cellulose and hemicellulose contents remained relatively unchanged.

Alkaline pre-treatment with sodium hydroxide (NaOH) can delignify by breaking the ester bonds cross-linkage present in lignin and xylan, which increases the porosity by reducing the crystallinity of the cellulose [151]. The results in Figure 3.1 showed that a maximum of 265 g/L reducing sugars was obtained at 1% NaOH while other concentrations resulted in concentrations lower than the control 229 g/L. Calcium hydroxide was less effective than sodium hydroxide with a maximum of 119 g/L. Alkali reacts with the natural acids in PW and when acid and a base react, it forms salts and other inhibitory compounds such as furfural and hydroxymethylfurfural (HMF) which is formed through dehydration of certain sugars [152]. Phenol and carboxylic acids are also formed which affects the sugar and ethanol production efficiency of the material [153]. This can explain why the samples treated with 0.5%, 3%, 5% NaOH and all concentrations of $Ca(OH)_2$ produced fewer sugars than the control. The crystallinity of cellulose can also affect the efficient production of sugars from biomass [154].

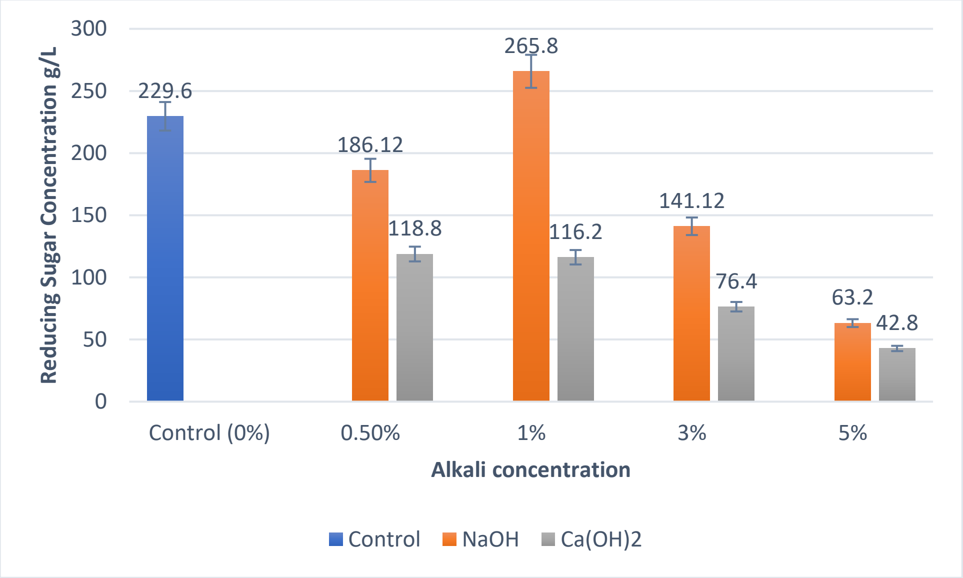


Fig. 3.1 Reducing Sugar concentration of pineapple waste after alkali pretreatment.

3.3.3 Effect of Sulphuric acid (H_2SO_4) and phosphoric acid (H_3PO_4)

In acidic pre-treatment, PW was treated with different concentrations of sulphuric acid (H_2SO_4) or phosphoric acid (H_3PO_4) for 7 days at 30 °C in a shaker incubator at 100 rpm. Sulphuric acid (H_2SO_4) and phosphoric acid (H_3PO_4) are widely used in acidic hydrolysis of biomass for ethanol production as they are relatively cheap and efficient to hydrolyze lignocellulose. The acidic pre-treatment mainly solubilizes the hemicellulose and part of the lignin and cellulosic material is exposed to enzymes for further saccharification [155].

In this study, different concentrations of H_2SO_4 and H_3PO_4 were made to evaluate the effect of mild and concentrated acidic pre-treatment on the production of reducing sugars. The results in Figure 3.2 show that the samples pre-treated with 0.5%, 1%, 3%, and 5% of H_2SO_4 and H_3PO_4 resulted in a lower concentration of reducing sugars compared to no pre-treatment. The highest amount of reducing sugar (191.1 g/L) was produced with 0.5% of H_2SO_4 followed by 166.1 g/L 0.5% H_3PO_4. Higher concentration of acids produced a lower amount of sugars presumably because some of the sugars are degraded into enzyme inhibiting by-products such as furfural, acetic acid, vanillin and some aldehydes [156-158]. This can explain why the control produced more sugars than the samples pre-treated with acids. The crystallinity of cellulose also affects the efficient production of sugars from the biomass [154].

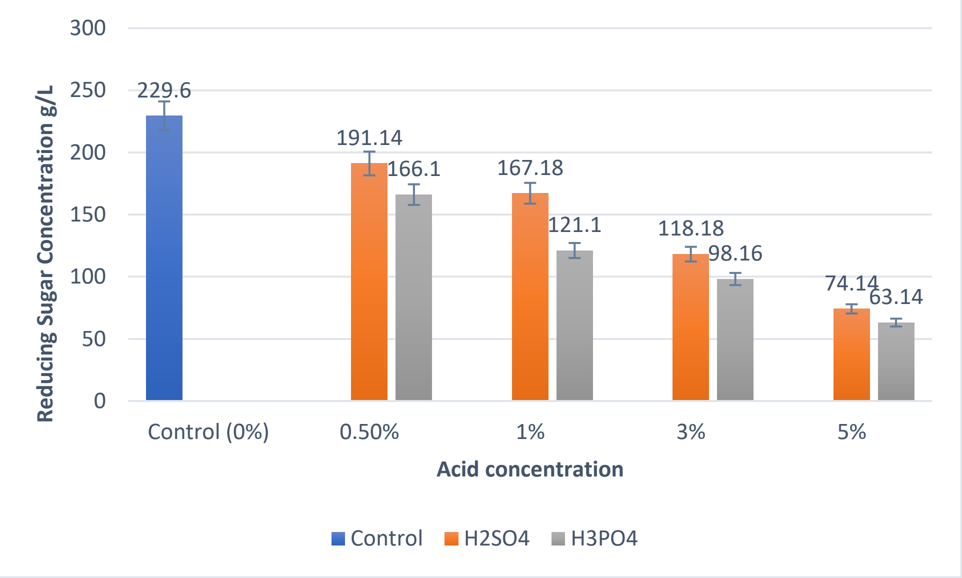


Fig. 3.2 Reducing Sugar concentration of pineapple waste after acidic pre-treatment.

Table 3.1. proximate analysis of samples before and after pre-treatment.

Samples		**Particle Size**	**pH**	**Moisture content**	**Cellulose**	**Hemicellulose**	**Lignin**
Before experiment		< 0.5 mm	3.7 (± 0.2)	42.2% ±0.34 (w/w)	21.1%±0.34 (w/w),	23.9% ± 0.29 (w/w)	5.5% ± 0.33 (w/w)
Control		< 0.5 mm	3.7 (± 0.2)	43.1% ±0.34 (w/w)	19.9% ± 0.31 (w/w)	21.4 % ± 0.19 (w/w)	5.4% ± 0.28 (w/w)
Alkali Treatment (NaOH)	0.5%	< 0.5 mm	4.1 (± 0.2)	42.3% ±0.24 (w/w)	20.3% ±0.17 (w/w)	23.0% ±0.18 (w/w)	4.8% ±0.29 (w/w)
	1.0%	< 0.5 mm	5.6 (± 0.2)	44.4% ±0.26 (w/w)	20.1% ±0.34 (w/w)	22.8% ±0.21 (w/w)	4.1% ±0.31 (w/w)
Acid Treatment (H_2SO_4)	0.5%	< 0.5 mm	2.9 (± 0.1)	41.6% ±0.34 (w/w)	20.6%±0.34 (w/w)	21.7%±0.34 (w/w)	5.1% ±0.31 (w/w)
	1.0%	< 0.5 mm	2.4 (± 0.3)	42.1% ±0.14 (w/w)	19.4%±0.34 (w/w)	20.4%±0.34 (w/w)	4.9 % ±0.31 (w/w)

3.3.4 HPLC Sugar Profiling after chemical pre-treatments

The HPLC characterization of monomeric sugars present in the hydrolysates from the untreated and acid and alkali pre-treated PW samples are presented in Table 3.2. The most abundant sugar in PW was glucose across all three samples with 34.9%, 36.1%, and 35.8% of total sugars, respectively in untreated, alkaline, and acidic treated samples. It is followed by sucrose with 19.6%, 24.3%, and 23.8%, respectively. Other sugars included xylose, fructose, sucrose, arabinose, and galactose and traces of mannose, fucose, and rhamnose which is consistent with the literature on PW [159].

Table 3.2. HPLC sugar profile in the hydrolysates.

Type of sugar	Control	1.0% NaOH pre-treatment	0.5 % H_2SO_4 pre-treatment
Glucose (%)	34.9	36.1	35.8
Xylose (%)	15.3	13.8	12.4
Fructose (%)	12.9	10.8	11.6
Sucrose (%)	19.6	24.3	23.8
Arabinose (%)	12.6	11.4	12.9
Galactose (%)	2.6	1.3	1.3
Mannose (%)	1.3	1.3	1.1
Fucose (%)	0.8	1.0	1.1

3.3.5 Effect of Enzymatic hydrolysis on pineapple waste

Enzymatic hydrolysis of lignocellulosic biomass for ethanol production is considered as one of the most promising approaches for reliable biofuel production. The hydrolysis of crystalline cellulose is slow because the substrate is insoluble and poorly accessible to enzymes. This experiment was conducted on a different batch of PW which explains why the initial concentration of reducing sugars is different than in the chemical pre-treatment experiments. The optimization of enzyme dosage was investigated, and it was found that 320µL of enzyme per 100mL of pineapple waste produced the highest concentration of reducing sugar with 111.86 g/L as shown in Fig. 3.3. That is a 79% increase compared to the control at 66g/L. Half the enzyme dosage (160 µL) of enzyme still resulted in a remarkable 101.18 g/L of reducing sugar after 24h of incubation. During the process of enzymatic hydrolysis of biomass, endo-β-glucanase first randomly cleaves the internal glycosidic bond of the cellulose chain, exposing the reducing or nonreducing ends for cellobiohydrolase or exo-β-glucanse to react and release cellobiose. β-glucosidase then hydrolyses the cellobiose into glucose to be further used in fermentation for ethanol production [160, 161]. The enzyme cellulase employed in this experiment was produced from *T. reesei* that secrets low levels of β − glucosidase, resulting in the accumaltion of cellobiose during hydrolysis [143, 162].

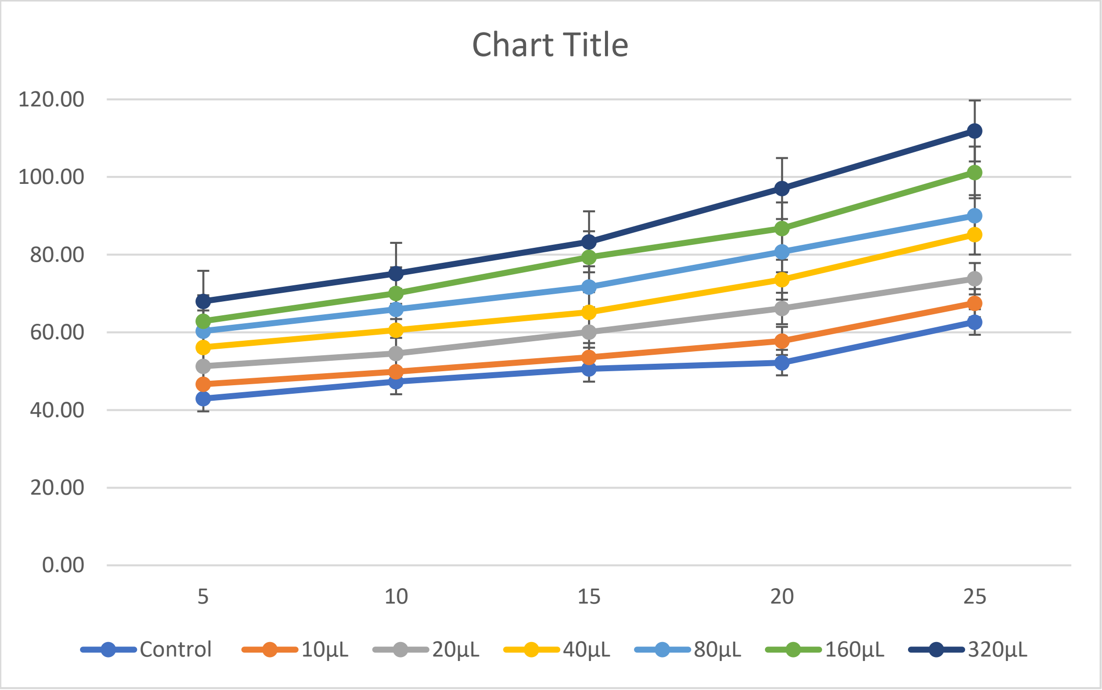


Fig. 3.3 Reducing Sugar concentration of pineapple waste after enzymatic pre-treatment

3.3.6 Alkali pre-treatment followed by enzymatic hydrolysis

To further increase the concentration of reducing sugars and bioethanol yield, the sequence of chemical pre-treatments followed by enzymatic hydrolysis of the PW was investigated. Based on the results from both chemical pre-treatments, four samples were selected to investigate the subsequent enzymatic hydrolysis. The highest concentration of reducing sugar obtained after chemical pre-treatments was 265.8 g/L using1% NaOH pre-treatment followed by the control (229.6 g/L), 0.5% H_2SO_4 pre-treatment (191.14 g/L), and 0.5% NaOH pre-treatment (186.12 g/L). These samples were then subjected to the enzymatic hydrolysis using 160 µL of commercial cellulase enzymes per 100 mL of pineapple waste and the results before and after the enzymatic treatment are listed in Table 3.3. The highest concentration of reducing sugar was 309.3g/L in the sample treated with 1% NaOH followed by enzymatic hydrolysis. There was a 16% increase due to the addition of enzymes compared to the alkali treatment alone demonstrating the benefits of applying enzymes following a chemical pre-treatment. The increase of reducing sugar in alkaline pre-treated samples was due to the dissociation of hydroxide ions (OH^-) and a high number of OH ions resulted in a greater release of reducing sugars. In the current experiment, alkaline pre-treated samples produced more sugar than acidic treated samples which is in line with a previous study by wang et al. (2016) [163].

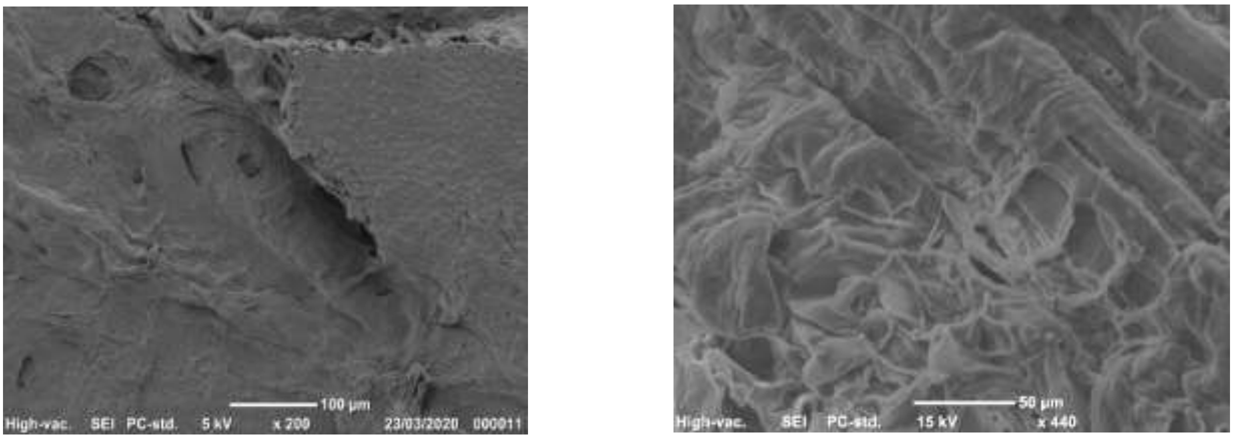
Table 3.3. Reducing sugar concentration after alkali pretreatment and alkali pre-treatment followed by enzymatic hydrolysis.

Sample	alkali pre-treatment	alkali pre-treatment followed by enzymatic hydrolysis (160 µL, 24 hrs)	% increase
Control	229.6g/L	274.4g/L	19%
0.5% NaOH	186.12 g/L	218.1g/L	23.5%
1% NaOH	265.8g/L	309.3g/L	16.5%
0.5% H_2SO_4	191.14 g/L	212.17g/L	23%

3.3.7 Scanning electron microscopy

To investigate the structural changes of PW before and after treatments, SEM images of the controlled and pre-treated are shown in Fig. 3.4. It can be observed that the morphological structure of the control and treated PW samples were significantly different from each other. Image 3.4A displays a relatively smooth, unscathed external structure. The external layer is composed of lignin, ash, hemicellulose that enfolded the inner structural cellulose fibers and different binding materials, which is consistent with previous studies on PW [164].

The alkaline and acidic treated samples (Figure 3.4B and 3.4C) show the break down the outermost structure of lignin which made the cellulose and hemicellulose more accessible to cellulolytic enzymes thereby enhancing the yield of fermentable sugars after enzymatic hydrolysis.



(3.4a). Untreated samples

(3.4b) 1.0% NaOH treated samples

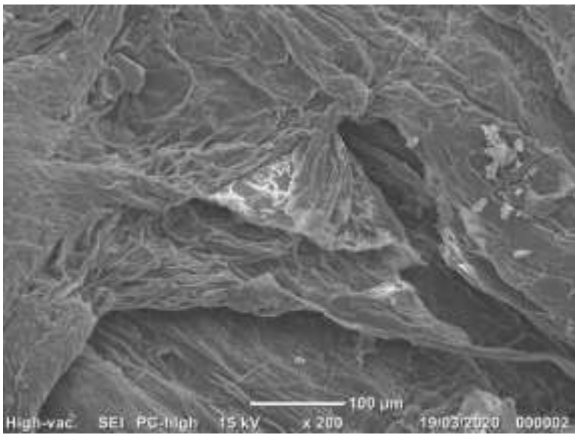


Fig. 3.4. Scanning electron micrographs of untreated and pre-treated pineapple waste.

3.3.8 Fermentation inhibitors:

The alkaline pre-treatment enhanced the production of reducing sugars by uncovering the cellulose, but the side effects of these pre-treatments are the formation of lignocellulose-derived by-products that inhibit the fermentation process. The inhibition problem becomes more significant as by-products accumulate when high solids loading is used to achieve concentrated sugars streams. The formation of inhibitory substances is dependent on the pre-treatment process. The alkaline pretreatment is aimed at removing lignin and minor part of hemicelluloses. Under alkaline conditions, the carbohydrates are better preserved, but some degradation of carbohydrates leads to the formation of carboxylic acids [165]. Figure 3.5 and

Table 3.4 show respectively the GC-MS chromatogram and compounds detected in the hydrolysate of PW treated with an alkaline solution. The results show the presence of acetic acid, hydroxy acids, formic acid, and phenolic compounds. The acid pre-treated PW samples showed the presence of furans like furfural and 5-hydroxymethylfurfural along with formic acid and acetic acid. Formic acid is one of the major inhibitory compounds present in the hydrolysates derived from lignocellulosic materials, its presence significantly affects the efficiency of conversation of sugars into bioethanol [166]. The acidic pre-treatment with sulphuric acid needs neutralization of acids which forms gypsum which inhibits the process of conversion of fermentable sugars into bioethanol [165].

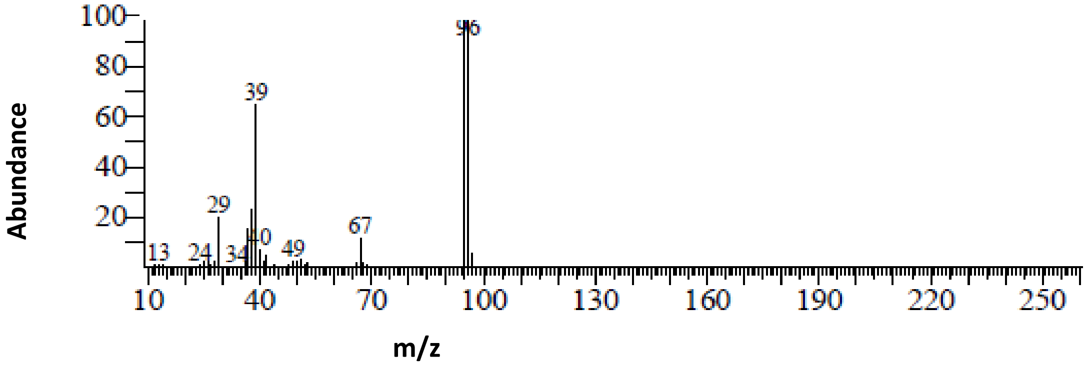


Fig. 3.5. The GC Spectrum of hydrolysate showing presence of inhibitors

Table 3.4: Compounds detected in the hydrolysate of alkaline treated samples.

peak Number	**Compound name**
13	Ethanoic acid
24	Methanecarboxylix acid
29	Hydroxyl acids
34	Ethylic acid
39	Acetic acid
49	Furancarboxaldehyde
67	Formic acid
96	5-hydroxymethylfurfural

3.3.9 Ethanol Fermentation:

The resulting hydrolysates from chemical pre-treatments were used for the fermentation of ethanol. As Fig. 3.6 shows, the highest amount of ethanol was produced by the control after 48 h of incubation reaching 9.45 ±0.94 g/L ethanol which then declined to 8.89±0.95 g/L after 72 h. A similar trend was observed in alkaline (1% NaOH) and acidic (0.5% H_2SO_4)pre-treated samples where 7.94±0.98 g/L and 7.83±1.04 g/L of ethanol was produced, respectively after 48 h, and declined to 6.79±0.92 g/L and 7.14±0.93 g/L respectively after 72 hours. This is greater than Gil and Maupoey et al. (2018) who reported 5.4 ±0.01 g/L after 48 h and Casabar et al. (2019) who reported a maximum of 5.98 ±1.01 g/L bioethanol from pineapple waste [167].

The remaining concentration of reducing sugars after the fermentation were found to be 41.2 ±3.98 g/L, 48.1 ±3.41 g/L, and 36.9 ±5.35 g/L in the control, alkaline and acidic samples, respectively, indicating a large fraction of non-fermentable sugars released from PW.

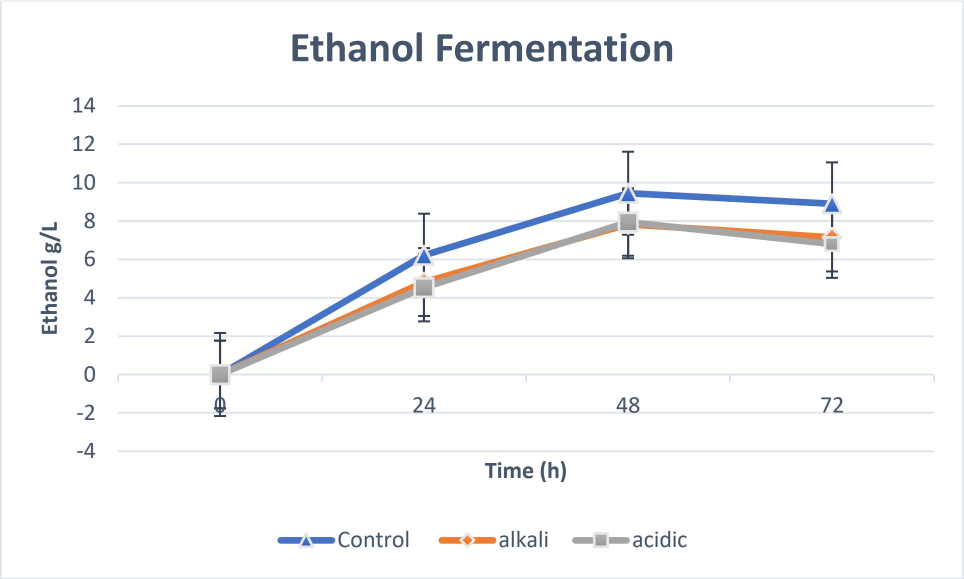


Fig. 3.6. Ethanol production following NaOH (1%) and H_2SO_4 (0.5%) pre-treatments of PW.

Theoretically, 1 g of fermentable sugars will produce 0.514 g of ethanol and 0.488 g of carbon dioxide [168]. Whoever, due to the formation of by-products such as glycerol and organic acids and cellular maintenance, the maximum real theoretical yield is around 0.485 g ethanol/ g of fermentable sugars[169]. It is well known that fermentation efficacy is the key parameter for the industry. Based on the results shown in Figure 5, the maximum yield was 3.05% which is significantly lower than the theoretical yield. This could be due to serval reasons including the presence of fermentation inhibitors like organic acids, furfural, and HMF in hydrolysates as well as non-fermentable sugars from PW.

3.4 Conclusions

Alkaline and acidic chemicals were used for the pre-treatment of pineapple waste (PW) before enzymatic hydrolysis and fermentation for ethanol production. The pre-treatment with 1% NaOH followed by the enzymatic hydrolysis resulted in the highest concentration of reducing sugars. The SEM analysis showed vast structural changes in pre-treated PW compared to untreated PW samples. The current study also suggests the yeast (*Saccharomyces cerevisiae*) used in the fermentation could produce up to 9.45 ±0.94 g/L bioethanol, but the yield was limited to 3.05% due to non-fermentable sugars and fermentation inhibitors. The findings of this study also suggest studying different pre-treatment or combinations of pre-treatment to enhance the production of fermentable sugars to make the ethanol production process more industrially feasible and efficient.

Declaration of competing interest.

The authors declare no conflict of interest.

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3.5 References:

1. Goula AM, Lazarides HN (2015) Integrated processes can turn industrial food waste into valuable food by-products and/or ingredients: The cases of olive mill and pomegranate wastes. Journal of Food Engineering 167:45-50

2. Roda A, De Faveri DM, Giacosa S, Dordoni R, Lambri M (2016) Effect of pre-treatments on the saccharification of pineapple waste as a potential source for vinegar production. J Clean Prod 112:4477-4484

3. Tran AV (2006) Chemical analysis and pulping study of pineapple crown leaves. Ind Crop Prod 24 (1):66-74. doi:10.1016/j.indcrop.2006.03.003

4. Australian Horticulture Statistics Handbook 2017/18 (2019).

5. Conesa C, Seguí L, Laguarda-Miró N, Fito P (2016) Microwaves as a pretreatment for enhancing enzymatic hydrolysis of pineapple industrial waste for bioethanol production. Food and Bioproducts Processing 100:203-213

6. Banerjee S, Ranganathan V, Patti A, Arora A (2018) Valorisation of pineapple wastes for food and therapeutic applications. Trends in Food Science & Technology 82:60-70. doi:https://doi.org/10.1016/j.tifs.2018.09.024

7. Silverstein RA, Chen Y, Sharma-Shivappa RR, Boyette MD, Osborne J (2007) A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. Bioresource Technol 98 (16):3000-3011

8. Rehman M, Kim I, Kim KH, Han J-I (2014) Optimization of sono-assisted dilute sulfuric acid process for simultaneous pretreatment and saccharification of rice straw. International Journal of Environmental Science and Technology 11 (2):543-550

9. Kapoor M, Raj T, Vijayaraj M, Chopra A, Gupta RP, Tuli DK, Kumar R (2015) Structural features of dilute acid, steam exploded, and alkali pretreated mustard stalk and their impact on enzymatic hydrolysis. Carbohydrate polymers 124:265-273

10. Kaur U, Oberoi HS, Bhargav VK, Sharma-Shivappa R, Dhaliwal SS (2012) Ethanol production from alkali-and ozone-treated cotton stalks using thermotolerant Pichia kudriavzevii HOP-1. Ind Crop Prod 37 (1):219-226

11. Wang M. LZ, Fang X., Wang L., Qu Y. (2012) Cellulolytic Enzyme Production and Enzymatic Hydrolysis for Second-Generation Bioethanol Production. In: Bai FW. LC, Huang H., Tsao G. (ed) Biotechnology in China III: Biofuels and Bioenergy. Advances in Biochemical Engineering Biotechnology,, vol 128. Springer,, Berlin, Heidelberg. doi:https://doi.org/10.1007/10_2011_131

12. Sassner P, Galbe, M., & Zacchi, G (2008) Techno-Economic Evaluation of Bioethanol Production from Three Different Lignocellulosic Materials. BIOMASS & BIOENERGY 32 (5):422-430. doi:https://doi.org/10.1016/j.biombioe.2007.10.014

13. Merino ST, Cherry J (2007) Progress and challenges in enzyme development for biomass utilization. Adv Biochem Eng Biotechnol 108:95-120. doi:10.1007/10_2007_066

14. Arianna Roda DMDF, Roberta Dordoni, Milena Lambri (2014) Vinegar Production from Pineapple Wastes –Preliminary Saccharification Trials. CHEMICAL ENGINEERING TRANSACTIONS 37:607-612. doi:DOI: 10.3303/CET1437102

15. Stelmock RL, Husby FM, Brundage AL (1985) Application of Van Soest Acid Detergent Fiber Method for Analysis of Shellfish Chitin1. Journal of Dairy Science 68 (6):1502-1506. doi:https://doi.org/10.3168/jds.S0022-0302(85)80989-9

16. MILLER GL (1959) Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry 31 (3):426-428

17. Sarkar N, Ghosh S, Bannerjee S, Aikat K (2012) Bioethanol production from agricultural wastes: An overview. Renewable Energy 37:19-27

18. Zhao X, Zhang L, Liu D (2010) Pretreatment of Siam weed stem by several chemical methods for increasing the enzymatic digestibility. Biotechnology Journal 5 (5):493-504. doi:https://doi.org/10.1002/biot.200900284

19. Gaurav Chaudhary, Lalit Kumar Singh, Ghosh. S (2012) Alkaline pretreatment methods followed by acid hydrolysis of Saccharum spontaneum for bioethanol production. Bioresource Technol 124:111-118. doi:http://dx.doi.org/10.1016/j.biortech.2012.08.067

20. van Putten R-J, van der Waal JC, de Jong E, Rasrendra CB, Heeres HJ, de Vries JG (2013) Hydroxymethylfurfural, A Versatile Platform Chemical Made from Renewable Resources. Chemical Reviews 113 (3):1499-1597. doi:10.1021/cr300182k

21. Klinke HB, Thomsen AB, BK. A (2004) Inhibition of ethanolproducing yeast and bacteria by degradation products produced during pre-treatment of biomass. Appl Microbiol Biotechnol 66 (1):10-26. doi:DOI: 10.1007/s00253-004-1642-2

22. Ishizaki H, Hasumi K (2014) Chapter 10 - Ethanol Production from Biomass. In: Tojo S, Hirasawa T (eds) Research Approaches to Sustainable Biomass Systems. Academic Press, Boston, pp 243-258. doi:https://doi.org/10.1016/B978-0-12-404609-2.00010-6

23. Bensah EC, Mensah M (2013) Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Technologies and Innovations. International Journal of Chemical Engineering 2013:719607. doi:10.1155/2013/719607

24. Jeong TS, Choi CH, Lee JY, Oh KK (2012) Behaviors of glucose decomposition during acid-catalyzed hydrothermal hydrolysis of pretreated Gelidium amansii. Bioresource Technol 116:435-440. doi:10.1016/j.biortech.2012.03.104

25. Jutakanoke R, Leepipatpiboon N, Tolieng V, Kitpreechavanich V, Srinorakutara T, Akaracharanya A (2012) Sugarcane leaves: Pretreatment and ethanol fermentation by

Saccharomyces cerevisiae. Biomass and Bioenergy 39:283-289. doi:https://doi.org/10.1016/j.biombioe.2012.01.018

26. Yi Y-B, Lee J-W, Choi Y-H, Park S-M, Chung C-H (2012) Simple process for production of hydroxymethylfurfural from raw biomasses of girasol and potato tubers. Biomass and Bioenergy 39:484-488. doi:https://doi.org/10.1016/j.biombioe.2012.01.011

27. Beatriz CordenunsiI FS-CMED-RAZMATMSBGBdSCCEBGE (2010) Carbohydrate composition of ripe pineapple (cv. perola)and the glycemic response in humans Food Science and Technology 30 (1):282-288

28. Fang X, Qin Y, Li X, Wang L, Wang T, Zhu M, Qu Y (2010) [Progress on cellulase and enzymatic hydrolysis of lignocellulosic biomass]. Sheng Wu Gong Cheng Xue Bao 26 (7):864-869

29. Dowe N (2009) Assessing cellulase performance on pretreated lignocellulosic biomass using saccharification and fermentation-based protocols. Methods Mol Biol 581:233-245. doi:10.1007/978-1-60761-214-8_15

30. Vinzant TB, Adney WS, Decker SR, Baker JO, Kinter MT, Sherman NE, Fox JW, Himmel ME (2001) Fingerprinting Trichoderma reesei hydrolases in a commercial cellulase preparation. Applied Biochemistry and Biotechnology 91 (1):99-107. doi:10.1385/ABAB:91-93:1-9:99

31. Wang M, Zhou D, Wang Y, Wei S, Yang W, Kuang M, Ma L, Fang D, Xu S, Du S-k (2016) Bioethanol production from cotton stalk: A comparative study of various pretreatments. Fuel 184:527-532. doi:https://doi.org/10.1016/j.fuel.2016.07.061

32. Hongjie Dai SO, Yue Huang, Huihua Huang (2018) Utilization of pineapple peel for production of nanocellulose and film application. Cellulose 25:1743-1756

33. Jönsson LJ, Martín C (2016) Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. Bioresource Technol 199:103-112. doi:https://doi.org/10.1016/j.biortech.2015.10.009

34. Oshoma CE, Greetham D, Louis EJ, Smart KA, Phister TG, Powell C, Du C (2015) Screening of Non- Saccharomyces cerevisiae Strains for Tolerance to Formic Acid in Bioethanol Fermentation. PLOS ONE 10 (8):e0135626. doi:10.1371/journal.pone.0135626

35. Ramaraj JTCYUR (2019) Fermentation of pineapple fruit peel wastes for bioethanol production. Biomass Conversion and Biorefinery 9:761-765 doi:https://doi.org/10.1007/s13399-019-00436-y

36. Yi-Huang Chang K-SC, Chien-Yu Chen, Chuan-Liang Hsu,Tsan-Chang Chang, and Hung-Der Jang. (2018) Enhancement of the Efficiency of Bioethanol Production by Saccharomyces cerevisiaevia Gradually Batch-Wise and Fed-Batch Increasing the Glucose Concentration. fermentation 4 (45):1-12. doi: https://doi.org/10.3390/fermentation4020045

37. Ricardo Soccol C, Faraco V, Karp S, Vandenberghe LPS, Thomaz-Soccol V, Woiciechowski A, Pandey A (2011) Chapter 5 - Lignocellulosic Bioethanol: Current Status and Future Perspectives. In: Pandey A, Larroche C, Ricke SC, Dussap C-G, Gnansounou E (eds) Biofuels. Academic Press, Amsterdam, pp 101-122. doi:https://doi.org/10.1016/B978-0-12-385099-7.00005-X

CHAPTER 4: Microwave-assisted acid and alkali pre-treatment of (Ananas comosus) pineapple waste to enhance reducing sugar production for ethanol production.

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Research Paper-II

Introduction Chapter 4

The manuscript presents the combined effect of physical and chemical pre-treatments on the pineapple waste for the production of reducing sugars to be further used in the fermentation for biofuel production. A range of different combinations of microwave heating time, microwave power along with different concentrations of acids and alkali followed by enzymatic hydrolysis was examined. A range of qualitative and quantitative experiments including total reducing sugars estimation, HPLC sugars profiling, determination of total phenolic content, and fermentation inhibitors including furfural and hydroxymethylfurfural to estimate the sugars produced and ethanol fermentation. Infrared thermography was used to detect the effect of microwave heating on PW. Scanning electron microscopy to examine the physical changes in biomass due to different microwave-assisted chemical pre-treatments. The mechanism of production of reducing sugars and ethanol production was predicted based on the results of the experiments conducted

Microwave-assisted acid and alkali pre-treatment of *(Ananas comosus)* pineapple waste to enhance reducing sugar production for ethanol production.

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Abstract:

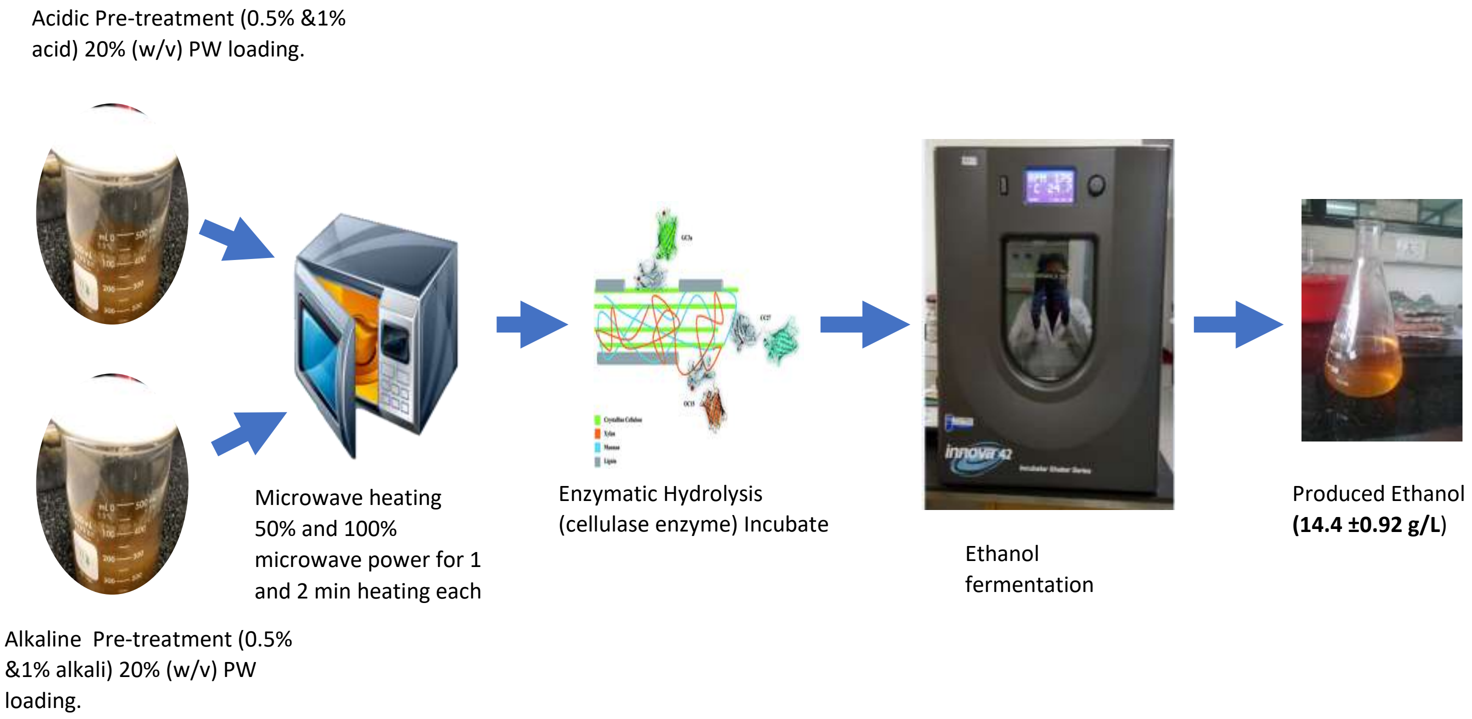
This study evaluates the microwave-assisted acidic and alkaline pre-treatment of pineapple waste (PW) for ethanol production. The effectiveness of microwave-assisted alkaline sodium hydroxide (NaOH) and acidic sulfuric acid (H_2SO_4) were studied using a concentration of 0.5% and 1% at a microwave power of 100% and 50% for 1 min and 2 min. The highest concentration of reducing sugars was 298.2g/L using 0.5% sulfuric acid and 100P for 1 minute. Microwave irradiation for a longer time (2 min) led to hydrothermal degradation of sugars and resulted in a lower amount of reducing sugar (231.6 ± 1.9 g/L). SEM and FTIR analysis showed significant structural changes in the control and pre-treated PW samples. 160 µL of commercial cellulase enzyme was used for further hydrolysis of pretreated samples. 1% H_2SO_4 pre-treatment with 2 min microwave heating at 100 power produced the highest amount of phenolic content of 0.918 ± 0.06 mg GAE/mL. GCMS spectrum of hydrolysate showed the presence of fermentation inhibitors such as furfural, levulinic acid, and other organic acids. A maximum concentration of 14.4 g/L of ethanol was produced after 48h fermentation.

Keywords: Microwave pre-treatment, Bioethanol, Saccharification, heat treatment,

Highlights:

- Microwave pre-treatment is an alternative to conventional heating methods
- Additional advantages of microwave-based pre-treatments include faster treatment time, selective processing, instantaneous control, and acceleration of the reaction rate.
- FTIR and SEM analysis showed the pineapple peel structure deteriorated after the pre-treatments.
- The highest amount of reducing sugars was produced by 0.5% sulfuric acid pretreatment at 100% microwave heating for 1 minute.

Graphical Abstract:



4.1 Introduction:

The growing demand for cleaner and sustainable energy has increased the demand for bioethanol production. Agricultural residues are a good substrate for the production of bioethanol. Australia produces over 76,000 tonnes of pineapple every year on average [52]. Up to 70-80% of the whole processed fruit end up as processing waste in form of peel, skin, and crown. Pineapple waste (PW) is a rich source of carbohydrates and potentially hydrolyzable cellulose and hemicellulose that can be used as feedstock for ethanol production [42, 164, 170]. Bioethanol is an important alternative to fossil fuels. The production of first-generation fuel ethanol from starch raw material may increase the risk of a food crisis in underdeveloped countries. The solution to this is second-generation biofuels from lignocellulosic biomass [171].

The lignocellulosic biomass conversion to biofuels has attracted much attention from researchers and the development of an efficient, cost-effective, and eco-friendly pre-treatment process is a major challenge in lignocellulosic biomass to biofuel conversion. pre-treatment Microwave (MW) irradiation has gain popularity due to its rapid internal heating capacity, instant control, and short treatment time. In 2016, Conesa et al reported that the use of microwave heating before enzymatic hydrolysis of PW increased the sugar yield? at intermediate power; however, the high power (1200W) and 20 minutes heating resulted in a lower yield of reducing sugars [138]. Questions have been raised based on the uneven distribution of microwave energy and the negative effects of overheating [172].

Pineapple waste consists of cellulose, hemicellulose, and lignin which is a non-carbohydrate polymer that acts as a barrier in the process of enzymatic hydrolysis. To enhance the enzymatic hydrolysis, it is important to disrupt the complex lignin structure and make cellulose and hemicellulose more accessible to enzymes to convert the complex polysaccharides to simple fermentable sugars [173].

Microwave heating is a promising pre-treatment of biomass for saccharification as it exploits thermal and specific effects in an aqueous environment [172]. Microwave heating is administered by Maxwell’s equations for electromagnetic waves, the interactions between microwaves and dielectric properties of a material, and the heat dissipation governed by heat and mass transfer. The electric field of commonly used irradiation frequency (240 MHz) oscillates 4.9x 10^9 times per second, and the sympathetic agitation generates heat [174]. The electromagnetic waves of microwave spanning a frequency range from (3x108 cycles/s) to (3x10^{11} cycles/s) with most domestic and industrial microwave work at 2.45 GHz [175]. Microwave produces the electromagnetic waves that interact with polar molecules and ions in material and results in thermal and non-thermal effects that drive the chemical, physical, and biological reactions in the samples [176]. Grounded biomass reduced particle size that positively reflected on the process and led to the increased specific surface area that helped in enhances enzyme accessibility for enzymatic hydrolysis [175].

The combination of microwave and acid or alkali pre-treatment has the potential to reach a high yield of fermentable sugars while maintaining a lower concentration of fermentation inhibitors, but the combination should be carefully optimized. Microwave-assisted alkaline and acid pre-treatments have been studied on rice straw [177], wheat and rye stillage [178, 179], catalpa sawdust [180], aloe vera rind [181], Spent coffee[182], and waste activated sludge, but there is a lack of studies carried out on pineapple waste.

So far, there has been only one study related to microwave-assisted alkali treatment of pineapple waste. Conesa et al in 2016 reported that microwave 6.375W/g for 5 seconds with 0.5 N NaOH pre-treatments for short exposure times (up to 60 s) significantly improved the yield of the enzymatic hydrolysis compared with non-pretreated waste. The greatest increase of fermentable (35.7%) and total sugars (33.5%) was obtained at 6.375 W/g for 5 s [183]. While the extended microwave irradiation caused degradation of sugars resulting in a lower yield of sugars as reported in earlier studies from the same authors.

However, the literature review showed there is no report on microwave-assisted acidic pretreatment of pineapple waste for bioethanol production. Furthermore, no study has compared these 2 methods. The microwave-assisted acid pre-treatment has the potential to solubilize the hemicellulose structure of PW to increases the accessibility of the enzymes in the enzymatic hydrolysis reaction. In contact with acids, Hemicellulose in biomass is hydrolyzed to xylose and other sugars and other sugars, altering the lignin structure [178]. Therefore, this study aimed to determine the effects of microwaves at different concentrations of acids and alkalies on the hydrolysis of polysaccharides contained in PW and to assess the effectiveness of the subsequent enzymatic hydrolysis for ethanol production from the resulting hydrolysate.

4.2 Materials and methods

4.2.1 Raw feedstock, chemicals, and micro-organisms

The feedstock used in this experiment is pineapple (*Ananas comosus)* waste (PW), which was collected from a local pineapple processing unit near Brisbane, Queensland Australia. The samples were collected at different times, from different batches of pineapple harvested at different times, and from different waste generation locations within the processing plant. All the samples were packed in bags and stored at -20°C until use.

The PW feedstock was characterized according to the acid detergent fiber, neutral detergent fiber, and acid detergent lignin (ADF-NDF-ADL) method by Goering and van Soest method 1970 [147]. Hemicellulose and cellulose were calculated using formulas as below.

Hemicellulose (%) = NDF (%) – ADF (%)

Cellulose (%) = NDF (%) – Hemicellulose (%) – Lignin (%)

A pH of 3.7 ± 0.2 was measured using a TPS benchtop pH meter. These samples were then used for the pre-treatment described below. PW samples were thawed, blended, and sieved through a 0.5mm mesh before each experiment. Moisture in the samples was measured using an OHAUS moisture balance (model MB120).

4.2.2 Microwave-assisted alkaline pre-treatment

A combined alkaline and microwave pre-treatment was carried out in a 2.45 GHz domestic SHARP R231ZS compact microwave (1200W) oven with a turntable plate. The tests were carried out in 250ml capped glass beaker containing 20% PW and 0.5% or 1% sodium hydroxide. The samples were subjected to microwave treatment for 1 or 2 minutes and at a power setting of 50% or 100%. Longer time exposure was not tested due to the scorching of the samples. The pre-treatment was carried out with 20% (w/v) PW biomass loading The initial pH was 5.2 and 6.9 in the 0.5% and 1% NaOH sample, respectively. Microwave based alkali. Water loss during microwave processing was determined by the differences in weight and was corrected by adding distilled water after the pre-treatment. After the pre-treatment, the pH of the samples was adjusted to 5.5 for enzymatic hydrolysis using 1 N HCl. Pre-treated samples were analyzed for reducing sugar (RS) as described by Miller et al. [148]

4.2.3 Microwave-assisted Acidic Pre-treatment

A combined acid and microwave pre-treatment was carried out according to the same conditions as for the alkali, except that the PW was mixed with 0.5% or 1% sulfuric acid (H_2SO_4). The initial pH was 3.2 and 2.9, respectively. Pretreated samples were analyzed for reducing sugar (RS) as described by Miller et al. 1959 [148].

4.2.4 Infrared Thermography

To determine the effects of the microwave heating, a FLIR C5 compact thermal camera (FLIR Systems, Estonia) was used with a spectral wavelength range from 8 µm to 14 µm and a 160x120 resolution (19,200 pixels). The IR sensors with thermal sensitivity of <70mK allowed us to monitor the temperature reached during microwave heating (maximum 400°C. An image of the top surface of the container was taken at the end of each microwave pre-treatment to shed more light on the link between localized temperature and hydrolysis of PW.

4.2.5 Enzymatic hydrolysis

Following the combined chemical and microwave pre-treatment, an enzymatic treatment was applied to the disrupted feedstock to further increase the sugar yield. Commercial cellulase enzyme from *Trichoderma reesei* was purchased from Sigma Aldrich (C2730 aqueous solution, ≥700 units/g). pre-treatment 100 mL of the pre-treated PW was mixed with 10 µg/mL of cellulase enzyme in 250-mL Erlenmeyer flasks at 30°C and pH 5.5 for 48 hours. Samples were then centrifuged for 10 min at 10,000 rpm, and filtered through a 0.45µm membrane filter. The filtered supernatant was then collected and analyzed for reducing sugar (RS) and fermentation inhibitors and compared with the untreated sample. The solid residue was stored at -20 °C and used for FTIR and SEM analysis. All the samples were done in triplicate.

4.2.6 Scanning electron microscope (SEM)

The surface morphology of all the samples was observed before and after the pre-treatment with a Scanning Electron Microscope (SEM) (JCM 6000 Benchtop, NO.ICM6000-4E). Before observation, the sample surface was coated with a thin, electric conductive gold film using an ion sputter coater (Model No. E1010; Hitachi Co., Japan).

4.2.7 Reducing Sugar concentration

The reducing sugar (RS) concentration was determined using the di-nitro salicylic acid (DNS) assay as described by Miller et al. 1959 [148]. This is based on the color reaction of 3,5-di-nitro salicylic acid and reducing sugar in alkaline solution and boiled water. The calibration curve was prepared using glucose as a standard with R^2= 0.9961 within the test ranges. 1 mL of sample was diluted with DI water and mixed with 1 mL of DNS solution in the test tube before boiling at 90 °C for 5 min. The absorbance of the sample was detected using a UV–vis spectrophotometer HACH DR 6000 series at a wavelength of 540 nm for the determination of total reducing sugar concentration. All the samples were done in triplicate and the standard deviation was reported as error bars on the graphs.

4.2.8 Profiling of sugars

The PW hydrolysates were analysed for sugar profiling using a High-Performance Liquid Chromatography (HPLC) (model 1600, waters, USA) with Refractive Index detector (model 2414, waters, USA). The hydrolysates were filtered through a 0.45µm membrane filter before running a 20 µl sample through a Carbohydrate High-Performance 4µm (4.6 mm x 250 mm cartridge) column at a flow rate of 1.3 mL/min using water: Acetonitrile (10:90) as mobile phase.

4.2.9 Determination of fermentation inhibitors

The presence of fermentation inhibitors including furfural (F) and hydroxymethylfurfural (HMF) and other organic acids in the liquid phase of the samples after pre-treatment were evaluated as described below along with phenolic contents.

4.2.10 Determination of phenolic content

The total phenolic content was measured using the method developed by Waterhouse *et al*. (2003) with Folin-Ciocalteu reagent (Sigma-Aldrich) and monohydrate gallic acid (Sigma-Aldrich) as the standard. Results were expressed as mg of gallic acid equivalents per mL of pineapple waste (mg GAE/mL).

4.2.11 Determination of furfural

The presence of furfural (F) and other acids in the liquid phase of the samples before and after pre-treatment were evaluated using A Shimadzu QP2010 Gas Chromatograph Mass spectrophotometer (GC-MS) (Shimadzu, Duisburg, Germany) using a Restek stabilwaxDA Column with the following program: oven 60 °C, hold time 0 min, increasing to 250 °C at a rate of 5°C min^{-1}, hold time 0 min. The injection temperature was 200 °C, the ion source temperature 250 °C; interface temperature 250 °C; solvent cut time 2 min; split ratio 5; flow control mode: linear velocity; pressure 115.9 kPa; total flow 14.2 mL/min; column flow 1.87 mL/min; linear velocity 49.9 cm/sec; purge flow 3 mL/min.

4.2.12 Ethanol fermentation

The yeast *Saccharomyces Cerevisiae in* powder form was provided by Lallemand Biofuels and Distilled Spirits (USA) under the name Thermosacc Dry. The yeast cells were activated in 100ml growth media containing 0.4 g yeast extract with 3g of glucose. The culture medium was then incubated at 35°C and 120 rpm for 12 hrs. The hydrolysate obtained after enzymatic saccharification was filtered and centrifuged (10000 rpm, 10 min) to remove the unhydrolyzed residue. Fermentation was done in a 250-mL Erlenmeyer flask with 75 mL hydrolysate at a pH of 5.6. This was inoculated with 2% S. cerevisiae (Angel Yeast Co., Ltd., P. R. China) and was incubated at 36 °C for 72 h. The ethanol concentration determination was done every 24 h of fermentation using viscometer. After completion of the fermentation, the fermented liquor was filtered through a 0.45µm membrane filter and analyzed using A Shimadzu QP2010 Gas Chromatograph Mass Spectrophotometer (GCMS) for ethanol concentration using the wax column the program settings were column oven temperature 100 °C, hold time 10 min, increasing to 250 °C at a rate of 5°C min^{-1}, hold time 0 min. The Injection Temperature was 150 °C, the Ion source Temperature 250 °C; Total Flow 14.2 ml/min; Column flow 1.87 ml/min; Linear Velocity 49.9 cm/sec; Purge flow 3 ml/min.

4.3 Results and Discussion

4.3.1 Microwave -alkali pre-treatment on sugar yield

Microwave-assisted chemical pre-treatments of lignocellulosic material enhances its hydrolysis and temperature plays a significant role during the pre-treatment [184]. Microwave alkali hydrolysis was performed with 0.5% and 1% sodium hydroxide, microwave exposure time of 1 min and 2 min, and microwave power of 50 and 100%. The concentration of reducing sugars following each condition is shown in Figure 4.1.

The microwave treatment increased the reducing sugar concentration from 170.2 g/L to 211.6 g/L after 1 minute and 268 g/L after 2 minutes which is a 24 % and 36% increase, respectively. Using 100% power was detrimental as it reduced the sugars concentration to 185 g/L.

0.5% NaOH at microwave heating for 2 min at 50% produced the highest amount of reducing sugars of 286.6 ± 2.2 g/L. This is a 44% increase compared to the control and 7% increase compared to microwave alone.

This correlated with a lower proportion of lignin and hemicellulose as shown in Table 4.1. It was also observed that increasing in the concentration of alkali to 1% and microwave irradiation 100% time to 2 minutes resulted in lower production of fermentable sugars. The combination of microwave and chemical required only 2 minutes compared to 7 days when using chemicals alone (Chapter 2) which is a significant advantage. This is in line with Mikulski et al (2019 who also observed that microwave heating shortens the time required for chemical pre-treatment, but does not significantly improve the cellulose hydrolysis efficiency at longer irradiation time. Therefore, the use of extended microwave heating is not recommended due to higher energy consumption [185].

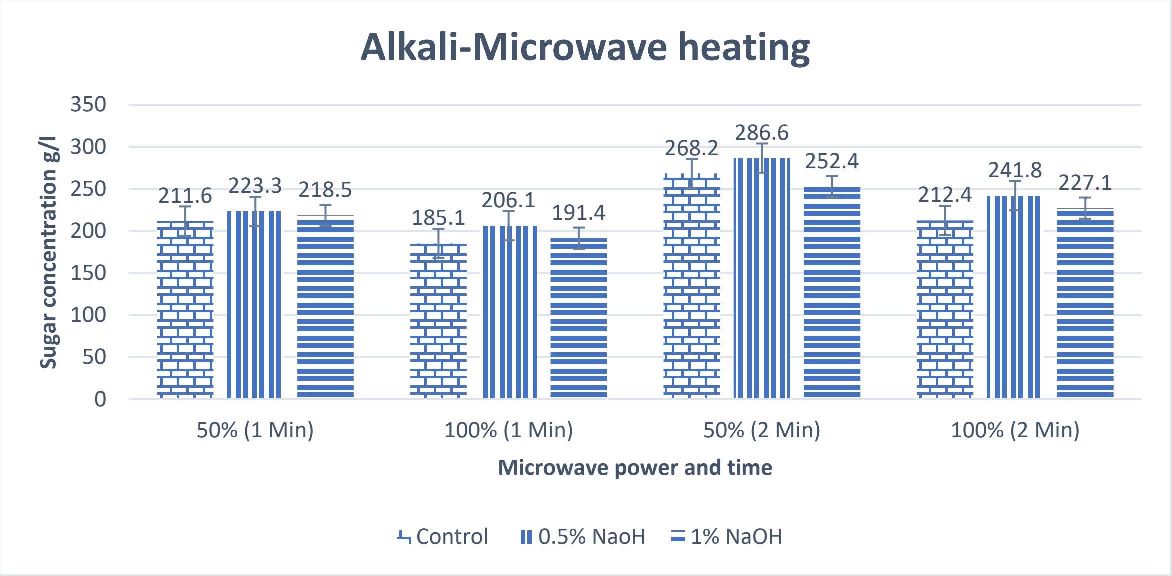


Fig. 4.1: Reducing sugar concentration after microwave-assisted alkaline pre-treatment.

4.3.2 Microwave-acid pre-treatment on sugar yield

The microwave-assisted acid pre-treatment was proposed to solubilize the hemicellulosic fraction of PW while the cellulose remains in the pretreated PW [186]. Microwave acid hydrolysis was performed with 0.5% and 1% sulphuric acid, microwave exposure time of 1 min and 2 min, and microwave power of 50 and 100%. The combination of, microwave heating time, the heat generated during microwave irradiation, and the sulfuric acid concentration were evaluated in this experiment. Figure 4.2 shows the amount of reducing sugar produced under each condition tested.

Microwave and 0.5% H_2SO_4 was found to increase the sugar concentration from 170.2 g/L up to 298 g/L using 100% power for 1 minute. Doubling the exposure time to 2 minutes did not result in greater concentration.

This is a 75% increase compared to the untreated sample and a 51% increase compared to microwave alone. These results are consistent with a lower proportion of hemicellulose and lignin following the experiment (Table 4.1).

An increase in microwave irradiation time from 1 minute to 2 minutes significantly reduced the concentration of sugars from 261.3 ± 2.8 g/L to 224.2 ± 2.5 g/L at 50% power and 298.2 ± 2.5 g/L to 231.6 ± 1.9 g/L at 100% power for 0.5% H_2SO_4 and a similar pattern was observed with 1% H_2SO_4. this may be due to the dehydration of sugars under excessive heat resulting in a lower concentration of sugars [187].

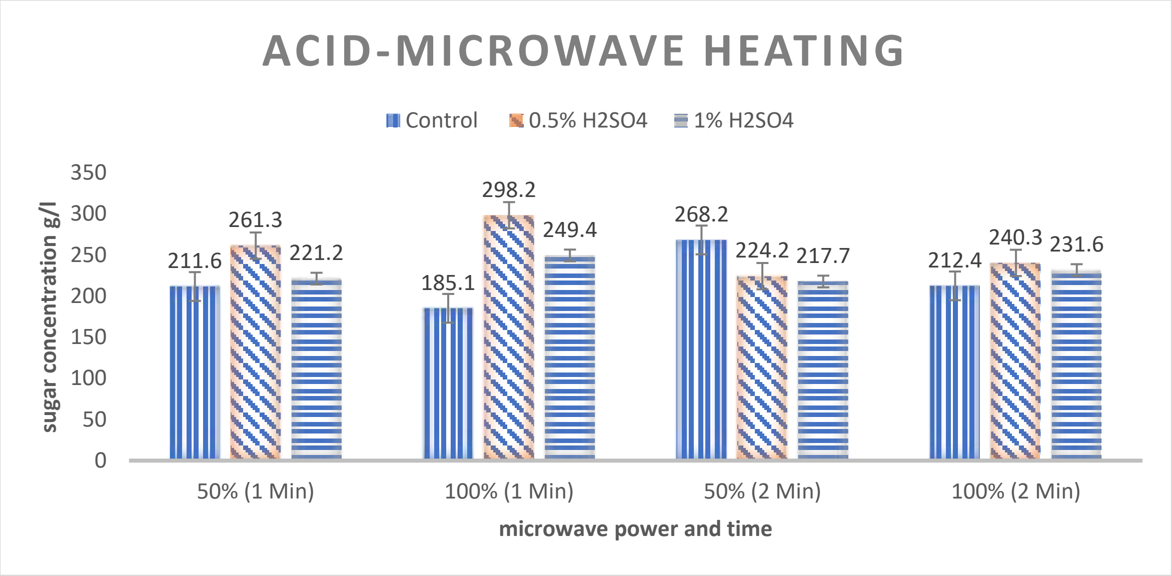


Fig. 4.2: Reducing sugar concentration after microwave-assisted acidic pre-treatment.

Table 4.1: Change in physical parameters in samples after each pre-treatment.

Samples		**Particle Size**	**pH**	**Moisture content**	**Cellulose**	**Hemicellulose**	**Lignin**
Before experiment		< 0.5 mm	3.7 (± 0.2)	42.2% ±0.34 (w/w)	21.1%±0.34 (w/w)	23.9% ± 0.29 (w/w)	5.5% ± 0.33 (w/w)
Control		< 0.5 mm	3.7 (± 0.2)	43.1% ±0.34 (w/w)	20.8 % ±0.41 (w/w)	23.3 % ±0.41 (w/w)	5.2% ± 0.19 (w/w)
Alkali Treatment	0.5 % NaOH 50% for 1min	< 0.5 mm	4.1 (± 0.2)	42.3% ±0.24 (w/w)	20.4 % ±0.41 (w/w)	21.3 % ±0.31 (w/w)	4.7 % ± 0.09 (w/w)
	0.5 % NaOH 100% for 1min	< 0.5 mm	5.6 (± 0.2)	42.4% ±0.26 (w/w)	20.1 % ±0.41 (w/w)	19.7 % ± 0.16 (w/w)	4.2% ± 0.26 (w/w)
Acid Treatment	0.5 % H_2SO_4 50% for 1min	< 0.5 mm	2.9 (± 0.1)	41.8% ±0.32 (w/w)	19.9 % ±0.41 (w/w)	20.5 % ±0.41 (w/w)	4.4 % ±0.41 (w/w)
	1.0 % H_2SO_4 100% for 1min	< 0.5 mm	2.4 (± 0.3)	42.1% ±0.14 (w/w)	19.7 % ±0.41 (w/w)	19.5% ±0.41 (w/w)	4.1 % ±0.41 (w/w)

4.3.3 Analysis of microwave-induced heating of pineapple waste by thermography

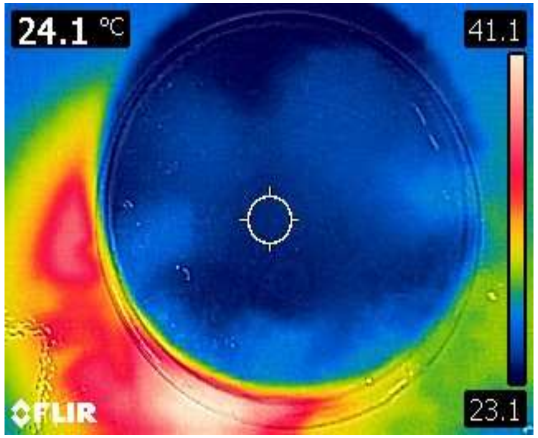
An infrared camera was used to determine the effect and efficiency of microwave heating on PW. The temperature of the samples during microwave pre-treatment in alkaline and acidic medium was evaluated via thermal images. Figure 4.3 shows the thermography data and the temperature in each image. As the thermography image was taken immediately after microwave pre-treatments, heat loss was considered insignificant as reported by some studies [188, 189]

In each thermograph, a circle indicates the maximum temperature and the temperature scale in each image. An increase in irradiation time and microwave power resulted in higher temperature and more uniform heat distribution, while shorter exposure time and lower power resulted in lower temperature and uneven heat distribution as can be visible in fig 3B. Need to explain what temperature was best based on reducing sugar analysis.

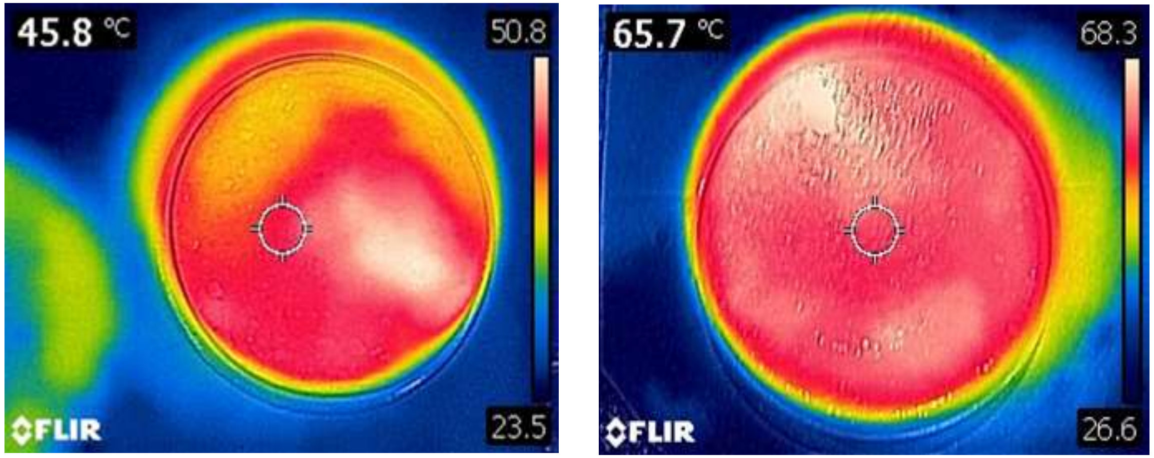
Figures 3B and 3C show a temperature of 46 and 66°C in the 0.5% NaOH pre-treated samples at 50% power for 1 and 2 minutes respectively. These temperatures corresponded to sugars concentrations of 223 and 286 g/L, respectively. Higher temperature was therefore beneficial for the hydrolysis of PW.

A temperature of 79°C in the center of the sample gave the maximum sugar concentration at 100% power, 1 minute, and 0.5% H_2SO_4 (Fig 3D), but a temperature of 10°C higher seems to become detrimental to the hydrolysis (Fig 3E). There is therefore a trade-off between chemical hydrolysis, the thermal effect, and the non-thermal effect during microwaving. Controlling the internal temperature of the sample using thermography may be more appropriate than exposure time to optimise the synergy between these effects.

This is in line with Kumar et al (2014) who reported that microwave irradiation for a shorter duration was more effective than extended irradiation which causes hydrothermal dehydration of samples and results in less efficient hydrolysis [190]. Microwave-assisted pretreatments not only increase the temperature, but also generate an electromagnetic field which creates the non-thermal effects that can also accelerate the destruction of the crystalline structure resulting in higher production of reducing sugars in a shorter time compared to other heating pre-treatments [191].

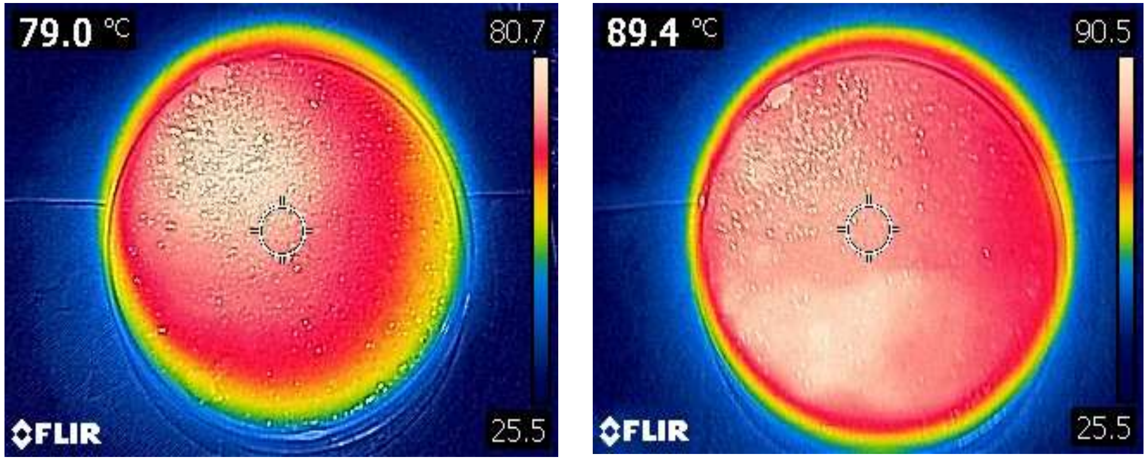


3A: No



3B: 50 % for 1 Min (0.5% NaOH)

3C: 50%for 2 Min (0.5% NaOH)



3D: 100% for 1 Min 0.5% H2SO4

3E: 100% for 2 Min 0.5% H_2SO_4

Figure 4.3. Thermographs of the bottom surface of glass beakers containing PW and microwave pretreatments of different samples.

4.3.4 Enzymatic Hydrolysis

The saccharification of PW using microwave heating and microwave-assisted chemical pre-treatments showed a significant increase in the production of fermentable sugars. To further increase the concentration of reducing sugars for ethanol production, enzymatic hydrolysis of saccharified waste was investigated. Three different samples were chosen based on the reducing sugar concentration from microwave-assisted chemical treatments.

The samples chosen were microwave only (50% for 2 min), 0.5% NaOH 50% for 2 min, and 0.5% H_2SO_4 100% for 1 min which produced 268.2g/L, 286.6g/L, and 298.2g/L of reducing sugars, respectively as shown in Table 4.2. For the enzymatic hydrolysis, 160 µL of commercial cellulase enzyme was pipetted based on results obtained previously with PW [192]. After 24 hrs of incubation, a significant increase was noted in all three samples (Table 4). The highest concentration of reducing sugars was 329.1 g/L in the samples treated with 0.5% H_2SO_4 100% for 1 min. The control samples recorded a 13.5% increase while 8.6% and 10.4% increases were recorded respectively in alkaline and acidic pre-treated samples. [160, 161].

During the process of enzymatic hydrolysis of biomass, endo-β-glucanase first randomly cleaves the internal glycosidic bond of the cellulose chain, exposing the reducing or nonreducing ends for cellobiohydrolase or exo-β-glucanse to react and release cellobiose. β-glucosidase then hydrolyses the cellobiose into glucose to be further used in fermentation for ethanol production [160, 161]. The enzyme cellulase employed in this experiment was produced from *T. reesei* that secrets low levels of $\beta-$glucosidase, resulting in the accumaltion of cellobiose during hydrolysis [143, 162]. The action of the cellulase complex consists of three groups of enzymes: endoglucanases, exoglucanases, and β-glucosidases are most probably the main responsible for glucose increase.

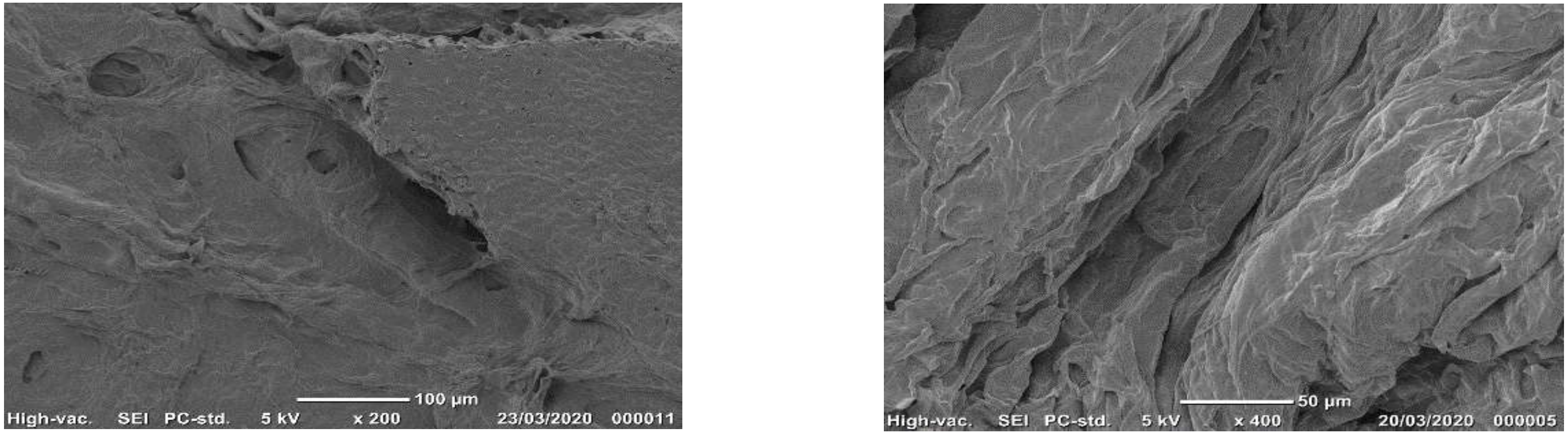
Table 4.2. Reducing sugar concentration after enzymatic hydrolysis.

Sample	Pre-treated samples	Pre-treated samples followed by enzymatic hydrolysis (160 µL, 24 hrs)	% increase
Microwave alone 50% for 2 min	268.2g/L	304.4g/L	13.5%
0.5% NaOH 50% for 2 min	286.6g/L	311.3g/L	8.6%
0.5% H_2SO_4 100% for 1 min	298.2g/L	329.1g/L	10.4%

4.3.5 Morphological Analysis by Scanning Electron Microscopy

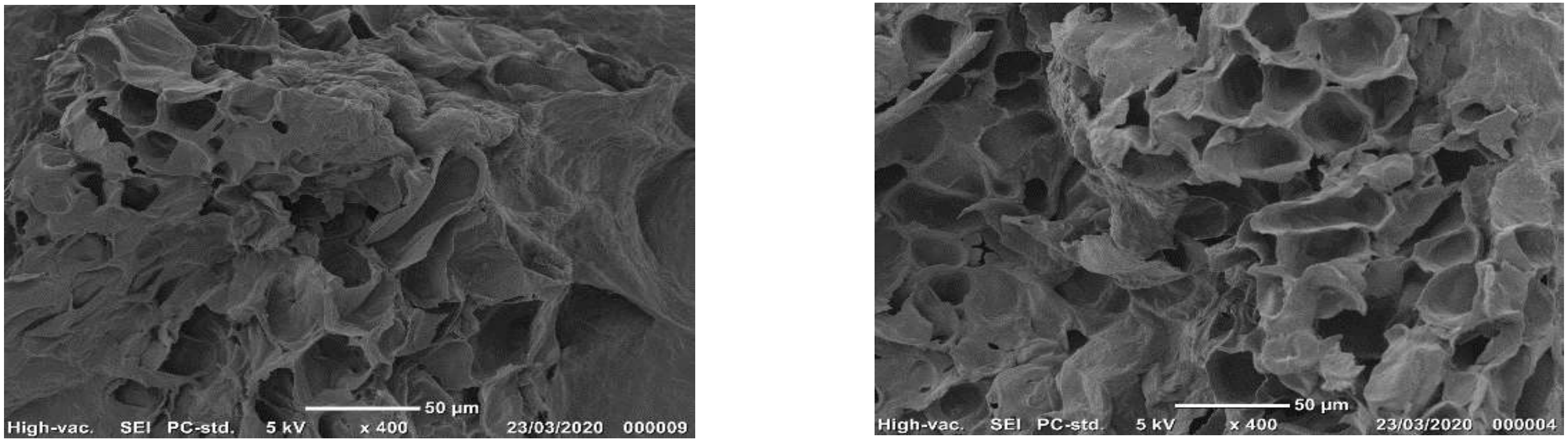
The structural changes that were induced by different chemical concentrations and microwave treatments were investigated by SEM in Fig 4.4. The SEM image of untreated samples (Fig 4.4A) revealed a relatively smooth, continuous and unscathed external structure. The external layer of pineapple peel is composed of lignin, ash, hemicellulose that enfolds the inner structural cellulose fibers and different binding materials, which is consistent with previous studies on PW reported by Hongjie et al in 2018 [164].

In Fig 4.4B, the PW samples treated with microwave showed some evidence of disruption in the external layer compared to the untreated sample. Figures 4C and 4D (0.5% alkaline treated samples 50% for 2 min and 0.5% acid treated samples 100% for 1 min) showed some evidence of pores in cell walls suggesting that chemical combined with microwaves destroyed cell wall components resulting in better hydrolysis and sugar release [183]. This is consistent with previous studies where structural changes in changes due to microwave led to improved saccharification of lignocellulosic feedstocks. [193, 194].



4.4A. Untreated samples

4.4B. Microwave only



4.4C. 0.5% Alkaline Treated samples 50% for 2 min

4.4D. 0.5% Acidic Treated samples 100% for 1 min

Fig. 4.4 Scanning Electron Micrographs of untreated and pre-treated pineapple waste samples

4.3.6 Sugar Profiling after enzymatic hydrolysis

The characterization of monomeric sugars present in the hydrolysates from the untreated and acid and alkali pre-treated PW samples are presented in Table 3. The most abundant sugar in PW was glucose across all three samples with 33.6%, 37.4%, and 39.3% of total sugars, respectively in microwave alone, microwave/alkaline, and microwave/acidic pre-treatments. Other sugars included xylose, fructose, sucrose, arabinose, and galactose and traces of mannose and fucose which is consistent with the literature on PW [159]. Among these sugars, only glucose, xylose, fructose, and sucrose can be used in the fermentation of ethanol, while the others are non-fermentable sugars.

Table 4.3. sugar profile in the hydrolysates.

Type of sugar	**Microwave alone 50% for 2 min.**	**0.5 % NaOH 50% for 2 min pre-treatment**	**0.5 % H_2SO_4 100% for 1 min pre-treatment**
Glucose (%)	33.6	37.4	39.3
Xylose (%)	16.1	14.2	15.7
Fructose (%)	13.4	11.9	12.4
Sucrose (%)	18.8	16.8	14.3
Arabinose (%)	12.4	14.3	12.7
Galactose (%)	2.6	3.5	2.8
Mannose (%)	1.9	1.1	1.6
Fucose (%)	1.2	0.8	1.2

4.3.7 Fermentation inhibitors

4.3.7.1 Total Phenolic Content

It can be concluded from Figure 4.5 that microwave heating caused an increase in the phenolic content of the saccharified samples. Higher microwave time, power, and concentration of chemicals led to higher TPC content with H2SO4 treatment producing more TPC than with NaOH The increase in phenolic content in samples might be due to the solubilization of lignin content of the feedstock as a result of microwave heating [195]. This also follows the pattern of results reported by Hu and Wen *et al*. [194]. 1% H_2SO_4 pre-treatment with 2 min microwave heating at 100% power produced the highest amount of phenolic content with 0.918 ± 0.06 mg GAE/mL. The major polyphenols reported in pineapple peel are gallic acids, catechin, epicatechin, ferulic acid [196]. The highest amount of polyphenols reported in pineapple peels were Gallic acid (31.76 mg/100 g dry extracts), catechin (58.51 mg/100 g), epicatechin (50.00 mg/100 g), and ferulic acid (19.50 mg/100 g) [197]. Whereas Adebo et al in 2020 reported the presence of a trace amount of phenolic content can inhibit the fermentation process [198].

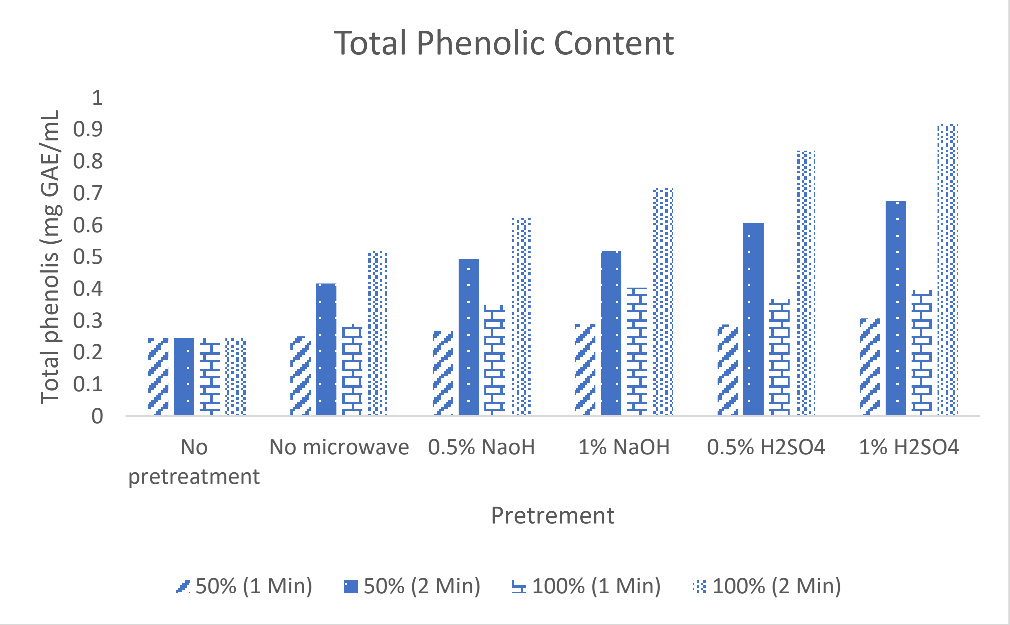


Fig. 4.5: Generation of fermentation inhibitors during combined microwave chemical pre-treatments.

4.3.7.2 Furfural and other organic acids

The use of microwave-assisted chemical pre-treatment showed a significant increase in the production of reducing sugars, but the side effect of the pre-treatment was the formation of inhibitors compounds such as furfural, furan-carboxaldehyde, and organic acids like acetic acid, levulinic acid, and formic acid as shown in Table 4.4. Microwave irradiation caused a temperature rise that can result in hydrothermal degradation of sugars leading to the formation of inhibitory compounds [178]. Figure 4.6 shows the GCMS chromatogram for the 0.5% H_2SO_4 100% and 1-minute sample which produced the highest sugar concentration after microwave-assisted heating followed by enzymatic hydrolysis. These compounds inhibit the growth of fermentation micro-organisms and thus reduces bioethanol production. However, there has been some evidence in the literature that *Saccharomyces cerevisiae* can tolerate the presence of furfural and other inhibitory compounds up to 2 g/L [199, 200].

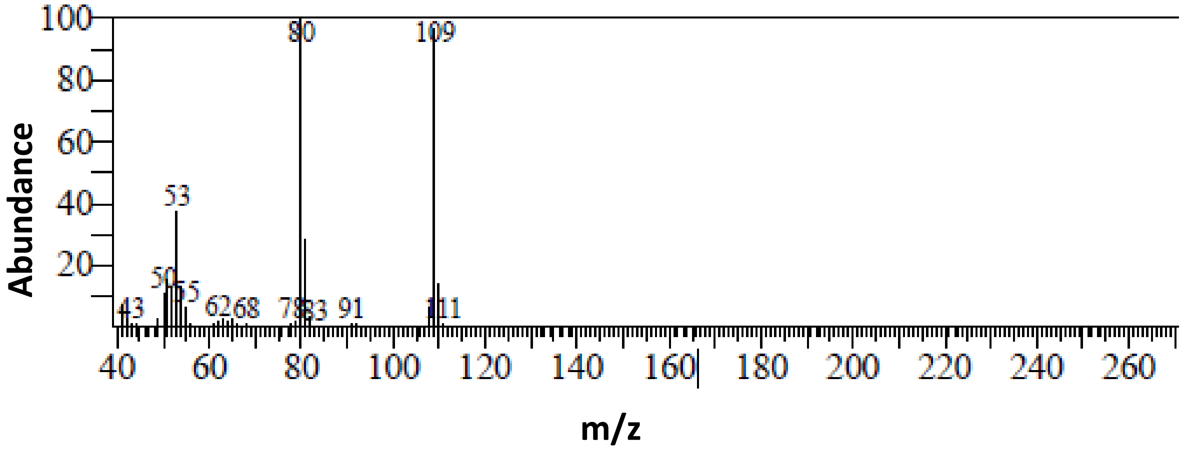


Fig. 4.6 GC Spectrum of hydrolysate showing presence of inhibitors

Table 4.4: Compounds detected in the (1% H2SO4, 100%P for 1 minute) treated samples

m/z peak	**Compound name**
43	Acetic acid
50	Furancarboxaldehyde
53	Formic acid
80	Furfural
109	Levulinic acid

4.3.8 Ethanol Fermentation:

The resulting hydrolysate from enzymatic hydrolysis (Table 4.4) was further used for ethanol fermentation. As shown in Fig. 4.7, the highest concentration of ethanol (14.4 g/L) was produced by PW samples treated with 0.5% H_2SO_4 followed by microwave treatment for 1 minute at 100% after 48 h of incubation. . Balat *et al.* in 2011 reported that exposing lignocellulosic substrates to microwave at various times and temperature can solubilize the substrate by breaking the network of exopolysaccharides which speeds up the chemical processes of conversion of fermentable sugars to ethanol [187].

However, microwave irradiation caused the hydrothermal degradation of sugars resulting in the formation of inhibitory compounds (Table 4.5) which limited the conversion of fermentable sugars to bioethanol. The presence of 0.918 ± 0.06 mg GAE/mL of TPC in samples are considered as a high concentration of inhibitors in fermentation media

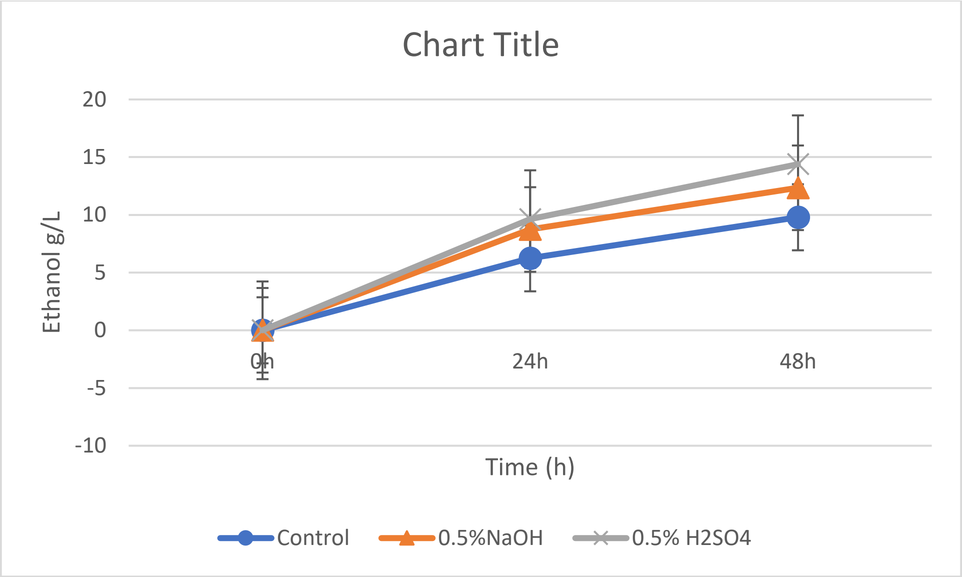


Fig. 4.7 Ethanol production following Microwave-assisted NaOH (1%) and H_2SO_4 (1%) pre-treatments of PW.

Theoretically, 1 g of fermentable sugars will produce 0.485 g/L of ethanol [169]. Table 4.5 shows the percentage of sugars in each sample which indicates all samples contain a minimum of 81.9%, 80.3%, and 81.7% of fermentable sugars in the control, 0.5% NaOH at 50% power for 2 min and 0.5% H2SO4 at 100% power for 1 min, respectively. The theoretical yield expected in this experiment was 134 g/L of ethanol whereas only 14.4 g/L was obtained, indicating the presence of inhibitory compound present in the fermentation media.

4.4 Conclusions:

The emerging microwave-assisted chemical pre-treatment technology is more efficient, rapid, and feasible for the production of reducing sugars from pineapple waste. In the present study different microwave heating powers (50% and 100%) respectively and different irradiation times (1 min and 2 min) have been studied along with the different concentrations (0.5% and 1.0%) of acid (H_2SO_4) and alkali (NaOH). Low microwave power and short exposure time (50% for 1 min) does not modify the sugar concentration significantly while; higher power (100%) and extending heating to 2 minutes caused the hydrothermal degradation of PW samples due to excessive heat which resulted in a lower concentration of reducing sugars. Infra-red thermography and SEM analysis also indicated the possible hydrothermal degradation of sugars resulted in a lower concentration of fermentable sugars at higher irradiation power and time.

The infrared thermography of the PW showed that an increase in irradiation time and microwave power results in higher temperature and even spread out of the heat, while shorter exposer time and lower power results in low temperature and uneven heating effects. The presence of fermentation inhibitors like phenolic compounds as a result of solubilization of lignin content of PW and formation of organic acids and furfural due to hydrothermal effects of degradation of sugars resulted in a lower amount of production of ethanol.

It can be concluded from this study that microwave pre-treatment using appropriate irradiation time and power enhanced the saccharification and that microwave-assisted chemical pretreatments could be used to improve the bioethanol yield of pineapple waste.

Declaration of competing interest.

The authors declare no conflict of interest.

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4.5 References:

Adebo, OA & Gabriela Medina-Meza, I 2020, 'Impact of Fermentation on the Phenolic Compounds and Antioxidant Activity of Whole Cereal Grains: A Mini Review', *Molecules (Basel, Switzerland)*, vol. 25, no. 4, p. 927.

Australian Horticulture Statistics Handbook 2017/18 2019, file:///C:/Users/u1092840/Downloads/AH15001-Australian-Horticulture-Statistics-Handbook-Fruit-190402.pdf >.

Balat, M 2011, 'Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review', *Energy Conversion and Management*, vol. 52, no. 2, pp. 858-75.

Banerjee, S, Ranganathan, V, Patti, A & Arora, A 2018, 'Valorisation of pineapple wastes for food and therapeutic applications', *Trends in Food Science & Technology*, vol. 82, pp. 60-70.

Beatriz CordenunsiI, FS-CMED-RAZMATMSBGBdSCCEBGE 2010, 'Carbohydrate composition of ripe pineapple (cv. perola)and the glycemic response in humans ', *Food Science and Technology*, vol. 30, no. 1, pp. 282-8.

Binod, PS, K.; Sindhu, R.; Janu, K.U.; Sukumaran, R.K.; and Pandey, A. 2012, 'Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse.', *Journal of Renewable Energy*, vol. 37, no. 1, pp. 109-16.

Birla, SL & Pitchai, K 2017, '18 - Simulation of microwave processes', in M Regier, et al. (eds), *The Microwave Processing of Foods (Second Edition)*, Woodhead Publishing, pp. 407-31.

Cantarella, M, Cantarella, L, Gallifuoco, A, Spera, A & Alfani, F 2004, 'Effect of inhibitors released during steam-explosion treatment of poplar wood on subsequent enzymatic hydrolysis and SSF', *Biotechnol Prog*, vol. 20, no. 1, pp. 200-6.

Conesa, C, Seguí, L, Laguarda-Miró, N & Fito, P 2016, 'Microwaves as a pretreatment for enhancing enzymatic hydrolysis of pineapple industrial waste for bioethanol production', *Food and Bioproducts Processing*, vol. 100, pp. 203-13.

Conesa, C, Seguí, L, Laguarda-Miró, N & Fito, P 2016, 'Microwave-assisted alkali pretreatment for enhancing pineapple waste saccharification', *Bioresources*, vol. 11, no. 3, pp. 6518-31.

de la Hoz, A, Díaz-Ortiz, Á & Moreno, A 2005, 'Microwaves in organic synthesis. Thermal and non-thermal microwave effects', *Chemical Society Reviews*, vol. 34, no. 2, pp. 164-78.

Dowe, N 2009, 'Assessing cellulase performance on pretreated lignocellulosic biomass using saccharification and fermentation-based protocols', *Methods Mol Biol*, vol. 581, pp. 233-45.

Fang, X, Qin, Y, Li, X, Wang, L, Wang, T, Zhu, M & Qu, Y 2010, '[Progress on cellulase and enzymatic hydrolysis of lignocellulosic biomass]', *Sheng Wu Gong Cheng Xue Bao*, vol. 26, no. 7, pp. 864-9.

Hong Zhang, RY, Yunze He, Ali Foudazi, Liang Cheng and Guiyun Tian 2017, 'A Review of Microwave Thermography Nondestructive Testing and Evaluation', *Sensors* vol. 17, pp. 1-33.

Hongjie Dai, SO, Yue Huang, Huihua Huang 2018, 'Utilization of pineapple peel for production of nanocellulose and film application', *Cellulose*, vol. 25, pp. 1743-56.

Hossain, MA & Rahman, SMM 2011, 'Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple', *Food Research International*, vol. 44, no. 3, pp. 672-6.

Hu, Z & Wen, Z 2008, 'Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment', *Biochemical Engineering Journal*, vol. 38, pp. 369-78.

Jackowiak, D, Bassard, D, Pauss, A & Ribeiro, T 2011, 'Optimisation of a microwave pretreatment of wheat straw for methane production', *Bioresour Technol*, vol. 102, no. 12, pp. 6750-6.

Jin, S, Zhang, G, Zhang, P, Li, F, Wang, S, Fan, S & Zhou, S 2016, 'Microwave assisted alkaline pretreatment to enhance enzymatic saccharification of catalpa sawdust', *Bioresource Technology*, vol. 221, pp. 26-30.

Kiran R Mahale, APT 2020, 'Effects of alkali, acidic and enzymatic pre-treatments on the ethanol fermentation of pineapple (Ananas comosus) processing waste. ', Originol reserach University of Southern Queensland

Klinke, HB, Thomsen, AB & Ahring, BK 2004, 'Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass', *Appl Microbiol Biotechnol*, vol. 66, no. 1, pp. 10-26.

Kumar, C, Joardder, M., Karim, A., Millar, G.J., Amin, Z., 2014, 'Temperature redistribution modelling during intermittentmicrowave connective heating', *Procedia Eng*, vol. 90, pp. 544-9.

Li, T, Shen, P, Liu, W, Liu, C, Liang, R, Yan, N & Chen, J 2014, 'Major Polyphenolics in Pineapple Peels and their Antioxidant Interactions', *International Journal of Food Properties*, vol. 17, no. 8, pp. 1805-17.

López-Linares, JC, García-Cubero, MT, Coca, M & Lucas, S 2021, 'Efficient biobutanol production by acetone-butanol-ethanol fermentation from spent coffee grounds with microwave assisted dilute sulfuric acid pretreatment', *Bioresource Technology*, vol. 320, p. 124348.

Mikulski, D & Kłosowski, G 2020, 'Microwave-assisted dilute acid pretreatment in bioethanol production from wheat and rye stillages', *Biomass and Bioenergy*, vol. 136, p. 105528.

Mikulski, D, Kłosowski, G, Menka, A & Koim-Puchowska, B 2019, 'Microwave-assisted pretreatment of maize distillery stillage with the use of dilute sulfuric acid in the production of cellulosic ethanol', *Bioresource Technology*, vol. 278, pp. 318-28.

MILLER, GL 1959, 'Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar', *Analytical Chemistry*, vol. 31, no. 3, pp. 426-8.

Nowicka, A, Zieliński, M & Dębowski, M 2020, 'Microwave support of the alcoholic fermentation process of cyanobacteria Arthrospira platensis', *Environmental Science and Pollution Research*, vol. 27, no. 1, pp. 118-24.

Puligundla, P, Oh, S-E & Mok, C 2016, 'Microwave-assisted pretreatment technologies for the conversion of lignocellulosic biomass to sugars and ethanol: a review', *Carbon letters*, vol. 17, no. 1, pp. 1-10.

Rajeswari, G, Arutselvy, B & Jacob, S 2020, 'Delignification of Aloe Vera Rind by Mild Acid Associated Microwave Pretreatment to Persuade Enhanced Enzymatic Saccharification', *Waste and Biomass Valorization*, vol. 11, no. 11, pp. 5965-75.

Ricardo Soccol, C, Faraco, V, Karp, S, Vandenberghe, LPS, Thomaz-Soccol, V, Woiciechowski, A & Pandey, A 2011, 'Chapter 5 - Lignocellulosic Bioethanol: Current Status and Future Perspectives', in A Pandey, et al. (eds), *Biofuels*, Academic Press, Amsterdam, pp. 101-22.

Robak, K & Balcerek, M 2020, 'Current state-of-the-art in ethanol production from lignocellulosic feedstocks', *Microbiological Research*, vol. 240.

Roberts B A, SCR 2005, 'Scale-up of Microwave-Assisted Organic Synthesis.', in LP Tierney J P (ed.), *Microwave Assisted Organic Synthesis*, Oxford, Blackwell, pp. 237-48.

Ruangviriyachai, C, Niwaswong, C, Kosaikanon, N, Chanthai, S & Chaimart, P 2010, 'Pineapple Peel Waste for Bioethanol Production', *Journal of Biotechnology*, vol. 150, pp. 10-.

Samanian N, MM 2016, 'Thermography, a new approach in food science studies: a review', *MOJ Food Process Technol*, vol. 2, no. 3, pp. 110-9.

Sarkar, N, Ghosh, SK, Bannerjee, S & Aikat, K 2012, 'Bioethanol production from agricultural wastes: an overview', *Renewable Energy*, vol. 37, no. 1, pp. 19-27.

Singh, R, Tiwari, S, Srivastava, M & Shukla, A 2014, 'Microwave Assisted Alkali Pretreatment of Rice Straw for Enhancing Enzymatic Digestibility', *Journal of Energy*, vol. 2014, p. 483813.

Stelmock, RL, Husby, FM & Brundage, AL 1985, 'Application of Van Soest Acid Detergent Fiber Method for Analysis of Shellfish Chitin1', *Journal of Dairy Science*, vol. 68, no. 6, pp. 1502-6.

Yu, KL, Chen, WH, Sheen, HK, Chang, JS, Lin, CS, Ong, HC, Show, PL & Ling, TC 2020, 'Bioethanol production from acid pretreated microalgal hydrolysate using microwave-assisted heating wet torrefaction', *Fuel*, vol. 279.

Zhu, S, Wu, Y, Yu, Z, Zhang, X, Li, H & Gao, M 2006, 'The effect of microwave irradiation on enzymatic hydrolysis of rice straw', *Bioresource Technology*, vol. 97, no. 15, pp. 1964-8.

Zhu, S, Wu, Y, Yu, Z, Zhang, X, Wang, C, Yu, F & Jin, S 2006, 'Production of ethanol from microwave-assisted alkali pretreated wheat straw', *Process Biochemistry*, vol. 41, no. 4, pp. 869-73.

CHAPTER 5: Effect of pyrolysis condition on pineapple (Ananas comosus) waste-derived biochar and its ability for arsenic removal.

Kiran R. Mahale[a], Mrak Lynch[b], Les Bowtell[c*].

Research Paper- III

Introduction

This manuscript explores the different aspects of the utilization of pineapple processing waste for the removal of arsenic from surface water. The manuscript presents the characteristics of pineapple biochar samples produced in a temperature range of 350-900 °C with a holding time of 2 h with and without a zeolite powder coating. A range of characterization methods was used to examine the effects of altering pyrolysis temperature on the surface chemistry and the physical characteristics of the samples. Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction, and scanning electron microscopy were carried out on samples. Different experimental conditions were examined for each of the inorganics As(III) and As(V) species by varying the initial concentration and contact time. The mechanisms of removal were examined based on the results of the SEM-EDS and FTIR analyses, where the composition of the As-loaded char was compared to the unloaded char, to detect structural changes.

Type of the Paper: Research Article

CHAPTER 5: Effect of pyrolysis condition on pineapple (Ananas comosus) waste-derived biochar and its ability for arsenic removal.

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Abstract:

Australia produces over 76 thousand tonnes of pineapple every year. Around 40-55% of total fruit weight goes to waste in the form of a crown, outer peel, core, and bottom slice. Pineapple waste then becomes a serious environmental issue to processing plants. This study aims to examine the effect of different pyrolysis temperatures and zeolite powder coating to achieve the highest sorption of arsenic from surface water. The dried pineapple peel was then pyrolyzed at 350 °C, 750 °C, and 900 °C at a holding time of 2 h in a limited oxygen environment with and without zeolite powder coating. A batch adsorption experiment was conducted to analyze the sorption effect which showed zeolite coating did not have any significant increase ($P > 0.05$) in arsenic adsorption, unlike fluoride adsorption. While increasing temperature dropped the char yield from 34.35% to 24.74%. A maximum of 83.54% of arsenic was absorbed by biochar made at 900°C without zeolite coating. Pineapple Biochar can provide a viable value-added waste stream that reduces landfill, and has a positive impact on water quality by adsorbing the heavy metals commonly found in surface and bore water in Australia.

Keywords: Pineapple, Biochar, Arsenic, Zeolite, Pyrolysis conditions,

5.1 *Introduction*

Contamination of groundwater, either from anthropogenic or natural sources has become a major environmental concern in different parts of the world. Over 200 million people from 105 countries and territories are at risk of potentially exposed to a high concentration of arsenic in groundwater from their domestic water supply [201]. Approximately 94% of the world's affected population belongs to the Asian continent [202]. Figure 1 shows the Global prediction of groundwater arsenic showing almost every country in the world has arsenic present in groundwater. The major arsenicosis-affected areas are along river basins across the world [203]. Arsenic is commonly found throughout Australian states and Territories [204]. The main source of As in the Australian environment is either natural or anthropogenic processes [204]. Arsenic can be easily dissolved in groundwater depending on pH, temperature, and redox conditions [205]. The solubility of arsenic decreases with the increase of pH value. Arsenic enters into the food chain through water [206, 207]. The guidelines from WHO has recommended a limit of arsenic in drinking water of 10 µg/L of arsenic [208]. Inorganic Arsenic (As) is the dominant form of As in groundwater represented by arsenite [As(III)] and arsenate [As(V)] [209]. Under reducing conditions, As(III) which is the most toxic and mobile phase of As is the dominant inorganic As species in groundwater [210].

Since water is the principal route through which As enters the food chain hence the human body [211]. Therefore removal of arsenic from ground and surface water is very important and tedious work. Many researchers have attempted removal of As using different methods such as nano-filtration, lime softening, coagulation/flocculation, electrochemical techniques, chemical precipitation, ion exchange, and membrane separation, and many others methods [212]. Australia also has a wide variety of feedstock available in all seasons. Agricultural waste is mainly composed of lignin, cellulose, hemicellulose, and other trace elements like lipids, proteins, hydrocarbon, and others [213]. This available feedstock can be used in the removal of As from ground and surface water. A thermal breakdown of feedstock biomass using pyrolysis technology in the presence of limited or no oxygen [212]. Many researchers have used biochar (activated carbon) for the removal of As including Wan et al (2021); [214]; magnetic biochar by Wang et al (2021) [215]; chitosan by Gerente et al 2010 [216]; metal oxides coated on the sand [217], Agricultural waste [218] biochar by Xue et al 2021, Mukherjee et al 2021 [219, 220].

Where the efficacy of different processes is being examined by the researchers in laboratory experiments and the field. Different factors determine the choice of proper arsenic treatments, including restrictions for soil and water treatment technologies, local and national authorities' requirements, a country's development stage, and local strategies for arsenic levels trendy plants and water system. Along these lines, there are various advances all over the world. The present study investigates the adsorption of As (III) on biochar derived from Pineapple processing waste. Where the biochar was made at different pyrolysis conditions at different temperature ranges from 350 °C to 900 °C for a holding time of 2 h. The pineapple-derived biochar was then divided into two groups for each temperature and been coated with zeolite powder to determine the efficacy of the zeolite powder on adsorption of As (III) on biochar from surface water.

The utilization of industrial food processing waste material for arsenic removal is of great importance to improve sustainability because of its cost-effectiveness, easy availability and it also helps industry in the waste disposal and reduces the carbon footprint. To the best of our knowledge, no research paper has discussed the potential role of pineapple peel-derived biochar and zeolite treated pineapple peel biochar for the removal of As from ground and surface water.

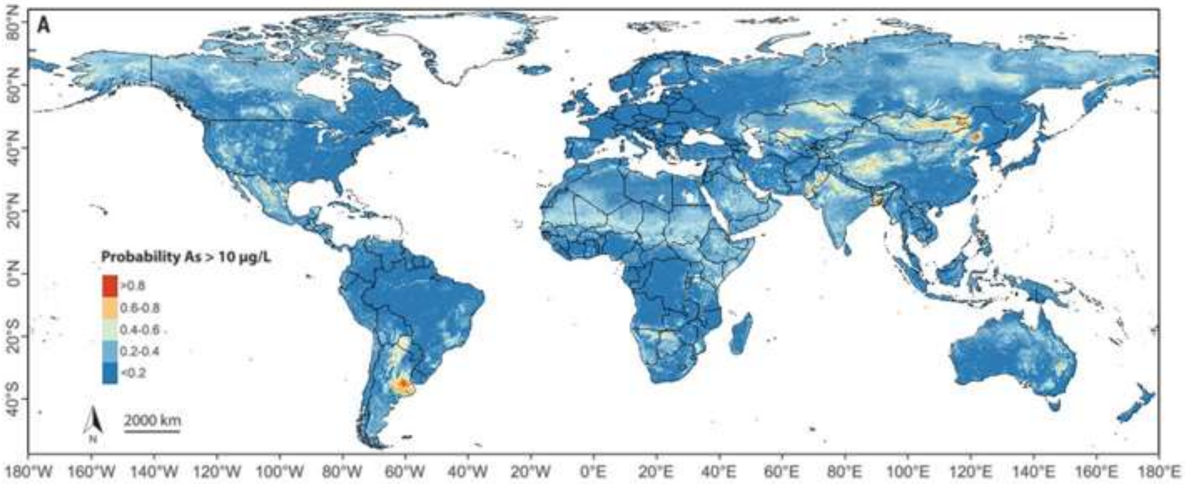


Figure 5.1. Global Prediction of groundwater arsenic (adopted from Podgorski& Berg et al 2020. [202])

5.2 *Materials and Methods*

5.2.1 *Biochar Preparation*

The feedstock used in this experiment is pineapple processing waste (PPW), which was collected from a local pineapple processing factory in Sandgate, Brisbane, Queensland Australia. The collected PPW was then cut into 2-3 cm segments and dried in oven at 60 °C, for 24 h. dried samples were then stored in air-tight bags to avoids further contamination. Frozen PPW was then used for feedstock characterization of treated and untreated PPW samples according to the acid detergent fiber, neutral detergent fiber, and acid detergent lignin (ADF-NDF-ADL) method by Goering and Van Soest method 1970 [147]. Hemicellulose and cellulose contents were calculated using formulas as below.

$$\text{Hemicellulose (\%)} = \text{NDF (\%)} - \text{ADF (\%)}$$

$$\text{Cellulose (\%)} = \text{NDF (\%)} - \text{Hemicellulose (\%)} - \text{Lignin (\%)}$$

Dried PPW samples were pyrolyzed in a limited oxygen environment using a Rio Grande brand kiln (model CS2 from USA). The pyrolysis process used a temperature ramp-up rate of 10 °C per min to three different temperature setpoints of 350 °C, 750 °C, and 900 °C at holding time 2 h. The holding time was applied to reduce the concentration of total organic matter released as reported by [221]. The produced biochar samples were crushed and sieved to an average particle size of 1-2 mm, then washed with deionized water repeatedly until a stable low electrical conductivity (EC) was reached indicating all mineralized ash was removed, before being sealed and stored for later use.

5.2.2 *Biochar modification*

Biochars exhibits good sorption properties; however, it can be additionally altered to improve its sorption efficiency. In the present study, the feedstock was pretreated with Zeolite powder to determine the efficacy of altered biochar for its sorption efficacy. The feedstock was sprinkled with water and then zeolite powder was added to the bin along with feedstock. Mixed properly and then pyrolyzed using the same condition described earlier in section 5.2.1. The zeolite used in this study was kindly provided by Zeolite industry Australia.

5.2.3 *FTIR Spectroscopy*

The FTIR spectroscopy has been used to examine the structural properties and characterization of functional groups on biochar and other sorbents/minerals surface. It helps to differentiate the functional groups of feedstock material and derived biochar, as well as determine changes in functional groups before and after As sorption. FTIR spectra were recorded between wavenumber of 4000cm-1 and 400cm-1 with an IRAffinity-1S FTIR spectrometer supplied by Shimadzu, at a basic resolution of 0.5 cm-1. Before FTIR analyses, all biochar samples were oven-dried overnight at 60 °C.

5.2.4 *Surface analysis by scanning electron microscope*

The morphology of the biochar particles was tested using a scanning electron microscope (SEM). All samples were fixed on tape and coated with gold-palladium particles to obtain clear images. The SEM images were visualized at 2000× magnification, 50-μm diameter, and 5 kV using an Apera S SEM (SEM (JEOL, model JSM-6000 plus).

5.2.5 *X-ray Diffractometer (XRD) and Raman Spectrometry*

XRD has been used to analyze the crystalline forms of powdered materials, by X-ray diffractometer with a Pixel detector using (XRD, Rigaku Smart Lab diffractometer) and Raman (Renishaw inVia Raman microscope) have been used to analyze the crystal structure of different types of carbon-containing compounds Crystallite sizes of the treated chars were determined from line broadening of the apatite (002) peak using the Scherrer calculator from Panalytical High Score Plus and a high purity crystalline apatite as a standard.

5.2.6 *Surface pH and EC*

Surface pH and EC were measured following the procedure reported in [222]. Approximately 0.2 g of each biochar sample was individually added to 100 mL of deionized water (pH adjusted to 7.0) and was then shaken on a Ratek rotary shaker for 30 min at 120 rpm. The pH and EC of the solutions were measured using Orion Star Bentop meters models A111 and A212, respectively.

5.2.7 *Proximate analysis*

Proximate analysis of the biochar samples, including moisture and ash content, volatile matter, and fixed carbon ratio was conducted following the procedure reported in Goering and van Soest et al. and [223] and [224]. One gram of each sample was placed in the OHAUS MB120 Moisture Balance at 105 °C to determine the moisture content. The dried samples were utilized to determine the volatile matter by calculating weight difference before and after heating samples in covered crucibles, using a programmable muffle furnace at 950 °C for 11 min. The same procedure was applied to determine the ash content; however, the furnace temperature was set at a lower temperature of 750 °C and the sample crucibles were not covered.

5.2.8 *Adsorption experiments*

The impact of pyrolysis temperature in the range of 350 °C to 900 °C on As(III) and As(V) adsorption capacity of the pineapple biochar was examined using synthetic samples prepared from high purity salts and stock samples with double distilled water. The removal capacity was calculated using the following formula:

$$R = C_0 - C_f / w * v \quad \text{--------} \quad (i)$$

Where R is the removal in mg/g, C0 is the initial concentration in mg/L, Cf is the final concentration in mg/L, v is the volume of the solution in L and w is the weight of the adsorbent in g.

5.2.9 *Batch adsorption experiment*

The adsorption experiment was carried out in 125 mL Erlenmeyer flasks. The initial concentration of Sodium Arsenate in the solution was 100ppm. 50 mL of the 100ppm As(V) solution and initial pH 7.0 was added to Erlenmeyer flasks. 0.5 g of biochar was then added to each flask respectively. Each flask was then sealed with aluminum foil and then shaken at 100 rpm at 25 °C in a shaker incubator (Innova series 42 Incubator shaker). The 15 mL samples were removed at a designated time (1, 5, 10, 20, 40, 60) minutes. 15 mL of fresh Sodium Arsenate 100 ppm solution was added to each flask after each sample was taken. Collected samples were then stored at -20 °C till further analysis using Atomic Absorption Spectroscopy (AAS).

5.3 Results and Discussion

5.3.1 Biochar modification

The prepared biochar from pineapple waste with and without zeolite has shown a significant difference in the yield of biochar (more than 1% in each temperature) and had slightly lowered the pH ($P < 0.5$) of the prepared biochar. The morphological changes in the biochar are visible in Figure 5.3.6.

5.3.2 Physicochemical properties of biochar

The prepared biochar from pineapple peel and modified biochar were analyzed for selected physicochemical properties as listed in table 5.1. The biochar yield decrease from 34.35% to 23.19 % as an increase in pyrolysis temperature from 350 °C to 900 °C. Decrease in yield of biochar due to decomposition of lignin and degradation of cellulose and hemicellulose content in pineapple peel due to increase in temperature [225]. An increase in temperature also resulted in an increase in ash content from 10.44% to 15.03% this is mainly due to organic matter loss from the residue and ash formed from mineral matter after carbonization [226]. The pH of the pineapple peel is highly acidic at 3.7 and increases in temperature resulted in the separation of ash content and formation of alkali salts from organic materials [226] which resulted in a significant increase in pH of all biochar. Kloss et al in 2012 reported an increase in temperature resulted in increases in “C” content and decreases in “H” and “O” content same trend was followed in this study this is due to dehydration and decarboxylation of cellulose and hemicellulose content present in a material during high temperature [227, 228]. Whereas there is no distinct trend for the effect of pyrolysis temperature on N content. With the escalation in a pyrolysis temperature, the C content increased whereas O content decreased which resulted in a reduction in the polarity of the samples this alines with the finding reported by Ahmad et al in 2012 [229].

Table 5.1. Characteristics of Pineapple peel and pyrolysis biochar at different conditions

Properties	Pineapple Peel	PB 350		PB 750		PB 900	
		Zeolite	No Zeolite	Zeolite	No Zeolite	Zeolite	No Zeolite
Yield (%)	00.00	34.35	33.28	28.74	27.42	24.74	23.19
Ash (%)	4.21 (± 0.6)	10.44	10.26	13.29	12.94	15.23	15.03
pH	3.7 (± 0.2)	9.2	9.3	9.6	9.7	10.5	10.8
C (%)	42.08	71.46	72.33	74.28	75.12	77.14	77.86
O (%)	47.23	13.04	13.12	11.13	11.15	9.34	9.38
N (%)	0.86	1.18	1.23	1.19	1.11	0.92	0.98
Cellulose	21.1%±0.34 (w/w)	Data Not available	Data Not available	Data Not available	Data Not available	Data Not available	Data Not available
Hemicellulose	23.9% ± 0.29 (w/w)	Data Not available	Data Not available	Data Not available	Data Not available	Data Not available	Data Not available
Lignin	5.5% ± 0.33 (w/w)	Data Not available	Data Not available	Data Not available	Data Not available	Data Not available	Data Not available

5.3.3 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy spectra were used to analyze the structural changes of biochar samples produced under different pyrolysis conditions. Deconvolution was used to determine the number and the position of the overlapped bands. The second spectral derivatives were used to provide a reasonable estimate of overlapped bands in a selected region *[230]*. FTIR spectra of biochar with different holding temperatures without zeolite powder PB350, PB750, and PB900 and zeolite coated biochar PBZ350, PBZ750, and PBZ900 are exhibited in figure 5.2.

Results revealed the influence of pyrolysis temperature on the surface chemistry of biochar. Spectra of all biochar samples provided evidence of hydrogen bonds, as carboxylic acid OH stretches at around 3400 cm-^{1} and OH at 960 cm-^{1}. Carboxylic groups are composed of C=O which can be detected around 1749- 1728 cm-^{1}. The broad peak around 3666 cm^{-1} observed due to the stretching vibration of the O-H groups and shows the presence of alcohol and phenols. The spectral peak, almost small at 2357 and 2333 cm^{-1} only at PB750 and PB900 could be due to asymmetric C-H stretching band which below to alkyl surface functional group such as methylene, methyl, and methoxy of biochar. The C=O stretching absorbance band observed at 1670 cm-1 on PB350 biochar represents non-ionic carboxyl surface functional moieties (-C=O, -COOH, and -COOCH3) which may associate with the carboxylic acid, due to high pyrolysis temperature for PC 750 and PB 900 spectral band almost disappeared. The bands at 1585- 1720cm–^{1} were associated with the presence of H2O and amide I. Increasing pyrolysis temperature led to a broader peak indicating the degradation of the amide compounds when temperature increased to 900 C. The effect of raising the pyrolysis temperature on the biochar structure could also be noticed in the formation of a new sharp peak at around 2013cm_1 (Fig. 5.2). This is likely to be due to the degradation of the protein available in the feedstock. And the formation of isocyanate (-NCO), thiocyanate(SCN_), and isothiocyanate groups (-N=C=S) [230]. The growing peak at 610-700 cm-1 with rising temperature suggests the presence of the (C=C-H) band (broad strong absorption) [231]. PB 350 peak Spectral band at 1556 cm-^{1} and PB 750 had medium stretching vibration at band 1556 cm-^{1} could be due to C=C functional ground stretching of alkene compound cases, PB 900 peaks spectral band was almost disappeared at high pyrolysis temperature. With increased temperature, the distinctive peaks such as O-H (3400 cm-^{1}), C-H (2920 cm-^{1}), and C=O (1650 cm-^{1}) are disappeared indicating the breakdown of cellulose and lignin, as well as the removal of hydrogen and oxygen in the carbonization, process [232].

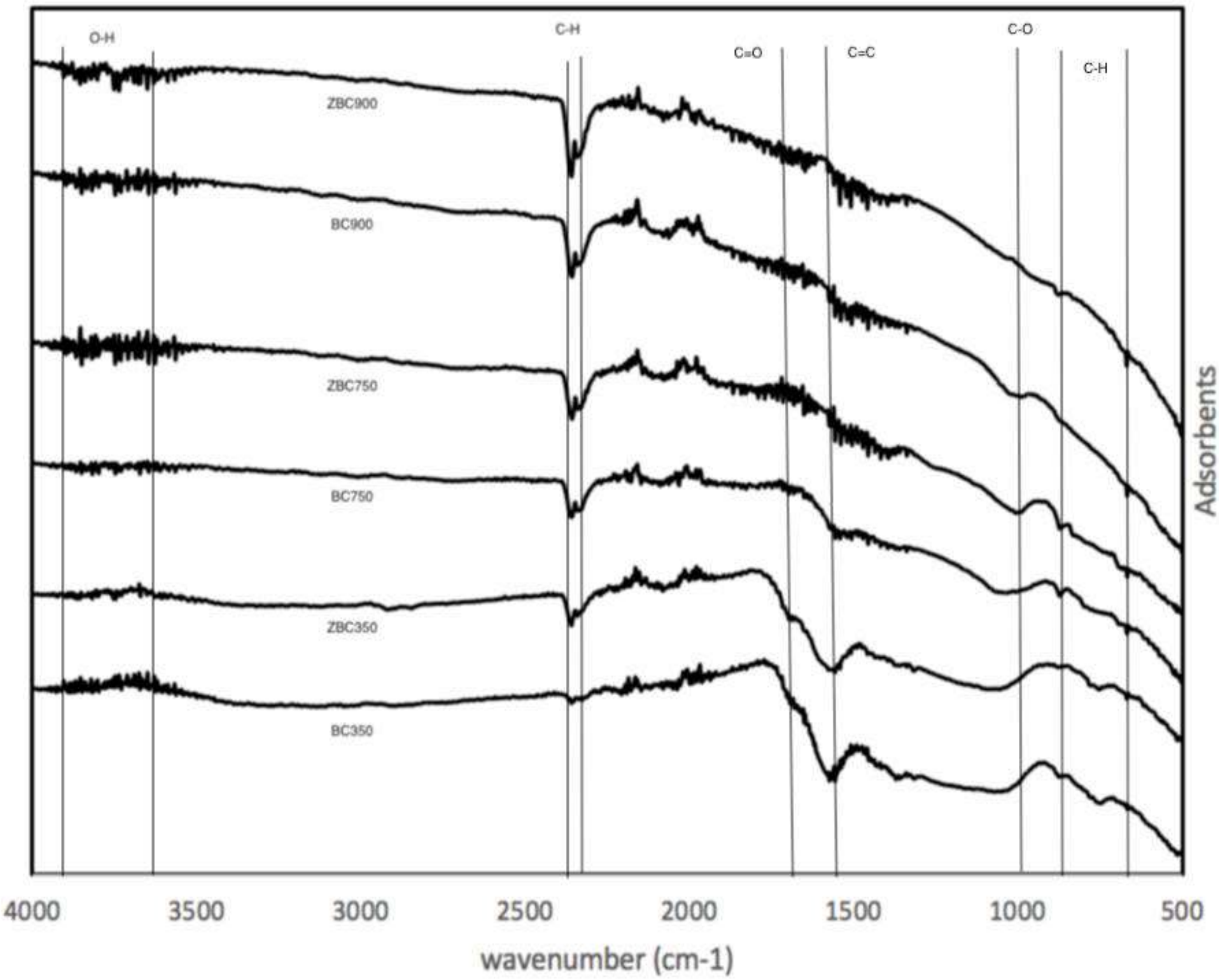
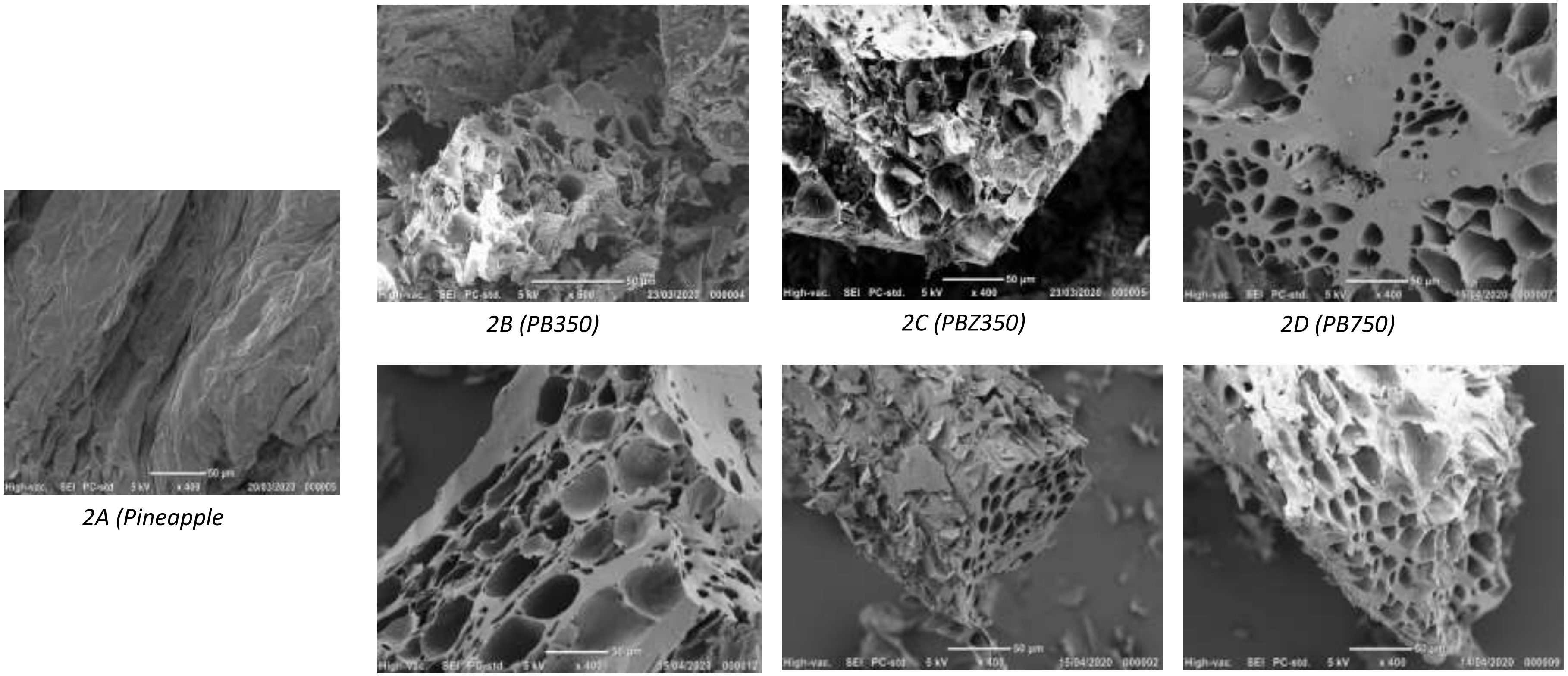


Figure 5.2. FTIR Absorbance spectra of Pineapple biochar

5.3.4 Scanning Electron Microscopy (SEM)

SEM analysis was employed to observe the surface morphology of samples. As shown in figure 5.2 There are obvious differences in the surface morphology among all the biochars with different pyrolysis conditions. The pineapple waste has a smooth surface and irregular structure as shown in (figure 2A). The biochar prepared at a lower temperature PB350 and PBZ350 (figure 2B and 2C) show the irregular structure and relatively fewer spores. This might be related to insufficient pyrolysis. When pyrolysis temperature reached 750 °C, the structure of the biochar shows loos, rough, and porous structure (Figure 2D and 2E). This also indicates the pattern reported by Fu et. al in 2016 where increased temperature, resulted in increase roughness and a more porous structure of biochar, which increased the surface area of the biochars *[233]*. Whereas the highest temperature of pyrolysis 900 °C showed (figure 2F and 2G) a more porous and rough surface. Similarly, the samples prepared at a higher temperature of 750 °C and 900 °C adsorbed the highest amount of Arsenic As (III).



2A (Pineapple

2B (PB350)

2C (PBZ350)

2D (PB750)

2E (PBZ750)

2F (PB900)

2G (PBZ900)

Figure. *5.3*. SEM images of Pineapple biochar with and without zeolite coating at different temperatures.

5.3.5 Raman Spectrometry

Raman Spectrum, which is sensitive to the structural characteristics of nano carbons, was used to analyze the carbon state within the biochar samples as shown in figure 5.4. Two characteristic bands located around 1330- 1370 cm^{-1} (D- band) and 1580-1620 cm^{-1} (G-band) were observed from the spectrum. The band at 1330- 1370 cm^{-1} (D- band) corresponds to the amorphous carbon, a reactive carbon that does not have any crystalline structure and may be stabilized dangling-π bond with hydrogen [234].

The band at 1580-1620 cm^{-1} (G-band) corresponds to a honeycomb-shaped carbon network formed by sp^2–hybridized C [235]. Both D and G bands represent the vibration mode of the aromatic ring carbon system (aromatic ≥ 6 rings) and E2g vibration mode of the sp2 bonded graphitic carbon structure [236, 237]. Similar results were reported by Kamonwan et al in 2020 in their recent studies where primary biomass constituents (cellulose, xylan, and lignin) began to form the aromatic rings at pyrolysis temperature 400 °C. Whereas in the present study PB350 and PBZ350 also showed the formation of aromatic carbon ring at pyrolysis temperature 350 °C whereas the Raman intensity is significantly lower than all other samples where pyrolysis temperature is well above 400 °C [238]. The Raman intensity of the zeolite-coated samples has significantly dropped in all three samples. The overlapped peak at 2750.3 - 2904.2 cm-1 might relate to the contribution of amorphous carbon structure in the product prepared by the pyrolysis methods.

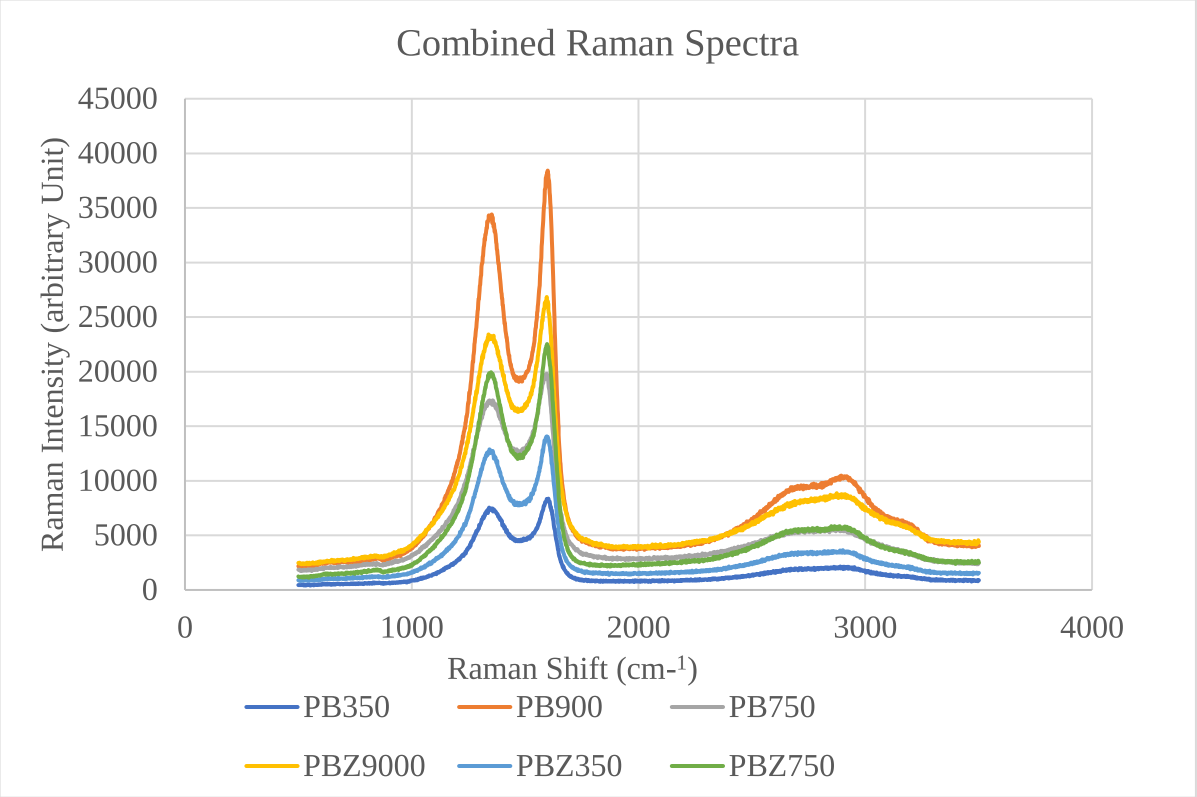


Fig. 5.4 Raman Spectra of pineapple peel.

5.3.6 X-ray diffractometer (XRD) spectral analysis

XRD was employed to analyze the crystalline forms of powdered biochar to get information about mineral phases in biochars samples *[212]*. patterns of PB and PBZ showed a predominant crystalline phase (figure 5.5) exhibit the typical hydroxyapatite structure, indicated by the appearance of its typical indices *[239]*. It was noticed in figure 5.3 as the temperature increases the height and width of the peaks decrease. This is due to the increase in the crystallinity of samples with an increase in pyrolysis temperature from 350 °C to 900 °C these results are aligned with previous studies published by Patel et al and Person et al in 2015 and 1995 respectively *[240, 241]*. The crystallite size of different pyrolysis conditions was measured as per the method described by Kang et al in 2018 and shown in figure 4 *[242]*. From figure 4 it can be easily noticed that crystallite size is increased as the increase in pyrolysis temperature whereas zeolite powder coated samples showed a slight increase than one without zeolite coating. The increase in crystallinity of biochar corresponds with a decrease in the carbon content of biochar which also similitude to data presented in table 5.1

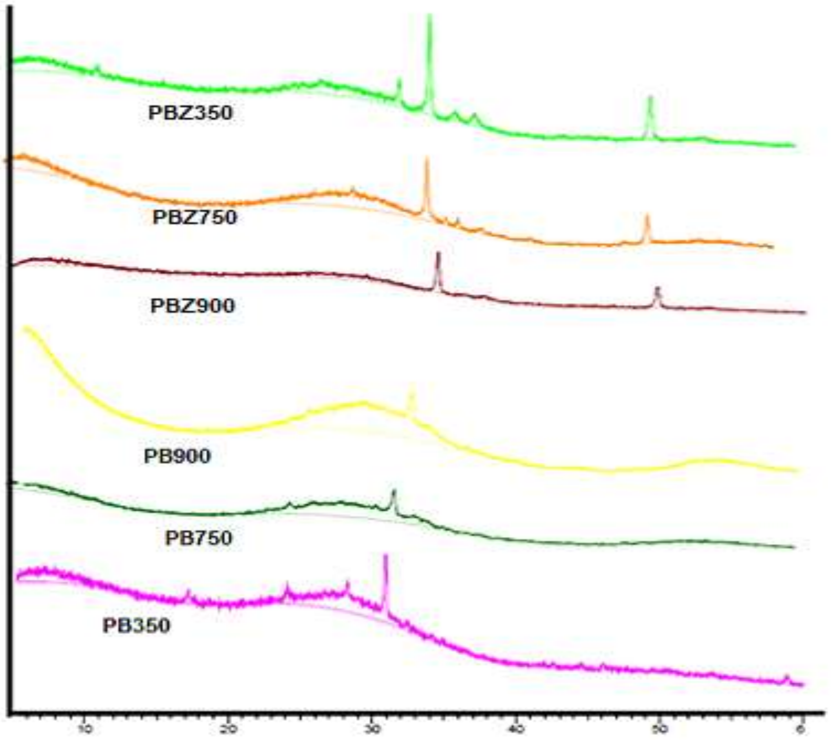


Figure 5.4. Effect of pyrolysis conditions on biochar crystallinity measured by XRD.

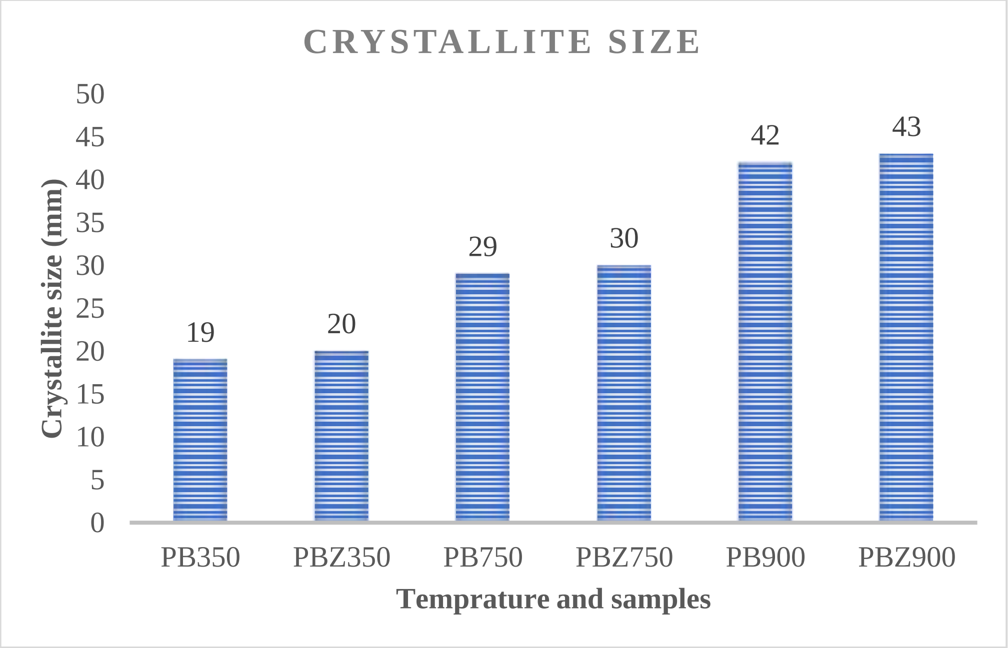


Figure 5.5. Crystallite size for bone char at different pyrolysis conditions

5.3.7 Application of Biochar as an adsorbent

To examine the effect of pyrolysis condition on biochar and its ability to absorb As (III) preliminary experiment batch absorption experiment was conducted keeping other experimental parameters like pH, the concentration of As(III) and biochar dose were kept constant. The arsenic adsorption capacity of the biochar samples at different pyrolysis conditions are presented in Figure 5.6 The results of the experiments show that the pyrolysis temperature and residence time have a substantial effect on As adsorption (P-value < 0.01). The optimum time for arsenic removal is determined by the graph by arsenic removal against time as shown in Figure 5.6 Biochar prepared at 350 °C with and without zeolite coating has shown very low removal capacity for As (III) (64.15%). whereas the biochar made at 900 °C showed the greatest removal capacity compared to other temperatures ie. 750 °C (83.16% and 71.25%) respectively.

There was a slight decrease observed in the removal capacity of As (III) in the biochar coated with zeolite powder as can be seen in figure 5.6. A significant difference in adsorption of As (III) is observed at 20 minutes in all samples. Where samples coated with zeolite powder shown significantly lower adsorption compared to biochar without zeolite powder. Increasing the temperature of pyrolysis increased the absorption of As (III) this is possibly due to the effect of pyrolysis temperature [243, 244] on the surface functional group as a described earlier section. Alkuradi et al in 2019 reported the mechanisms for removal of As (III) in biochar is surface complexation, precipitation, or ligand exchange [245]. The main factor that was found to affect removal capacity was charring temperature and holding time. At high charring pyrolysis temperatures, a higher pH, ash content, CEC, and negative surface charge were observed. The effect on acidic functional groups was the opposite, while the basic functional groups were not affected significantly by pyrolysis temperature.

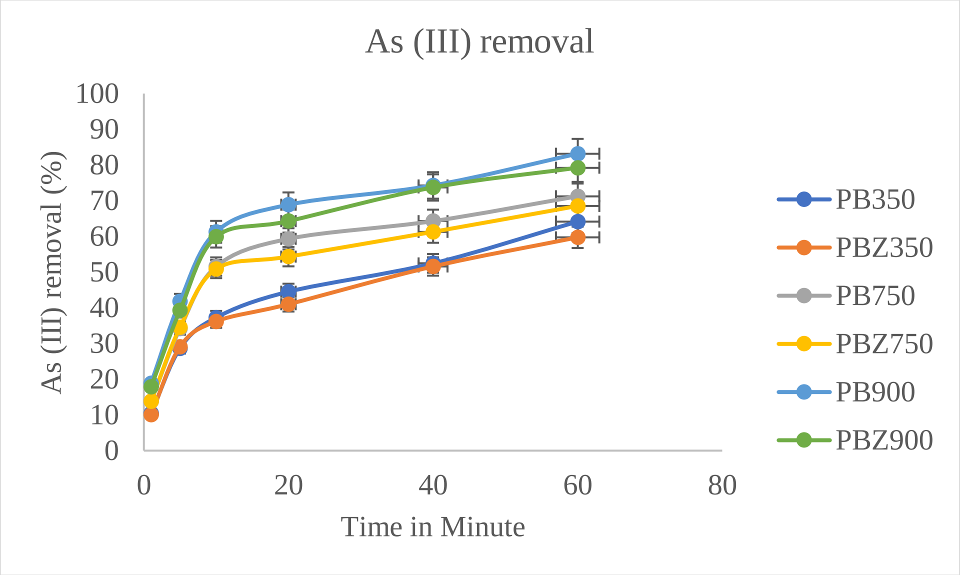


Fig. 5.6 Percentage Arsenic As (III) removal

5.3.8 Environmental implications

Biochar can be the best alternative as an adsorbent of organic impurities from surface water compared to activated carbon. lesser energy requirements and no pre-oriented requirements of activation of char are the advantages of biochar over activated carbon. Transforming the easily available and abundance of waste biomass by pyrolysis into biochar provides a beneficial way to reuse the materials and helps to reduce the carbon footprint. In the present study biochar produced at 750 °C was effective in removing Arsenic As (III) from water samples. Whereas the literature has shown evidence where biochar has made a great impact in reducing greenhouse gas emissions and global warming, reducing soil nutrient leaching losses, sequester atmospheric carbon into the soil, increasing agricultural productivity, reducing the bioavailability of environmental pollutants [246-248].

5.4 Conclusions:

Many researchers have been researching on development of adsorption of arsenic from groundwater. Different types of materials have been tested and improved to perform better adsorption. Most studies were at preliminary stages, and there is still a gap between the identification of novel adsorbents and feasibility studies for practical applications.

In the current experiment, the effect of pyrolytic temperature on biochar properties and arsenic adsorptive behavior of pineapple peel-derived biochar was studied along with zeolite coating for enhancement of sorption activity. The zeolite coating does not have any positive impact on adsorption activity. The increasing temperature gradually improved the yield, pore size, surface functional groups, carbon content, and pH.

The biochar was derived from pineapple peel and successfully utilized for As (III) sorption. The properties of the PB were significantly affected by pyrolytic temperature. Increased pyrolysis temperature increased pH, ash content, and crystallized size. With increased pyrolysis temperature resulted in increase roughness and a more porous structure of biochar, which increased the surface area of the biochars. Zeolite powder coating did not improve or affected the sorption of As. Among all the biochar PB900 had a maximum sorption capacity of As (III) which was 83.54% indicates that the pineapple biochar can be used as effective adsorbing material for removing arsenic from surface water.

Author Contributions:

Kiran R. Mahale formulated the existing research gaps, prepared the abstract, and outlined and composed the manuscript. The Contribution made by the candidate is 80%. Dr. Mark Lynch, and Dr. Les Bowtell, assisted with the manuscript compilation, editing, and critical revision. Overall, their contribution to this manuscript is 20%.

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“The authors declare no conflict of interest.”

5.5 References

1. Murcott, S., *Arsenic Contamination in the World*. 2012, London, United Kingdom IWA Publishing
2. Podgorski, J. and M. Berg, *Global threat of arsenic in groundwater.* Science, 2020. **368**(6493): p. 845
3. Scott Fendorf1, H.A.M., Alexander van Geen, *Spatial and temporal variations of groundwater arsenic in South and Southeast Asia.* Science, 2010. **328**(5982): p. 1123-1127.
4. Smith, E., et al., *Arsenic in Australian environment: an overview.* J Environ Sci Health A Tox Hazard Subst Environ Eng, 2003. **38**(1): p. 223-39.
5. Nordstrom, D.K., *Worldwide occurrences of arsenic in ground water*. 2002, American Association for the Advancement of Science.
6. Mandal, B.K. and K.T. Suzuki, *Arsenic round the world: a review.* Talanta, 2002. **58**(1): p. 201-235.
7. Chen, Y., et al., *Arsenic exposure at low-to-moderate levels and skin lesions, arsenic metabolism, neurological functions, and biomarkers for respiratory and cardiovascular*

diseases: Review of recent findings from the Health Effects of Arsenic Longitudinal Study (HEALS) in Bangladesh. Toxicology and Applied Pharmacology, 2009. **239**(2): p. 184-192.

8. *Arsenic in Drinking-water* W.H. Organization, Editor. 2011, World Health Organization. p. 1-24.
9. Rehman I., I.M., Ali L., Muhammad S., Din I.U., Yaseen M., Ullah H., *Potentially toxic elements' occurrence and risk assessment through water and soil of Chitral urban environment, Pakistan: a case study.* Environ. Geochem. Health, 2020: p. 1-14.
10. Mohammed Abdul, K.S., et al., *Arsenic and human health effects: A review.* Environmental Toxicology and Pharmacology, 2015. **40**(3): p. 828-846.
11. Shankar, S., U. Shanker, and Shikha, *Arsenic Contamination of Groundwater: A Review of Sources, Prevalence, Health Risks, and Strategies for Mitigation.* The Scientific World Journal, 2014. **2014**: p. 304524.
12. Amen, R., et al., *A critical review on arsenic removal from water using biochar-based sorbents: The significance of modification and redox reactions.* Chemical Engineering Journal, 2020. **396**: p. 125195.
13. Carneiro, M.A., et al., *Current Trends of Arsenic Adsorption in Continuous Mode: Literature Review and Future Perspectives.* Sustainability, 2021. **13**(3): p. 1186.
14. Wan, C., et al., *Pilot-scale combined adsorption columns using activated carbon and zeolite for hazardous trace elements removal from wastewater of entrained-flow coal gasification.* Process Safety and Environmental Protection, 2021. **147**: p. 439-449.
15. Wang, L., et al., *Performance and mechanisms for remediation of Cd(II) and As(III) co-contamination by magnetic biochar-microbe biochemical composite: Competition and synergy effects.* Science of the Total Environment, 2021. **750**.
16. Gérente, C., et al., *Removal of arsenic(V) onto chitosan: From sorption mechanism explanation to dynamic water treatment process.* Chemical Engineering Journal, 2010. **158**(3): p. 593-598.
17. G.M. Sawood, S.G., Arsenic remediation of the waste water using adsorbent: a review. Int. J. Eng. Technol., Sci. Res., 2018. 5: p. 1054-1070.
18. Anastopoulos, I., et al., Agricultural biomass/waste as adsorbents for toxic metal decontamination of aqueous solutions. Journal of Molecular Liquids, 2019. 295: p. 111684.
19. Xue, K.H., et al., Enhanced As(III) transformation and removal with biochar/SnS2/phosphotungstic acid composites: Synergic effect of overcoming the electronic inertness of biochar and W2O3(AsO4)2 (As(V)-POMs) coprecipitation. Journal of Hazardous Materials, 2021. 408.
20. Mukherjee, S., et al., Efficacy of agricultural waste derived biochar for arsenic removal: Tackling water quality in the Indo-Gangetic plain. Journal of Environmental Management, 2021. 281.
21. Stelmock, R.L., F.M. Husby, and A.L. Brundage, Application of Van Soest Acid Detergent Fiber Method for Analysis of Shellfish Chitin1. Journal of Dairy Science, 1985. 68(6): p. 1502-1506.
22. Alkurdi, S.S.A., et al., Effect of pyrolysis conditions on bone char characterization and its ability for arsenic and fluoride removal. Environmental Pollution, 2020. 262: p. 114221.

23. Dang T, M.P., Fitzpatrick R, Mosley LM, Assessment of the Binding of Protons, Al and Fe to Biochar at Different pH Values and Soluble Metal Concentrations. Water, 2018. 10(1): p. 55.
24. Stelmock RL, H.F., Brundage AL, Application of Van Soest Acid Detergent Fiber Method for Analysis of Shellfish Chitin1. Journal of Dairy Science, 1985. 68(6): p. 1502-1506.
25. D1762-84, A., AnnualBookofASTMStandards. Vol. D1762-84.
26. Parthasarathy, P., K.S. Narayanan, and L. Arockiam, Study on kinetic parameters of different biomass samples using thermo-gravimetric analysis. Biomass and Bioenergy, 2013. 58: p. 58-66.
27. Cao, X. and W. Harris, Properties of dairy-manure-derived biochar pertinent to its potential use in remediation. Bioresour Technol, 2010. 101(14): p. 5222-8.
28. Keiluweit, M., et al., Dynamic Molecular Structure of Plant Biomass-Derived Black Carbon (Biochar). Environmental Science & Technology, 2010. 44(4): p. 1247-1253.
29. Keiluweit, M., et al., Dynamic molecular structure of plant biomass-derived black carbon (biochar). Environ Sci Technol, 2010. 44(4): p. 1247-53.
30. Ahmad, M., et al., Effects of pyrolysis temperature on soybean stover- and peanut shell-derived biochar properties and TCE adsorption in water. Bioresour Technol, 2012. 118: p. 536-44.
31. C., S.B., Fundamentals of Fourier Transform Infrared Spectroscopy. 2011: CRC press. .
32. Stuart, B., Infrared Spectroscopy Kirk-Othmer Encyclopedia of Chemical Technology,, 2000: p. 1-18.
33. Deng, H., Yu, H., Chen, M., and Ge, C., Sorption of atrazine in tropical soil by biochar prepared from cassava waste. Bioresource 2014. 9(4): p. 6627-6643.
34. Bomin Fu, C.G., Lin Yue, Jiwei Luo, Dan Feng, Hui Deng, and Huamei Yu, Characterization of Biochar Derived from Pineapple Peel Waste and Its Application for Sorption of Oxytetracycline from Aqueous Solution. bioresources, 2016. 11(1): p. 9017-9035.
35. Ho, M.P. and A.K.T. Lau, 12 - Amorphous carbon nanocomposites, in Fillers and Reinforcements for Advanced Nanocomposites, Y. Dong, R. Umer, and A.K.-T. Lau, Editors. 2015, Woodhead Publishing. p. 309-328.
36. Zhang, K., et al., Characterization of biochar derived from rice husks and its potential in chlorobenzene degradation. Carbon, 2018. 130: p. 730-740.
37. Chamseddine Guizani, K.H., Lionel Limousy, Mejdi Jeguirim, New insights on the structural evolution of biomass char upon pyrolysis as revealed by the Raman spectroscopy and elemental analysis. Carbon letters, 2017. 119: p. 519-521.
38. Smith, M.W., Pecha, B., Helms, G., Scudiero, L., Garcia-Perez, M, Chemical and morphological evaluation of chars produced from primary biomass constituents: cellulose, xylan, and lignin. Biomass and Bioenergy, 2017. 104: p. 17-35.
39. Aup-Ngoen, K. and M. Noipitak, Effect of carbon-rich biochar on mechanical properties of PLA-biochar composites. Sustainable Chemistry and Pharmacy, 2020. 15.
40. Rojas-Mayorga, C.K., Mendoza-Castillo,D.I.,Bonilla-Petriciolet,A.,Silvestre Albero, J.,, Tailoring the adsorption behavior of bonechar for heavy metal removal from aqueous solution. Adsoption Science Technol, 2016. 34: p. 368-387.
41. Patel, S., Han,J.,Qiu,W.,Gao,W.,, Synthesis and characterisation of mesoporous bone char obtained by pyrolysis of animal bones, for environmental application J. Environ.Chem.Eng., 2015. 3 p. 2368-2377.

42. Person, A., Bocherens,H.,Sali ege, J.-F.,Paris,F.,Zeitoun,V.,Gerard,M.,. Early diagenetic evolution of bone phosphate : an X-raydiffractometry analysis. J. Archaeol.Sci, 1995. 22: p. 211-221
43. Kang, D.-S., Lee,S.-M.,Lee,S.-H.,Roh,J.-S.,, X-raydiffraction analysis of the crystallinity of phenolic resin-derived carbon as a function of the heating rate during the carbonization process. Carbon letters, 2018. 27: p. 108-111.
44. Fan, J., et al., Enhanced As (V) Removal from Aqueous Solution by Biochar Prepared from Iron-Impregnated Corn Straw. Journal of Chemistry, 2018. 2018: p. 5137694.
45. Ma, X., et al., Study of Biochar Properties by Scanning Electron Microscope – Energy Dispersive X-Ray Spectroscopy (SEM-EDX). Communications in Soil Science and Plant Analysis, 2016. 47(5): p. 593-601.
46. Susan S.A. Alkurdi, R.A.A.-J., JochenBundschuh, Les Bowtell, Stafford McKnight, Effect of pyrolysis conditions on bone char characterization and its ability for arsenic and fluoride removal. Environmental Pollution, 2020. 262: p. 114221
47. Oni, B.A., O. Oziegbe, and O.O. Olawole, Significance of biochar application to the environment and economy. Annals of Agricultural Sciences, 2019. 64(2): p. 222-236.
48. Luo, Y., et al., Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biology and Biochemistry, 2011. 43(11): p. 2304-2314.
49. Awad, Y.M., et al., Carbon and nitrogen mineralization and enzyme activities in soil aggregate-size classes: Effects of biochar, oyster shells, and polymers. Chemosphere, 2018. 198: p. 40-48.

CHAPTER 6: Conclusion and Recommendations

This chapter summarizes the contribution of this thesis and discusses the recommendations for future work.

6.1 Conclusion

The ever-increasing demand for fossil fuel and an increasing amount of food waste both are challenges for mankind. Due to its tropical aroma and exotic flavors, pineapple is one of the popular fruits among all age groups. More than 50 % of the fruit is used for manufacturing processed items such as juice, slices, hames, jellies, and so on. The waste generated by the processing industry is almost 50% of wet fruit weight. This thesis focuses on the value addition of food waste generated from the pineapple processing industry to meet the ever-increasing demand for fossil fuels and other environmental issues like the presence of heavy metals in surface or groundwater.

This study investigates the efficacy of pineapple processing waste in the production of fermentable sugars and then fermentation of sugars into ethanol as an alternative for fossil fuels. The further remains solid residues from ethanol production then further used in pyrolysis and produced biochar and the biochar was used in the removal of Arsenic As (III) from surface water. The present studies in this thesis show in detail why pretreatment of pineapple waste is important for the production of higher sugars and efficacy of pineapple biochar in the removal of As (III) from surface water.

In Chapter 3, in the first stage of the experiments, pineapple waste was treated with different concentrations of alkaline and acidic solutions to determine the effect of different conditions like pH, acids, and alkaline solutions. The samples were treated for 7 days before enzymatic hydrolysis to further enhance sugar production followed by fermentation for ethanol production. The pre-treatment with 1% NaOH followed by the enzymatic hydrolysis resulted in the highest concentration of reducing sugars. The SEM analysis showed vast structural changes in pre-treated PW compared to untreated PW samples. The current study also suggests the yeast (*Saccharomyces cerevisiae*) used in the fermentation could produce up to 9.45 ±0.94 g/L bioethanol, but the yield was limited to 3.05% due to non-fermentable sugars and fermentation inhibitors. The findings of this study also suggest studying different pre-treatment or combination of pre-treatment to enhance the production of fermentable sugars to make the ethanol production process more industrially feasible and efficient.

As it was concluded from the first study that the alkaline and acidic solutions were not so effective in the production of ethanol due to a long-standing time of 7 days it was recommended to find an alternative method to decrease the reaction time and increase the efficacy of the production of fermentable sugars. Different options were considered including mechanical pressure cruising, microwave heating, ultra-sonication treatment, an explosion of high voltage for a short duration, and so on. Based on the literature search and to make it more efficient and Industrial feasible the option of ever-emerging microwave-assisted heating and combined microwave heating with chemical pre-treatments were carried out.

Chapter 3 investigated the efficacy of different acids and alkaline solutions in the extraction of fermentable sugars from pineapple processing waste. The pre-treatment time was 0 minutes to 7 days. Once the dose of acid and alkali was optimized, further investigation was done to reduce the time of pre-treatment. Combinations of chemical and physical pre-treatment were investigated in the form of micro-wave assisted acid and alkaline treatment. The finding and conclusions of the study as below.

In Chapter 4, Microwave-assisted chemical pre-treatment technology is more efficient, rapid, and feasible for the production of reducing sugars from pineapple waste. In the present study different microwave heating powers (50% and 100%) respectively and different irradiation times (1 min and 2 min) have been studied along with the different concentrations (0.5% and 1.0%) of acid (H_2SO_4) and alkali (NaOH). Low microwave power and short exposure time (50% for 1 min) does not modify the sugar concentration significantly while; higher power (100%) and extending heating to 2 minutes caused the hydrothermal degradation of PW samples due to excessive heat which resulted in a lower concentration of reducing sugars. Infra-red thermography and SEM analysis also indicated the possible hydrothermal degradation of sugars resulted in a lower concentration of fermentable sugars at higher irradiation power and time.

The infrared thermography of the PW showed that an increase in irradiation time and microwave power results in higher temperature and even spread out of the heat, while shorter exposer time and lower power results in low temperature and uneven heating effects. The presence of fermentation inhibitors like phenolic compounds as a result of solubilization of lignin content of PW and formation of organic acids and furfural due to hydrothermal effects of degradation of sugars resulted in a lower amount of production of ethanol. It was concluded from this study that microwave pre-treatment using appropriate irradiation time and power enhanced the saccharification and that microwave-assisted chemical pretreatments could be used to improve the bioethanol yield of pineapple waste.

The solid residues that remain after the ethanol production had so many different applications like can be used in anaerobic fermentation for production of methane gas, can be used in the production of degradable pots for plantation to be used in the nursery, as garden mulching and straight as fertilizer. While all these are conventional usage the present study took an innovative approach to utilize the leftover in production of biochar and then use biochar for removal of heavy metals like arsenic As (III) from surface water.

The solid residues of pineapple waste from ethanol production were further used to investigate to removal of arsenic using biochar. The solid residues were pyrolyzed at different temperature with zeolite coating to remove arsenic from ground and surface water the findings of the study are as below.

In Chapter 5, Is the first stage of the experiments, biochar was produced at different pyrolysis temperatures (350 – 900 °C) in absence of oxygen at holding time for 2 h. The characterization of each biochar sample was examined using a wide range of analytical and technical procedures. The Scanning Electron Microscopy (SEM) images provided information about the morphology of the surface, showing that it was rough with an undefined geometry. It was found that the biochar derived from pineapple peel and successfully utilized for As (III) sorption. The properties of the PB were significantly affected by pyrolytic temperature. Generally, the factors that affected the removal capacity of both of the contaminates were in the following order: pyrolysis temperature > residence time. Increased pyrolysis temperature increased pH, ash content, and crystallized size. With increased pyrolysis temperature resulted in increase roughness and a more porous structure of biochar, which increased the surface area of the biochars. Zeolite powder coating did not improve or affected the sorption of As. Among all the biochar PB900 had a maximum sorption capacity of As (III) which was 83.54% indicates that the pineapple biochar can be used as effective adsorbing material for removing arsenic from surface water.

6.2 Recommendations

The results obtained in this study show that pineapple waste can be used as feedstock for the production of reducing sugars. The optimized pretreatment with different acids and alkaline solution followed by enzymatic hydrolysis significantly improved the fermentable sugar production. Microwave irradiation significantly improved the reaction time from 7 days to 1 minute; paving the way for more applications on an industrial scale. However, several aspects were identified for further exploration to improve the performance of these waste materials for environmental remediation. The following aspects are worthy of further investigation in future work:

1. In this study, only two alkaline solutions namely Sodium hydroxide (NaOH) and Calcium hydroxide Ca(OH)2, and two acidic solutions Sulphuric acid (H2SO4) and phosphoric acid (H3PO4) were investigated. It is recommended that the effect of other alkaline solutions like Calcium Carbonate ($CaCO_3$), Ammonium Hydroxide (NH_4OH), etc could be investigated.
2. Similarly, only two acidic solutions Sulphuric acid (H2SO4) and phosphoric acid (H3PO4) were investigated. It is recommended that the effect of other acidic solutions like Hydrochloric acid (HCl), Nitric acid (HNO_3), Acetic acid (CH_3COOH), etc could be investigated. The effect of other pre-treatments like Organic solvents, Ionic liquids, Ammonium fiber explosion could be investigated.
3. It is evinced that the resulting sugar content could not be converted into ethanol. It will be fascinating to investigate the further development of other value-added products like Polyhydroxybutyrate (PHB) as bio-derived and biodegradable plastics. The resulting hydrolysate could be used in the fermentation of other organic acids like acetic acid,

lactic acid, citric acid, succinic acid which are of more interest in the pharmaceutical and food industry.

4. The application of pineapple derived biochar to remove arsenic from surface water has been investigated at a lab-scale only on surface water. It would be of interest to further investigate the removal of mechanism and removal of other heavy metals like Fluoride (F^-) and others.

Appendix A: Conference Proceedings part of this thesis:

Proceeding I:

Mahale, Kiran R., and Burey, Paulomi (Polly) (2018) *Emerging technologies for extraction of bioactives and polysaccharides from tropical fruit waste.* In: 2nd ISEKI Food Waste Recovery Workshop 2018, 3-5 July 2018, Stuttgart, Germany

Proceeding II:

Mahale, Kiran R., and Trzcinski Antoine P. (2019). In: *The application of winery waste (grape pomace) in the production of bioactive compounds and bioethanol,* 1st Food Technology and Nutritional Science Workshop 2019, 11-13 November 2019, Vienna, Austria.

Proceeding I:

Emerging technologies for extraction of bioactive and polysaccharides from tropical fruit waste

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Keywords: Shockwave technology, mango, pineapple, polyphenols, anthocyanins, carotenoids

Abstract

The use of emerging technologies to valorize food waste is of growing interest, as the amount of food waste globally is very high. Useful bioactive compounds and functional ingredients can be liberated with the application of sequential separation and extraction processes as outlined in the Universal Recovery Strategy.

Here we detail initial work done on analysing the yield of bioactive compounds and pectin from tropical fruit wastes via a control of thawed frozen waste compared with 2 processes:

1. Freeze drying combined with aqueous, solvent, and acid extraction,

2. Hydrodynamic shockwave technology, combined with aqueous, solvent and acid extraction.

Our waste materials came from tropical fruit processing lines based in Queensland, Australia, and included both mango and pineapple skin and adhered flesh. Compounds of interest from the mango skin and flesh included polyphenols, carotenoids, anthocyanins and pectin, while compounds in pineapple skin and flesh included carotenoids, polyphenols and bromelain. The

byproduct of all extractions was primarily cellulose and was of interest for bioplastic development.

We present here our initial conclusions on whether hydrodynamic shockwave technology can enhance accessibility of bioactive compounds and separation of the different components of tropical fruit wastes.

Proceeding II:

The application of winery waste (grape pomace) in the production of bioactive compounds and bioethanol

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Keywords: Food waste, winery waste, renewable energy, ethanol, biochar, polyphenols, anthocyanins, carotenoids

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Abstract:

Australia produces approximately 150,000 tons of unutilized biomass in the form of grape pomace as a processing waste from the winery industry. Grapes and grape waste are a rich source of polyphenols including flavonoids, anthocyanins, minerals, and dietary fibers (non-starch polysaccharides) which are required for nutrition and health, and the development of the human body. In particular, the bioactive compounds extracted from grape pomace/marc are rich in therapeutic properties, which can help to reduce the risk of many chronic diseases like cardiovascular disease. The bioactive compounds extracted from grape pomace also act as antioxidants as well as anticancer, antitumor, and anti-inflammatory agents. These also are known for their anti-viral and anti-inflammatory activities, cardiovascular protective activity. Here we detail initial work done on analysing the yield of bioactive compounds and other potential renewable energy sources including the production of ethanol, production of methane gas, and biochar for environmental, agricultural, and energy storage applications.

Different methods were used to extract the bio-actives from grape waste (solvent-acid extraction, sonication, freeze-drying, aqueous extraction method). whereas for ethanol production, different pre-treatments were used (acid, alkaline pre-treatment, ultrasonication, water bath, heat treatment, microwave heating, enzymatic hydrolysis) to enhance the reducing sugar production for ethanol fermentation. The conventional pyrolysis method was used to produced biochar for the removal of heavy metals from surface water.

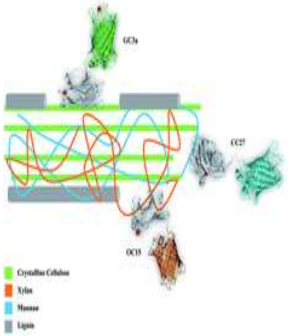
We present here our initial conclusion on how different approaches can be used to generate different value-added products from winery waste for a wide range of applications.

Graphical Abstract Research paper 1:

Acidic Pre-treatment (0-7 days) (0.5-5%) 20% (w/v) PW loading.

Alkaline Pre-treatment (0-7 days) (0.5-5%) 20% (w/v) PW loading

Incubate for 7 days 30 °C 100 rpm.



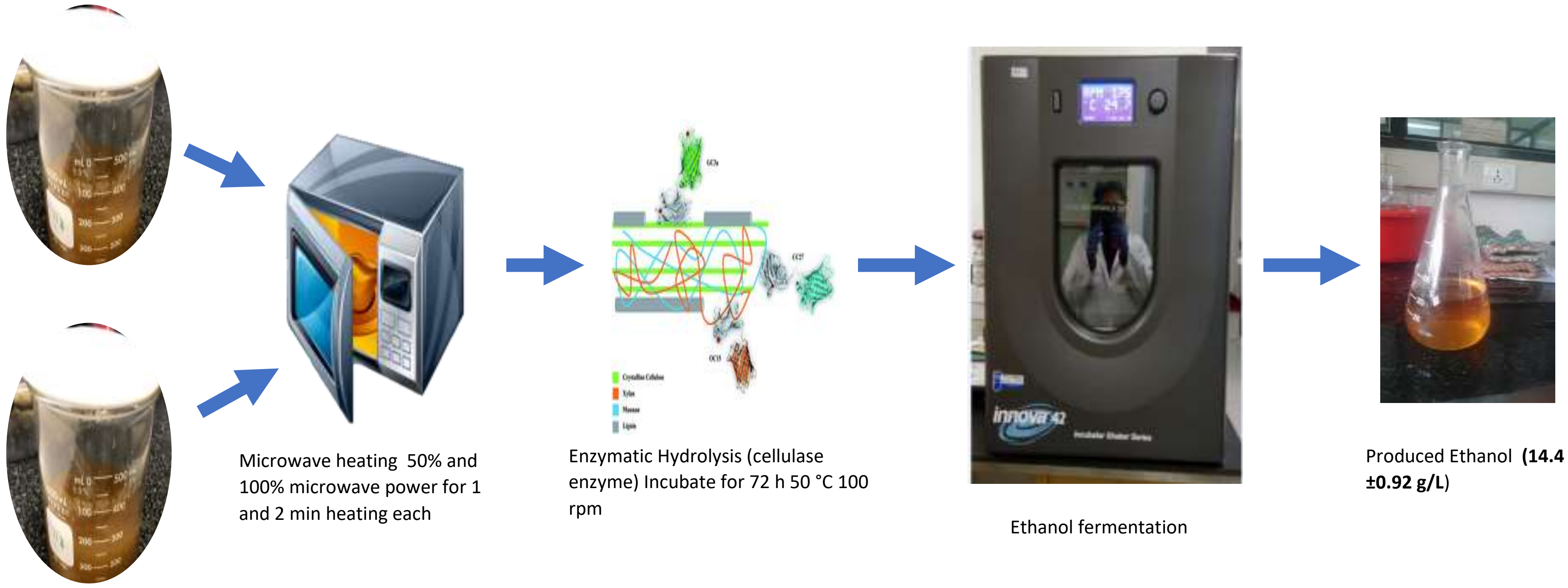
Enzymatic Hydrolysis (cellulase enzyme) Incubate for 72 h 50 °C 100 rpm

Ethanol fermentation

Produced Ethanol (9.45 ±0.94 g/L)

Graphical Abstract Research paper 2:

Acidic Pre-treatment (0.5% &1% acid) 20% (w/v) PW loading.



Microwave heating 50% and 100% microwave power for 1 and 2 min heating each

Enzymatic Hydrolysis (cellulase enzyme) Incubate for 72 h 50 °C 100 rpm

Ethanol fermentation

Produced Ethanol **(14.4 ±0.92 g/L)**

Alkaline Pre-treatment (0.5% &1% alkali) 20% (w/v) PW loading.

Refernces of thesis:

1. Kiran, E.U., A.P. Trzcinski, and Y. Liu, Platform chemical production from food wastes using a biorefinery concept. Journal of Chemical Technology and Biotechnology, 2015. 90(8): p. 1364-1379.

2. Energy, D.o.t.E.a. Tackling australia's food waste: Deapartment of the Environment and Energy, Fedral Government of Australia. 2018 [cited 2018 05/03/2018]; Available from: http://www.environment.gov.au/protection/waste-resource-recovery/food-waste.

3. Pham, T.P.T., et al., Food waste-to-energy conversion technologies: Current status and future directions. Waste Management, 2015. 38: p. 399-408.

4. Vigano, J., A.P.D. Machado, and J. Martinez, Sub- and supercritical fluid technology applied to food waste processing. Journal of Supercritical Fluids, 2015. 96: p. 272-286.

5. Kiran, E.U., et al., Bioconversion of food waste to energy: A review. Fuel, 2014. 134: p. 389-399.

6. M.Galanakis, C., Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. Trends in Food Science & Technology, 2012. 26(2): p. 68-87.

7. Schieber, A., F.C. Stintzing, and R. Carle, By-products of plant food processing as a source of functional compounds - recent developments. Trends in Food Science & Technology, 2001. 12(11): p. 401-+.

8. Mirabella, N., V. Castellani, and S. Sala, Current options for the valorization of food manufacturing waste: a review. Journal of Cleaner Production, 2014. 65: p. 28-41.

9. Hegde, S., J.S. Lodge, and T.A. Trabold, Characteristics of food processing wastes and their use in sustainable alcohol production. Renewable & Sustainable Energy Reviews, 2018. 81: p. 510-523.

10. Girotto, F., L. Alibardi, and R. Cossu, Food waste generation and industrial uses: A review. Waste Management, 2015. 45: p. 32-41.

11. Paritosh, K., et al., Food Waste to Energy: An Overview of Sustainable Approaches for Food Waste Management and Nutrient Recycling. Biomed Research International, 2017.

12. Nazzaro, F., et al., Recovery of biomolecules of high benefit from food waste. Current Opinion in Food Science, 2018. 22: p. 43-54.

13. Sonja Djtlas Jasna. Čanadanovic-Brunet, G.C., By-products of ruits processing as a source of phytochemicals. Chemical Industry & Chemical Engineering Quarterly 2009. 15(4): p. 191-202.

14. Ren, Y.Y., et al., A comprehensive review on food waste anaerobic digestion: Research updates and tendencies. Bioresource Technology, 2018. 247: p. 1069-1076.

15. Li, L., et al., Anaerobic digestion of food waste: A review focusing on process stability. Bioresource Technology, 2018. 248: p. 20-28.

16. Muhammad Waqasa, M.R., Muhammad Daud Khanb, Abdul-Sattar Nizamia, Conversion of Food Waste to Fermentation Products. Encyclopedia of Food Security and Sustainability, 2019. 1: p. 501-509.

17. Ma, H.Z., et al., Recovery of lactic acid and other organic acids from food waste ethanol fermentation stillage: Feasibility and effects of substrates. Separation and Purification Technology, 2019. 209: p. 223-228.

18. W.Babbitt, T.A.T.a.C., Introduction to Sustainable Food Waste-To-energy Systems, in Sustainable Food Waste-to-Energy Systems, T.A.T.a.C. W.Babbitt, Editor. 2018, Academic Press. p. 1-10.

19. team, A.E.S.p., Australian Energy Update 2017, D.o.t.E.a. Energy, Editor. 2017, Department of the Environment and Energy, Government of Australia CANBERRA.

20. Roger Farrell, Australia Biofuels Annual 2017. 2017, USDA Foreign Agricultural Service Canberra. p. 1-21.

21. Mckenzie, S., Australia Bioenery policies and status of implementation 2018 updates in IEA Bioenergy L. Pelkmans, Editor. 2018, IEA Bioenergy.

22. O'Connell, D.D., Biofuels in Australia — issues and prospects. 2007.

23. Australia, B.A.o. Biodiesel Plants in Australia. 2012 [cited 2019 08/02/2019]; Available from: http://www.biofuelsassociation.com.au/~biofuels/images/stories/pdf/biodieselmap.pdf.

24. Kim, J.S., Y.Y. Lee, and T.H. Kim, A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresource Technology, 2016. 199: p. 42-48.

25. Sindhu, R., P. Binod, and A. Pandey, Biological pretreatment of lignocellulosic biomass - An overview. Bioresource Technology, 2016. 199: p. 76-82.

26. Sharma, H.K., C.B. Xu, and W.S. Qin, Biological Pretreatment of Lignocellulosic Biomass for Biofuels and Bioproducts: An Overview. Waste and Biomass Valorization, 2019. 10(2): p. 235-251.

27. Mathew, A.K., et al., An evaluation of dilute acid and ammonia fiber explosion pretreatment for cellulosic ethanol production. Bioresource Technology, 2016. 199: p. 13-20.

28. Ravindran, R. and A.K. Jaiswal, A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities. Bioresource Technology, 2016. 199: p. 92-102.

29. da Silva, A.R.G., C.E.T. Ortega, and B. Rong, Techno-economic analysis of different pretreatment processes for lignocellulosic-based bioethanol production. Bioresource Technology, 2016. 218: p. 561-570.

30. Espinoza-Acosta, J.L., et al., Ionic Liquids and Organic Solvents for Recovering Lignin from Lignocellulosic Biomass. Bioresources, 2014. 9(2): p. 3660-3687.

31. Park, J., et al., Use of mechanical refining to improve the production of low-cost sugars from lignocellulosic biomass. Bioresource Technology, 2016. 199: p. 59-67.

32. Serna, L.V.D., C.E.O. Alzate, and C.A.C. Alzate, Supercritical fluids as a green technology for the pretreatment of lignocellulosic biomass. Bioresource Technology, 2016. 199: p. 113-120.

33. Zhang, K., Z.J. Pei, and D.H. Wang, Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review. Bioresource Technology, 2016. 199: p. 21-33.

34. Li, H.Q., et al., Microwave irradiation - A green and efficient way to pretreat biomass. Bioresource Technology, 2016. 199: p. 34-41.

35. Tran, A.V., Chemical analysis and pulping study of pineapple crown leaves. Industrial Crops and Products, 2006. 24(1): p. 66-74.

36. Loudon, J.C., The Different Modes of Cultivating the Pineapple, from its First Introduction to Europe to the Late Improvements of T.A. 1822: Knight Esq. London: Houlgman Hurst Resorme Brown.

37. María Gloria Lobo, M.S., Overview of pineapple production, postharvest physiology, processing and nutrition, in Handbook of pineapple technology, M.G.L.a.R.E. Paull, Editor. 2016, Wileys: Chichester, West Sussex. p. 1-15.

38. siddiq, M.G.L.a.M., Overview of pineapple production, postharvest physiology in Handbook of pineapple technology M.G.L.a.R.E. Paull, Editor. 2017, John wiley and Sones p. 1-15.

39. Wadhwa, M., & Bakshi, S., Utilization of fruit and vegetable wastes as livestock feed and as substrate for generation of other value-added products, H.P.S. Makkar, Editor. 2013. p. 1-67.

40. Gorinstein, S., et al., Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. Journal of Nutritional Biochemistry, 1999. 10(6): p. 367-371.

41. Barretto, L.C.D., et al., Characterization and extraction of volatile compounds from pineapple (Ananas comosus L. Merril) processing residues. Food Science and Technology, 2013. 33(4): p. 638-645.

42. Banerjee, S., et al., Valorisation of pineapple wastes for food and therapeutic applications. Trends in Food Science & Technology, 2018. 82: p. 60-70.

43. Nor, M.Z.M., et al., Characteristic properties of crude pineapple waste extract for bromelain purification by membrane processing. Journal of Food Science and Technology-Mysore, 2015. 52(11): p. 7103-7112.

44. Parfitt, J., M. Barthel, and S. Macnaughton, Food waste within food supply chains: quantification and potential for change to 2050. Philosophical Transactions of the Royal Society B-Biological Sciences, 2010. 365(1554): p. 3065-3081.

45. Miranda, R.C. and D.W. Schaffner, Virus risk in the food supply chain. Current Opinion in Food Science, 2019. 30: p. 43-48.

46. Sogi, E.D.a.D.S., Value added processing and utilization of pineapple by-products, in Handbook of pineapple technology, D.M.G.L.a.D.R.E. Paull, Editor. 2017, Wiley p. 196-220.

47. Food waste footprint impacts on natural resources 2013, Food And Agriculture Organization of United Nations. p. 1-63.

48. María Elena Sánchez Pardo1*, M.E.R.C., Rosalva Mora Escobedo1, Epifanio Jiménez García1 Chemical Characterisation of the Industrial Residues of the Pineapple (Ananas comosus). Journal of Agricultural Chemistry and Environment, 2014. 3: p. 53-56.

49. Banerjee, J., et al., Bioactives from fruit processing wastes: Green approaches to valuable chemicals. Food Chemistry, 2017. 225: p. 10-22.

50. Hemalatha, R.A., S, Physicochemical constituents of pineapple pulp and waste. . Journal of Chemical and Pharmaceutical Research, 2013. 5(2): p. 240-242.

51. Collins, J., The Pineapple: Botany, Cultivation and Utilisation. 1960: Interscience Publishers, New York.

52. Australian Horticulture Statistics Handbook 2017/18 2019. p. 116.

53. Pineapples. 2018 [cited 2019 15/05/2019]; Available from: https://www.daf.qld.gov.au/business-priorities/agriculture/plants/fruit-vegetable/fruit-vegetable-crops/pineapples.

54. Castillo-Gonzalez, E., et al., Pre-Composting and Vermicomposting of Pineapple (Ananas Comosus) and Vegetable Waste. Applied Sciences-Basel, 2019. 9(17).

55. AophatChoonutMakornSaejongKanokphornSangkharak, The Production of Ethanol and Hydrogen from Pineapple Peel by Saccharomyces Cerevisiae and Enterobacter Aerogenes. Energy Procedia, 2014. 52: p. 242-249.

56. M, A.A.a.H., Characterisation of solid and liquid pineapple waste. Reaktor 2008. 12: p. 48-52.

57. Shalini Gaur Rudra*, J.N., Neetu Jakhar and Charanjit Kaur FOOD INDUSTRY WASTE: MINE OF NUTRACEUTICALS. International Journal of Science, Environment and Technology 2015. 4(1): p. 205-229.

58. ATUL UPADHYAY, JEEWAN PRAVA LAMA and SHINKICHI TAWATA, Utilization of Pineapple Waste: A Review. Journal of Food Science and Technology-Nepal 2010. 6: p. 10-18.

59. Roda, A. and M. Lambri, Food uses of pineapple waste and by-products: a review. International Journal of Food Science and Technology, 2019. 54(4): p. 1009-1017.

60. Ueno, T., et al., Lactic acid production using two food processing wastes, canned pineapple syrup and grape invertase, as substrate and enzyme. Biotechnology Letters, 2003. 25(7): p. 573-577.

61. Jin, B., et al., Production of lactic acid and fungal biomass by Rhizopus fungi from food processing waste streams. Journal of Industrial Microbiology & Biotechnology, 2005. 32(11-12): p. 678-686.

62. Idris, A. and W. Suzana, Effect of sodium alginate concentration, bead diameter, initial pH and temperature on lactic acid production from pineapple waste using immobilized Lactobacillus delbrueckii. Process Biochemistry, 2006. 41(5): p. 1117-1123.

63. Nakanishi, K., et al., L-Lactic Acid Production from Canned Pineapple Syrup by Rapid Sucrose Catabolizing Lactobacillus paracasei NRIC 0765. Food Science and Technology Research, 2010. 16(3): p. 239-246.

64. Aziman, S.N., H.H. Tumari, and N.A.M. Zain, Determination of Lactic Acid Production by Rhizopus Oryzae in Solid State Fermentation of Pineapple Waste. Jurnal Teknologi, 2015. 77(31): p. 95-102.

65. Sawant, O., et al., Fungal Citric Acid Production Using Waste Materials: A Mini-Review. Journal of Microbiology Biotechnology and Food Sciences, 2018. 8(2): p. 821-828.

66. Dhillon, G.S., et al., Recent Advances in Citric Acid Bio-production and Recovery. Food and Bioprocess Technology, 2011. 4(4): p. 505-529.

67. Tran, C.T., L.I. Sly, and D.A. Mitchell, Selection of a strain of Aspergillus for the production of citric acid from pineapple waste in solid-state fermentation. World Journal of Microbiology & Biotechnology, 1998. 14(3): p. 399-404.

68. Kareem, S.O., Akpan, I. and Alebiowu, O. O., Production of citric acid by Aspergillus niger using pineapple waste. Malaysian Journal of Microbiology, 2010. 6(2): p. 161-165.

69. Kumar, D., et al., Utilisation of fruits waste for citric acid production by solid state fermentation. Process Biochemistry, 2003. 38(12): p. 1725-1729.

70. Imandi, S.B., et al., Application of statistical experimental designs for the optimization of medium constituents for the production of citric acid from pineapple waste. Bioresource Technology, 2008. 99(10): p. 4445-4450.

71. R, D., Overview of pharmaceutical excipients used in tablets and capsules. 2008.

72. Norela Jusoh, N.O., Ani Idris, Alina Nasruddin, Characterization of Liquid Pineapple Waste as Carbon Source for Production of Succinic Acid. Jurnal Teknologi 2014. 69(4): p. 11-13.

73. Ferone, M., et al., Efficient succinic acid production from high-sugar-content beverages by Actinobacillus succinogenes. Biotechnology Progress, 2019. 35(5).

74. Fokom-Domgue, J., et al., Performance of alternative strategies for primary cervical cancer screening in sub-Saharan Africa: systematic review and meta-analysis of diagnostic test accuracy studies. Bmj-British Medical Journal, 2015. 351.

75. Roda, A., et al., Metabolite profiling and volatiles of pineapple wine and vinegar obtained from pineapple waste. Food Chemistry, 2017. 229: p. 734-742.

76. Roda, A., et al., Pineapple Wines Obtained from Saccharification of Its Waste with Three Strains of Saccharomyces cerevisiae. Journal of Food Processing and Preservation, 2017. 41(5).

77. deLima, V.L.A.G., T.L.M. Stamford, and A.A. Salgueiro, Citric acid production from pineapple waste by solid state fermentation using Aspergillus niger. Arquivos De Biologia E Tecnologia, 1995. 38(3): p. 773-783.

78. Patagundi, B.I. and B.B. Kaliwal, Biotechnological Production of Citric Acid Using Different Substrates by Aspergillus Niger ATCC 26550. Research Journal of Biotechnology, 2008: p. 196-206.

79. Aruna, T.E., Production of value-added product from pineapple peels using solid state fermentation. Innovative Food Science & Emerging Technologies, 2019. 57.

80. Khedkar, M.A., et al., Sustainable biobutanol production from pineapple waste by using Clostridium acetobutylicum B 527: Drying kinetics study. Bioresource Technology, 2017. 225: p. 359-366.

81. Tilay, A., et al., Preparation of ferulic acid from agricultural wastes: Its improved extraction and purification. Journal of Agricultural and Food Chemistry, 2008. 56(17): p. 7644-7648.

82. Ong Khai Lun, T.B.W. and, and L.S. Ling, Pineapple cannery waste as a potential substrate for microbial biotranformation to produce vanillic acid and vanillin. International Food Research Journal, 2014. 21(3): p. 953-958.

83. Ou, S.Y. and K.C. Kwok, Ferulic acid: pharmaceutical functions, preparation and applications in foods. Journal of the Science of Food and Agriculture, 2004. 84(11): p. 1261-1269.

84. Wang, B.H. and J.P. Ou-Yang, Pharmacological actions of sodium ferulate in cardiovascular system. Cardiovascular Drug Reviews, 2005. 23(2): p. 161-172.

85. Trzcinski, A.P., Enzyme production from food waste in Biofules from Food Waste A.P. Trzcinski, Editor. 2018, CRC Place New York p. 49-68.

86. Kavuthodi, B. and D. Sebastian, Biotechnological valorization of pineapple stem for pectinase production by Bacillus subtilis BKDS1: Media formulation and statistical optimization for submerged fermentation. Biocatalysis and Agricultural Biotechnology, 2018. 16: p. 715-722.

87. Ramesh Chander Kuhad , R.G., and Ajay Singh, Microbial Cellulases and Their Industrial Applications. Enzyme Reserach 2011: p. 1-11.

88. Mohite, B.V. and S.V. Patil, A novel biomaterial: bacterial cellulose and its new era applications. Biotechnology and Applied Biochemistry 2013. 61(2): p. 101-110.

89. Cacicedo, M.L., et al., Progress in bacterial cellulose matrices for biotechnological applications. Bioresour Technol, 2016. 213: p. 172-180.

90. Budiman Anwar, B.B., I. Made Arcana,, Isolation of Cellulose Nanocrystals from Bacterial Cellulose Produced from Pineapple Peel Waste Juice as Culture Medium,. Procedia Chemistry, 2015. 16: p. 279-284.

91. Itxaso Algar, S.C.M.F., Gurutz Mondragon, Cristina Castro, and N.G. Clara Garcia-Astrain, Alona Retegi, Arantxa Eceiza, Pineapple Agroindustrial Residues for the Production of High Value Bacterial Cellulose with Different Morphologies. Journal of Applied Polymer science 2015. 132: p. 41237(1-8).

92. Zoya Manzoor, A.N., Hamid Mukhtar, Ikram Haq, Bromelain: Methods of Extraction, Purification and Therapeutic Applications. BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY, 2016. 59: p. 1-16.

93. Marialice Pinto Coelho Silvestre, et al., Effect of pH and Temperature on the Activity of Enzymatic Extracts from Pineapple Peel. Food Bioprocess Technol, 2012. 5: p. 1824-1831.

94. Hossain MM, L.S., Kim IH., Effects of bromelain supplementation on growth performance, nutrient digestibility, blood profiles, faecal microbial shedding, faecal score and faecal noxious gas emission in weanling pigs. Veterinarni Medicina., 2015. 60(10): p. 544-552.

95. Babu, B.R., N.K. Rastogi, and K.S.M.S. Raghavarao, Liquid-liquid extraction of bromelain and polyphenol oxidase using aqueous two-phase system. Chemical Engineering and Processing, 2008. 47(1): p. 83-89.

96. Novaes, L.C., et al., Polymer-based alternative method to extract bromelain from pineapple peel waste. Biotechnol Appl Biochem, 2013. 60(5): p. 527-35.

97. Ketnawa S, R.S., Chaiwut P., Two phase partitioning and collagen hydrolysis of bromelain from pineapple peel Nang Laecultivar. J Biochem Eng. , 2010. 52(3): p. 205-211.

98. Ketnawa S, C.P., Rawdkuen S., Aqueous Two-phase Extraction of Bromelain from Pineapple Peels (‘PhuLae’ cultv.) and Its Biochemical Properties. Food Sci Biotechnol., 2011. 20(5): p. 1219-1226.

99. Lopes, F.L.G., et al., Concentration by Membrane Separation Processes of a Medicinal Product Obtained from Pineapple Pulp. Brazilian Archives of Biology and Technology, 2009. 52(2): p. 457-464.

100. M.B.Doko, V.B., J.Casadebaig, L. Cavailles, M.Jacob. , Preparation of proteolytic enzyme extracts from Ananas comosus L., Merr. fruit juice using semipermeable membrane, ammonium sulfate extraction, centrifugation and freeze-drying processes. International Journal of Pharmaceutics, 1991. 76(3): p. 199-206.

101. Chaurasiya, R.S., et al., Efficacy of reverse micellar extracted fruit bromelain in meat tenderization. Journal of Food Science and Technology-Mysore, 2015. 52(6): p. 3870-3880.

102. Hebbar HU, H.A., Sumana B, Raghavarao KSMS, Reverse micellar extraction of bromelain from pineapple (ananascomosus l. merryl) waste: scale-up, reverse micelles characterization and mass transfer studies. Sep Sci Technol, 2011. 46(10): p. 1656-1664.

103. Kumar, S., A.B. Hemavathi, and H.U. Hebbar, Affinity based reverse micellar extraction and purification of bromelain from pineapple (Ananas comosus L. Merryl) waste. Process Biochemistry, 2011. 46(5): p. 1216-1220.

104. Nie, H.L., et al., Purification of bromelain using immobilized metal affinity membranes. Journal of Biotechnology, 2008. 136: p. S416-S416.

105. Nadzirah, K.Z., et al., Efficacy of selected purification techniques for bromelain. International Food Research Journal 2013. 20(1): p. 43-46.

106. Kong, X.Z., H.M. Zhou, and H.F. Qian, Enzymatic hydrolysis of wheat gluten by proteases and properties of the resulting hydrolysates. Food Chemistry, 2007. 102(3): p. 759-763.

107. Watanabe, M., et al., Novel method for producing hypoallergenic wheat flour by enzymatic fragmentation of the constituent allergens and its application to food processing. Bioscience Biotechnology and Biochemistry, 2000. 64(12): p. 2663-2667.

108. Hage, D.S., et al., Pharmaceutical and biomedical applications of affinity chromatography: Recent trends and developments. Journal of Pharmaceutical and Biomedical Analysis, 2012. 69: p. 93-105.

109. Sunantha Ketnawa, S.R., Application of Bromelain Extract for Muscle Foods Tenderization. Food and Nutrition Sciences, 2011. 2: p. 393-401.

110. Benucci, I., et al., Bromelain from pineapple stem in alcoholic-acidic buffers for wine application. Food Chemistry, 2011. 124(4): p. 1349-1353.

111. C.Ramalingam, R.S.a.N.N.I., Isolation and characterization of Bromelain from pineapple (Ananas Comosus) and comparing its anti-browning activity on apple juice with commercial antibrowning agents. Elixir Food Science, 2012. 45: p. 7822-7826.

112. Anupama and P. Ravindra, Value-added food: Single cell protein. Biotechnology Advances, 2000. 18(6): p. 459-479.

113. Zhou, Y.-M., et al., Recycling of orange waste for single cell protein production and the synergistic and antagonistic effects on production quality. Journal of Cleaner Production, 2019. 213: p. 384-392.

114. Shahla Samadi, M.M., Ghasem Najafpour, Production of Single Cell Protein from Sugarcane Bagasse by Saccharomyces cerevisiae in Tray Bioreactor P A P E R I N F O Paper history. Enzyme Technology and Commercial Enzyme Production, 2016. 29(8): p. 1029-1036.

115. Gabriel, A.Y., et al., Production of single cell protein from cereal by-products. Agricultural Wastes, 1981. 3(3): p. 229-240.

116. Zacharof, M.-P., Grape Winery Waste as Feedstock for Bioconversions: Applying the Biorefinery Concept. Waste and Biomass Valorization, 2017. 8(4): p. 1011-1025.

117. Rashad, M.M., S.A. Moharib, and E.W. Jwanny, Yeast conversion of mango waste or methanol to single cell protein and other metabolites. Biological Wastes, 1990. 32(4): p. 277-284.

118. Nigam, J.N., Single cell protein from pineapple cannery effluent. World Journal of Microbiology & Biotechnology, 1998. 14(5): p. 693-696.

119. Nasir, I.M., T.I.M. Ghazi, and R. Omar, Production of biogas from solid organic wastes through anaerobic digestion: a review. Applied Microbiology and Biotechnology, 2012. 95(2): p. 321-329.

120. Barz, M., M.K. Delivand, and K. Dinkier, Agricultural Wastes - A Promising Source for Biogas Production in Developing Countries of the Tropical and Subtropical Regions. Revista Forestal Mesoamerica Kuru-Rfmk, 2019. 16(38): p. 2-12.

121. Rani, D.S. and K. Nand, Ensilage of pineapple processing waste for methane generation. Waste Management, 2004. 24(5): p. 523-528.

122. Namsree, P., et al., Anaerobic digestion of pineapple pulp and peel in a plug-flow reactor. Journal of Environmental Management, 2012. 110: p. 40-47.

123. Nurul Shafiqah Rosli, S.I., Azmir Md Dom and Nik Norsyahariati Nik Daud, Potential of pineapple waste extract (PWE) as co-substrate in anaerobic digestion of rice straw washwater (RSWW): enhancement of biogas production, in Lecture Notes in Civil Engineering. 2019, Springer p. 1479-1493.

124. Cahyari, K., et al., Biohydrogen Production from Pineapple Waste: Effect of Substrate Concentration and Acid Pretreatment. 3rd International Conference on Global Sustainability and Chemical Engineering (Icgsce), 2018. 358.

125. Han, S.K. and H.S. Shin, Biohydrogen production by anaerobic fermentation of food waste. International Journal of Hydrogen Energy, 2004. 29(6): p. 569-577.

126. Reungsang, A. and C. Sreela-or, Bio-Hydrogen Production from Pineapple Waste Extract by Anaerobic Mixed Cultures. Energies, 2013. 6(4): p. 2175-2190.

127. Wang, C.H., P.J. Lin, and J.S. Chang, Fermentative conversion of sucrose and pineapple waste into hydrogen gas in phosphate-buffered culture seeded with municipal sewage sludge. Process Biochemistry, 2006. 41(6): p. 1353-1358.

128. Dey, P., et al., Lignocellulosic bioethanol production: prospects of emerging membrane technologies to improve the process – a critical review. Reviews in Chemical Engineering, 2020. 36(3): p. 333-367.

129. J. Itelima, F.O., E. Onwuliri, Isaac Onyimba, and S. Oforji, Bio-Ethanol Production from Banana, Plantain and Pineapple Peels by Simultaneous Saccharification and Fermentation Process. International Journal of Environmental Science and Development, 2013. 4(2): p. 213-216.

130. Choonut, A., M. Saejong, and K. Sangkharak, The Production of Ethanol and Hydrogen from Pineapple Peel by Saccharomyces Cerevisiae and Enterobacter Aerogenes. Energy Procedia, 2014. 52: p. 242-249.

131. Gil, L.S. and P.F. Maupoey, An integrated approach for pineapple waste valorisation. Bioethanol production and bromelain extraction from pineapple residues. Journal of Cleaner Production, 2018. 172: p. 1224-1231.

132. Hans, M., et al., A review on bioprocessing of paddy straw to ethanol using simultaneous saccharification and fermentation. Process Biochemistry, 2019. 85: p. 125-134.

133. Guilhen, S.N., et al., Pyrolytic temperature evaluation of macauba biochar for uranium adsorption from aqueous solutions. Biomass and Bioenergy, 2019. 122: p. 381-390.

134. Kwak, J.-H., et al., Biochar properties and lead (II) adsorption capacity depend on feedstock type, pyrolysis temperature, and steam activation. Chemosphere, 2019. 231: p. 393-404.

135. Wang, C., et al., Sorption behavior of Cr(VI) on pineapple-peel-derived biochar and the influence of coexisting pyrene. International Biodeterioration & Biodegradation, 2016. 111: p. 78-84.

136. Goula, A.M. and H.N. Lazarides, Integrated processes can turn industrial food waste into valuable food by-products and/or ingredients: The cases of olive mill and pomegranate wastes. Journal of Food Engineering, 2015. 167: p. 45-50.

137. Roda, A., et al., Effect of pre-treatments on the saccharification of pineapple waste as a potential source for vinegar production. Journal of cleaner production, 2016. 112: p. 4477-4484.

138. Conesa, C., et al., Microwaves as a pretreatment for enhancing enzymatic hydrolysis of pineapple industrial waste for bioethanol production. Food and Bioproducts Processing, 2016. 100: p. 203-213.

139. Silverstein, R.A., et al., A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. Bioresource technology, 2007. 98(16): p. 3000-3011.

140. Rehman, M., et al., Optimization of sono-assisted dilute sulfuric acid process for simultaneous pretreatment and saccharification of rice straw. International Journal of Environmental Science and Technology, 2014. 11(2): p. 543-550.

141. Kapoor, M., et al., Structural features of dilute acid, steam exploded, and alkali pretreated mustard stalk and their impact on enzymatic hydrolysis. Carbohydrate polymers, 2015. 124: p. 265-273.

142. Kaur, U., et al., Ethanol production from alkali-and ozone-treated cotton stalks using thermotolerant Pichia kudriavzevii HOP-1. Industrial Crops and Products, 2012. 37(1): p. 219-226.

143. Wang M., L.Z., Fang X., Wang L., Qu Y., Cellulolytic Enzyme Production and Enzymatic Hydrolysis for Second-Generation Bioethanol Production, in Biotechnology in China III: Biofuels and Bioenergy. Advances in Biochemical Engineering Biotechnology,, L.C. Bai FW., Huang H., Tsao G., Editor. 2012, Springer,: Berlin, Heidelberg.

144. Sassner, P., Galbe, M., & Zacchi, G, Techno-Economic Evaluation of Bioethanol Production from Three Different Lignocellulosic Materials. BIOMASS & BIOENERGY, 2008. 32(5): p. 422-430.

145. Merino, S.T. and J. Cherry, Progress and challenges in enzyme development for biomass utilization. Adv Biochem Eng Biotechnol, 2007. 108: p. 95-120.

146. Arianna Roda, D.M.D.F., Roberta Dordoni, Milena Lambri, Vinegar Production from Pineapple Wastes –Preliminary Saccharification Trials. CHEMICAL ENGINEERING TRANSACTIONS, 2014. 37: p. 607-612.

147. Stelmock, R.L., F.M. Husby, and A.L. Brundage, Application of Van Soest Acid Detergent Fiber Method for Analysis of Shellfish Chitin1. Journal of Dairy Science, 1985. 68(6): p. 1502-1506.

148. MILLER, G.L., Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry, 1959. 31(3): p. 426-428.

149. Sarkar, N., et al., Bioethanol production from agricultural wastes: An overview. Renewable Energy, 2012. 37: p. 19-27.

150. Zhao, X., L. Zhang, and D. Liu, Pretreatment of Siam weed stem by several chemical methods for increasing the enzymatic digestibility. Biotechnology Journal, 2010. 5(5): p. 493-504.

151. Gaurav Chaudhary, Lalit Kumar Singh, and S. Ghosh., Alkaline pretreatment methods followed by acid hydrolysis of Saccharum spontaneum for bioethanol production. Bioresource Technology, 2012. 124: p. 111-118.

152. van Putten, R.-J., et al., Hydroxymethylfurfural, A Versatile Platform Chemical Made from Renewable Resources. Chemical Reviews, 2013. 113(3): p. 1499-1597.

153. Klinke HB, Thomsen AB, and A. BK., Inhibition of ethanolproducing yeast and bacteria by degradation products produced during pre-treatment of biomass. Appl Microbiol Biotechnol, 2004. 66(1): p. 10-26.

154. Ishizaki, H. and K. Hasumi, Chapter 10 - Ethanol Production from Biomass, in Research Approaches to Sustainable Biomass Systems, S. Tojo and T. Hirasawa, Editors. 2014, Academic Press: Boston. p. 243-258.

155. Bensah, E.C. and M. Mensah, Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Technologies and Innovations. International Journal of Chemical Engineering, 2013. 2013: p. 719607.

156. Jeong, T.S., et al., Behaviors of glucose decomposition during acid-catalyzed hydrothermal hydrolysis of pretreated Gelidium amansii. Bioresource technology, 2012. 116: p. 435-440.

157. Jutakanoke, R., et al., Sugarcane leaves: Pretreatment and ethanol fermentation by Saccharomyces cerevisiae. Biomass and Bioenergy, 2012. 39: p. 283-289.

158. Yi, Y.-B., et al., Simple process for production of hydroxymethylfurfural from raw biomasses of girasol and potato tubers. Biomass and Bioenergy, 2012. 39: p. 484-488.

159. Beatriz CordenunsiI, F.S.-C.M.E.D.-R.A.Z.M.A.T.M.S.B.G.B.d.S.C.C.E.B.G.E., Carbohydrate composition of ripe pineapple (cv. perola)and the glycemic response in humans Food Science and Technology, 2010. 30(1): p. 282-288.

160. Fang, X., et al., [Progress on cellulase and enzymatic hydrolysis of lignocellulosic biomass]. Sheng Wu Gong Cheng Xue Bao, 2010. 26(7): p. 864-9.

161. Dowe, N., Assessing cellulase performance on pretreated lignocellulosic biomass using saccharification and fermentation-based protocols. Methods Mol Biol, 2009. 581: p. 233-45.

162. Vinzant, T.B., et al., Fingerprinting Trichoderma reesei hydrolases in a commercial cellulase preparation. Applied Biochemistry and Biotechnology, 2001. 91(1): p. 99-107.

163. Wang, M., et al., Bioethanol production from cotton stalk: A comparative study of various pretreatments. Fuel, 2016. 184: p. 527-532.

164. Hongjie Dai, S.O., Yue Huang, Huihua Huang, Utilization of pineapple peel for production of nanocellulose and film application. Cellulose, 2018. 25: p. 1743-1756.

165. Jönsson, L.J. and C. Martín, Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. Bioresource Technology, 2016. 199: p. 103-112.

166. Oshoma, C.E., et al., Screening of Non- Saccharomyces cerevisiae Strains for Tolerance to Formic Acid in Bioethanol Fermentation. PLOS ONE, 2015. 10(8): p. e0135626.

167. Ramaraj, J.T.C.Y.U.R., Fermentation of pineapple fruit peel wastes for bioethanol production. Biomass Conversion and Biorefinery, 2019. 9: p. 761-765

168. Yi-Huang Chang, K.-S.C., Chien-Yu Chen, Chuan-Liang Hsu,Tsan-Chang Chang, and Hung-Der Jang., Enhancement of the Efficiency of Bioethanol Production by Saccharomyces cerevisiaevia Gradually Batch-Wise and Fed-Batch Increasing the Glucose Concentration. fermentation, 2018. 4(45): p. 1-12.

169. Ricardo Soccol, C., et al., Chapter 5 - Lignocellulosic Bioethanol: Current Status and Future Perspectives, in Biofuels, A. Pandey, et al., Editors. 2011, Academic Press: Amsterdam. p. 101-122.

170. Australia, BAo 2012, *Biodiesel Plants in Australia*, Biofuels Association of Australia, viewed 08/02/2019, <http://www.biofuelsassociation.com.au/~biofuels/images/stories/pdf/biodieselmap.pdf>.

171 da Silva, ARG, Ortega, CET & Rong, B 2016, 'Techno-economic analysis of different pretreatment processes for lignocellulosic-based bioethanol production', *Bioresource Technology*, vol. 218, pp. 561-70.

Energy, DotEa 2018, *Tackling australia's food waste: Deapartment of the Environment and Energy, Fedral Government of Australia*, viewed 05/03/2018, <http://www.environment.gov.au/protection/waste-resource-recovery/food-waste>.

Espinoza-Acosta, JL, Torres-Chavez, PI, Carvajal-Millan, E, Ramirez-Wong, B, Bello-Perez, LA & Montano-Leyva, B 2014, 'Ionic Liquids and Organic Solvents for Recovering Lignin from Lignocellulosic Biomass', *Bioresources*, vol. 9, no. 2, pp. 3660-87.

Girotto, F, Alibardi, L & Cossu, R 2015, 'Food waste generation and industrial uses: A review', *Waste Management*, vol. 45, pp. 32-41.

Hegde, S, Lodge, JS & Trabold, TA 2018, 'Characteristics of food processing wastes and their use in sustainable alcohol production', *Renewable & Sustainable Energy Reviews*, vol. 81, pp. 510-23.

Kim, JS, Lee, YY & Kim, TH 2016, 'A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass', *Bioresource Technology*, vol. 199, pp. 42-8.

Kiran, EU, Trzcinski, AP & Liu, Y 2015, 'Platform chemical production from food wastes using a biorefinery concept', *Journal of Chemical Technology and Biotechnology*, vol. 90, no. 8, pp. 1364-79.

Kiran, EU, Trzcinski, AP, Ng, WJ & Liu, Y 2014, 'Bioconversion of food waste to energy: A review', *Fuel*, vol. 134, pp. 389-99.

Li, HQ, Qu, YS, Yang, YQ, Chang, SL & Xu, J 2016, 'Microwave irradiation - A green and efficient way to pretreat biomass', *Bioresource Technology*, vol. 199, pp. 34-41.

Li, L, Peng, XY, Wang, XM & Di Wu, D 2018, 'Anaerobic digestion of food waste: A review focusing on process stability', *Bioresource Technology*, vol. 248, pp. 20-8.

M.Galanakis, C 2012, 'Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications', *Trends in Food Science & Technology*, vol. 26, no. 2, pp. 68-87.

Ma, HZ, Yue, SY, Li, HG, Wang, QH & Tu, MB 2019, 'Recovery of lactic acid and other organic acids from food waste ethanol fermentation stillage: Feasibility and effects of substrates', *Separation and Purification Technology*, vol. 209, pp. 223-8.

Mathew, AK, Parameshwaran, B, Sukumaran, RK & Pandey, A 2016, 'An evaluation of dilute acid and ammonia fiber explosion pretreatment for cellulosic ethanol production', *Bioresource Technology*, vol. 199, pp. 13-20.

Mckenzie, S 2018, *Australia Bioenery policies and status of implementation 2018 updates* IEA Bioenergy IEA Bioenergy, https://www.ieabioenergy.com/wp-content/uploads/2018/10/CountryReport2018_Australia_final.pdf>.

Mirabella, N, Castellani, V & Sala, S 2014, 'Current options for the valorization of food manufacturing waste: a review', *Journal of Cleaner Production*, vol. 65, pp. 28-41.

Muhammad Waqasa, MR, Muhammad Daud Khanb, Abdul-Sattar Nizamia 2019, 'Conversion of Food Waste to Fermentation Products', *Encyclopedia of Food Security and Sustainability*, vol. 1, pp. 501-9.

Nazzaro, F, Fratianni, F, Ombra, MN, d'Acierno, A & Coppola, R 2018, 'Recovery of biomolecules of high benefit from food waste', *Current Opinion in Food Science*, vol. 22, pp. 43-54.

O'Connell, DD 2007, *Biofuels in Australia — issues and prospects*, CSW – 44A, RIRaD Corporation, http://biomassproducer.com.au/wp-content/uploads/2013/11/BiofuelsinAustralia_CSIRO.pdf>.

Paritosh, K, Kushwaha, SK, Yadav, M, Pareek, N, Chawade, A & Vivekanand, V 2017, 'Food Waste to Energy: An Overview of Sustainable Approaches for Food Waste Management and Nutrient Recycling', *Biomed Research International*.

Park, J, Jones, B, Koo, B, Chen, X, Tucker, M, Yu, JH, Pschorn, T, Venditti, R & Park, S 2016, 'Use of mechanical refining to improve the production of low-cost sugars from lignocellulosic biomass', *Bioresource Technology*, vol. 199, pp. 59-67.

Pham, TPT, Kaushik, R, Parshetti, GK, Mahmood, R & Balasubramanian, R 2015, 'Food waste-to-energy conversion technologies: Current status and future directions', *Waste Management*, vol. 38, pp. 399-408.

Ravindran, R & Jaiswal, AK 2016, 'A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities', *Bioresource Technology*, vol. 199, pp. 92-102.

Ren, YY, Yu, M, Wu, CF, Wang, QH, Gao, M, Huang, QQ & Liu, Y 2018, 'A comprehensive review on food waste anaerobic digestion: Research updates and tendencies', *Bioresource Technology*, vol. 247, pp. 1069-76.

Roger Farrell 2017, *Australia Biofuels Annual 2017*, ASI1712, USDA Foreign Agricultural Service Canberra, https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Biofuels%20Annual_Canberra_Australia_8-15-2017.pdf>.

Schieber, A, Stintzing, FC & Carle, R 2001, 'By-products of plant food processing as a source of functional compounds - recent developments', *Trends in Food Science & Technology*, vol. 12, no. 11, pp. 401-+.

Serna, LVD, Alzate, CEO & Alzate, CAC 2016, 'Supercritical fluids as a green technology for the pretreatment of lignocellulosic biomass', *Bioresource Technology*, vol. 199, pp. 113-20.

Sharma, HK, Xu, CB & Qin, WS 2019, 'Biological Pretreatment of Lignocellulosic Biomass for Biofuels and Bioproducts: An Overview', *Waste and Biomass Valorization*, vol. 10, no. 2, pp. 235-51.

Sindhu, R, Binod, P & Pandey, A 2016, 'Biological pretreatment of lignocellulosic biomass - An overview', *Bioresource Technology*, vol. 199, pp. 76-82.

Sonja Djtlas Jasna. Čanadanovic-Brunet, GC 2009, 'By-products of ruits processing as a source of phytochemicals', *Chemical Industry & Chemical Engineering Quarterly*, vol. 15, no. 4, pp. 191-202.

Sundaram, V & Muthukumarappan, K 2016, 'Influence of AFEX (TM) pretreated corn stover and switchgrass blending on the compaction characteristics and sugar yields of the pellets', *Industrial Crops and Products*, vol. 83, pp. 537-44.

team, AESp 2017, *Australian Energy Update 2017*, DotEa Energy, Department of the Environment and Energy, Government of Australia CANBERRA, viewed 8/1/2019, <https://www.energy.gov.au/sites/default/files/energy-update-report-2017.pdf>.

Vigano, J, Machado, APD & Martinez, J 2015, 'Sub- and supercritical fluid technology applied to food waste processing', *Journal of Supercritical Fluids*, vol. 96, pp. 272-86.

W.Babbitt, TATaC 2018, ' Introduction to Sustainable Food Waste-To-energy Systems', in TATaC W.Babbitt (ed.), *Sustainable Food Waste-to-Energy Systems*, Academic Press, vol. 1, pp. 1-10.

Zhang, K, Pei, ZJ & Wang, DH 2016, 'Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review', *Bioresource Technology*, vol. 199, pp. 21-33.

Adebo, OA & Gabriela Medina-Meza, I 2020, 'Impact of Fermentation on the Phenolic Compounds and Antioxidant Activity of Whole Cereal Grains: A Mini Review', *Molecules (Basel, Switzerland)*, vol. 25, no. 4, p. 927.

Australian Horticulture Statistics Handbook 2017/18 2019, file:///C:/Users/u1092840/Downloads/AH15001-Australian-Horticulture-Statistics-Handbook-Fruit-190402.pdf >.

Balat, M 2011, 'Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review', *Energy Conversion and Management*, vol. 52, no. 2, pp. 858-75.

Banerjee, S, Ranganathan, V, Patti, A & Arora, A 2018, 'Valorisation of pineapple wastes for food and therapeutic applications', *Trends in Food Science & Technology*, vol. 82, pp. 60-70.

Beatriz CordenunsiI, FS-CMED-RAZMATMSBGBdSCCEBGE 2010, 'Carbohydrate composition of ripe pineapple (cv. perola)and the glycemic response in humans ', *Food Science and Technology*, vol. 30, no. 1, pp. 282-8.

Binod, PS, K.; Sindhu, R.; Janu, K.U.; Sukumaran, R.K.; and Pandey, A. 2012, 'Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse.', *Journal of Renewable Energy*, vol. 37, no. 1, pp. 109-16.

Birla, SL & Pitchai, K 2017, '18 - Simulation of microwave processes', in M Regier, et al. (eds), *The Microwave Processing of Foods (Second Edition)*, Woodhead Publishing, pp. 407-31.

Cantarella, M, Cantarella, L, Gallifuoco, A, Spera, A & Alfani, F 2004, 'Effect of inhibitors released during steam-explosion treatment of poplar wood on subsequent enzymatic hydrolysis and SSF', *Biotechnol Prog*, vol. 20, no. 1, pp. 200-6.

Conesa, C, Seguí, L, Laguarda-Miró, N & Fito, P 2016, 'Microwaves as a pretreatment for enhancing enzymatic hydrolysis of pineapple industrial waste for bioethanol production', *Food and Bioproducts Processing*, vol. 100, pp. 203-13.

Conesa, C, Seguí, L, Laguarda-Miró, N & Fito, P 2016, 'Microwave-assisted alkali pretreatment for enhancing pineapple waste saccharification', *Bioresources*, vol. 11, no. 3, pp. 6518-31.

de la Hoz, A, Díaz-Ortiz, Á & Moreno, A 2005, 'Microwaves in organic synthesis. Thermal and non-thermal microwave effects', *Chemical Society Reviews*, vol. 34, no. 2, pp. 164-78.

Dowe, N 2009, 'Assessing cellulase performance on pretreated lignocellulosic biomass using saccharification and fermentation-based protocols', *Methods Mol Biol*, vol. 581, pp. 233-45.

Fang, X, Qin, Y, Li, X, Wang, L, Wang, T, Zhu, M & Qu, Y 2010, '[Progress on cellulase and enzymatic hydrolysis of lignocellulosic biomass]', *Sheng Wu Gong Cheng Xue Bao*, vol. 26, no. 7, pp. 864-9.

Hong Zhang, RY, Yunze He, Ali Foudazi, Liang Cheng and Guiyun Tian 2017, 'A Review of Microwave Thermography Nondestructive Testing and Evaluation', *Sensors* vol. 17, pp. 1-33.

Hongjie Dai, SO, Yue Huang, Huihua Huang 2018, 'Utilization of pineapple peel for production of nanocellulose and film application', *Cellulose*, vol. 25, pp. 1743-56.

Hossain, MA & Rahman, SMM 2011, 'Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple', *Food Research International*, vol. 44, no. 3, pp. 672-6.

Hu, Z & Wen, Z 2008, 'Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment', *Biochemical Engineering Journal*, vol. 38, pp. 369-78.

Jackowiak, D, Bassard, D, Pauss, A & Ribeiro, T 2011, 'Optimisation of a microwave pretreatment of wheat straw for methane production', *Bioresour Technol*, vol. 102, no. 12, pp. 6750-6.

Jin, S, Zhang, G, Zhang, P, Li, F, Wang, S, Fan, S & Zhou, S 2016, 'Microwave assisted alkaline pretreatment to enhance enzymatic saccharification of catalpa sawdust', *Bioresource Technology*, vol. 221, pp. 26-30.

Kiran R Mahale, APT 2020, 'Effects of alkali, acidic and enzymatic pre-treatments on the ethanol fermentation of pineapple (Ananas comosus) processing waste. ', Originol reserach University of Southern Queensland

Klinke, HB, Thomsen, AB & Ahring, BK 2004, 'Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass', *Appl Microbiol Biotechnol*, vol. 66, no. 1, pp. 10-26.

Kumar, C, Joardder, M., Karim, A., Millar, G.J., Amin, Z., 2014, 'Temperature redistribution modelling during intermittentmicrowave connective heating', *Procedia Eng*, vol. 90, pp. 544-9.

Li, T, Shen, P, Liu, W, Liu, C, Liang, R, Yan, N & Chen, J 2014, 'Major Polyphenolics in Pineapple Peels and their Antioxidant Interactions', *International Journal of Food Properties*, vol. 17, no. 8, pp. 1805-17.

López-Linares, JC, García-Cubero, MT, Coca, M & Lucas, S 2021, 'Efficient biobutanol production by acetone-butanol-ethanol fermentation from spent coffee grounds with microwave assisted dilute sulfuric acid pretreatment', *Bioresource Technology*, vol. 320, p. 124348.

Mikulski, D & Kłosowski, G 2020, 'Microwave-assisted dilute acid pretreatment in bioethanol production from wheat and rye stillages', *Biomass and Bioenergy*, vol. 136, p. 105528.

Mikulski, D, Kłosowski, G, Menka, A & Koim-Puchowska, B 2019, 'Microwave-assisted pretreatment of maize distillery stillage with the use of dilute sulfuric acid in the production of cellulosic ethanol', *Bioresource Technology*, vol. 278, pp. 318-28.

MILLER, GL 1959, 'Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar', *Analytical Chemistry*, vol. 31, no. 3, pp. 426-8.

Nowicka, A, Zieliński, M & Dębowski, M 2020, 'Microwave support of the alcoholic fermentation process of cyanobacteria Arthrospira platensis', *Environmental Science and Pollution Research*, vol. 27, no. 1, pp. 118-24.

Puligundla, P, Oh, S-E & Mok, C 2016, 'Microwave-assisted pretreatment technologies for the conversion of lignocellulosic biomass to sugars and ethanol: a review', *Carbon letters*, vol. 17, no. 1, pp. 1-10.

Rajeswari, G, Arutselvy, B & Jacob, S 2020, 'Delignification of Aloe Vera Rind by Mild Acid Associated Microwave Pretreatment to Persuade Enhanced Enzymatic Saccharification', *Waste and Biomass Valorization*, vol. 11, no. 11, pp. 5965-75.

Ricardo Soccol, C, Faraco, V, Karp, S, Vandenberghe, LPS, Thomaz-Soccol, V, Woiciechowski, A & Pandey, A 2011, 'Chapter 5 - Lignocellulosic Bioethanol: Current Status and Future Perspectives', in A Pandey, et al. (eds), *Biofuels*, Academic Press, Amsterdam, pp. 101-22.

Robak, K & Balcerek, M 2020, 'Current state-of-the-art in ethanol production from lignocellulosic feedstocks', *Microbiological Research*, vol. 240.

Roberts B A, SCR 2005, 'Scale-up of Microwave-Assisted Organic Synthesis.', in LP Tierney J P (ed.), *Microwave Assisted Organic Synthesis*, Oxford, Blackwell, pp. 237-48.

Ruangviriyachai, C, Niwaswong, C, Kosaikanon, N, Chanthai, S & Chaimart, P 2010, 'Pineapple Peel Waste for Bioethanol Production', *Journal of Biotechnology*, vol. 150, pp. 10-.

Samanian N, MM 2016, 'Thermography, a new approach in food science studies: a review', *MOJ Food Process Technol*, vol. 2, no. 3, pp. 110-9.

Sarkar, N, Ghosh, SK, Bannerjee, S & Aikat, K 2012, 'Bioethanol production from agricultural wastes: an overview', *Renewable Energy*, vol. 37, no. 1, pp. 19-27.

Singh, R, Tiwari, S, Srivastava, M & Shukla, A 2014, 'Microwave Assisted Alkali Pretreatment of Rice Straw for Enhancing Enzymatic Digestibility', *Journal of Energy*, vol. 2014, p. 483813.

Stelmock, RL, Husby, FM & Brundage, AL 1985, 'Application of Van Soest Acid Detergent Fiber Method for Analysis of Shellfish Chitin1', *Journal of Dairy Science*, vol. 68, no. 6, pp. 1502-6.

Yu, KL, Chen, WH, Sheen, HK, Chang, JS, Lin, CS, Ong, HC, Show, PL & Ling, TC 2020, 'Bioethanol production from acid pretreated microalgal hydrolysate using microwave-assisted heating wet torrefaction', *Fuel*, vol. 279.

Zhu, S, Wu, Y, Yu, Z, Zhang, X, Li, H & Gao, M 2006, 'The effect of microwave irradiation on enzymatic hydrolysis of rice straw', *Bioresource Technology*, vol. 97, no. 15, pp. 1964-8.

Zhu, S, Wu, Y, Yu, Z, Zhang, X, Wang, C, Yu, F & Jin, S 2006, 'Production of ethanol from microwave-assisted alkali pretreated wheat straw', *Process Biochemistry*, vol. 41, no. 4, pp. 869-73.

1. Murcott, S., *Arsenic Contamination in the World*. 2012, London, United Kingdom IWA Publishing
2. Podgorski, J. and M. Berg, *Global threat of arsenic in groundwater.* Science, 2020. **368**(6493): p. 845.
3. Scott Fendorf1, H.A.M., Alexander van Geen, *Spatial and temporal variations of groundwater arsenic in South and Southeast Asia.* Science, 2010. **328**(5982): p. 1123-1127.
4. Smith, E., et al., *Arsenic in Australian environment: an overview.* J Environ Sci Health A Tox Hazard Subst Environ Eng, 2003. **38**(1): p. 223-39.
5. Nordstrom, D.K., *Worldwide occurrences of arsenic in ground water*. 2002, American Association for the Advancement of Science.
6. Mandal, B.K. and K.T. Suzuki, *Arsenic round the world: a review.* Talanta, 2002. **58**(1): p. 201-235.
7. Chen, Y., et al., *Arsenic exposure at low-to-moderate levels and skin lesions, arsenic metabolism, neurological functions, and biomarkers for respiratory and cardiovascular diseases: Review of recent findings from the Health Effects of Arsenic Longitudinal Study (HEALS) in Bangladesh.* Toxicology and Applied Pharmacology, 2009. **239**(2): p. 184-192.
8. *Arsenic in Drinking-water* W.H. Organization, Editor. 2011, World Health Organization. p. 1-24.
9. Rehman I., I.M., Ali L., Muhammad S., Din I.U., Yaseen M., Ullah H., *Potentially toxic elements' occurrence and risk assessment through water and soil of Chitral urban environment, Pakistan: a case study.* Environ. Geochem. Health, 2020: p. 1-14.
10. Mohammed Abdul, K.S., et al., *Arsenic and human health effects: A review.* Environmental Toxicology and Pharmacology, 2015. **40**(3): p. 828-846.
11. Shankar, S., U. Shanker, and Shikha, *Arsenic Contamination of Groundwater: A Review of Sources, Prevalence, Health Risks, and Strategies for Mitigation.* The Scientific World Journal, 2014. **2014**: p. 304524.
12. Amen, R., et al., *A critical review on arsenic removal from water using biochar-based sorbents: The significance of modification and redox reactions.* Chemical Engineering Journal, 2020. **396**: p. 125195.
13. Carneiro, M.A., et al., *Current Trends of Arsenic Adsorption in Continuous Mode: Literature Review and Future Perspectives.* Sustainability, 2021. **13**(3): p. 1186.
14. Wan, C., et al., *Pilot-scale combined adsorption columns using activated carbon and zeolite for hazardous trace elements removal from wastewater of entrained-flow coal gasification.* Process Safety and Environmental Protection, 2021. **147**: p. 439-449.
15. Wang, L., et al., *Performance and mechanisms for remediation of Cd(II) and As(III) co-contamination by magnetic biochar-microbe biochemical composite: Competition and synergy effects.* Science of the Total Environment, 2021. **750**.
16. Gérente, C., et al., *Removal of arsenic(V) onto chitosan: From sorption mechanism explanation to dynamic water treatment process.* Chemical Engineering Journal, 2010. **158**(3): p. 593-598.
17. G.M. Sawood, S.G., *Arsenic remediation of the waste water using adsorbent: a review.* Int. J. Eng. Technol., Sci. Res., 2018. **5**: p. 1054-1070.
18. Anastopoulos, I., et al., *Agricultural biomass/waste as adsorbents for toxic metal decontamination of aqueous solutions.* Journal of Molecular Liquids, 2019. **295**: p. 111684.

19. Xue, K.H., et al., *Enhanced As(III) transformation and removal with biochar/SnS2/phosphotungstic acid composites: Synergic effect of overcoming the electronic inertness of biochar and W2O3(AsO4)2 (As(V)-POMs) coprecipitation.* Journal of Hazardous Materials, 2021. **408**.
20. Mukherjee, S., et al., *Efficacy of agricultural waste derived biochar for arsenic removal: Tackling water quality in the Indo-Gangetic plain.* Journal of Environmental Management, 2021. **281**.
21. Stelmock, R.L., F.M. Husby, and A.L. Brundage, *Application of Van Soest Acid Detergent Fiber Method for Analysis of Shellfish Chitin1.* Journal of Dairy Science, 1985. **68**(6): p. 1502-1506.
22. Alkurdi, S.S.A., et al., *Effect of pyrolysis conditions on bone char characterization and its ability for arsenic and fluoride removal.* Environmental Pollution, 2020. **262**: p. 114221.
23. Dang T, M.P., Fitzpatrick R, Mosley LM, *Assessment of the Binding of Protons, Al and Fe to Biochar at Different pH Values and Soluble Metal Concentrations.* Water, 2018. **10**(1): p. 55.
24. Stelmock RL, H.F., Brundage AL, *Application of Van Soest Acid Detergent Fiber Method for Analysis of Shellfish Chitin1.* Journal of Dairy Science, 1985. **68**(6): p. 1502-1506.
25. D1762-84, A., *AnnualBookofASTMStandards*. Vol. D1762-84.
26. Parthasarathy, P., K.S. Narayanan, and L. Arockiam, *Study on kinetic parameters of different biomass samples using thermo-gravimetric analysis.* Biomass and Bioenergy, 2013. **58**: p. 58-66.
27. Cao, X. and W. Harris, *Properties of dairy-manure-derived biochar pertinent to its potential use in remediation.* Bioresour Technol, 2010. **101**(14): p. 5222-8.
28. Keiluweit, M., et al., *Dynamic Molecular Structure of Plant Biomass-Derived Black Carbon (Biochar).* Environmental Science & Technology, 2010. **44**(4): p. 1247-1253.
29. Keiluweit, M., et al., *Dynamic molecular structure of plant biomass-derived black carbon (biochar).* Environ Sci Technol, 2010. **44**(4): p. 1247-53.
30. Ahmad, M., et al., *Effects of pyrolysis temperature on soybean stover- and peanut shell-derived biochar properties and TCE adsorption in water.* Bioresour Technol, 2012. **118**: p. 536-44.
31. C., S.B., *Fundamentals of Fourier Transform Infrared Spectroscopy.* 2011: CRC press. .
32. Stuart, B., *Infrared Spectroscopy* Kirk-Othmer Encyclopedia of Chemical Technology,, 2000: p. 1-18.
33. Deng, H., Yu, H., Chen, M., and Ge, C., *Sorption of atrazine in tropical soil by biochar prepared from cassava waste.* Bioresource 2014. **9**(4): p. 6627-6643.
34. Bomin Fu, C.G., Lin Yue, Jiwei Luo, Dan Feng, Hui Deng, and Huamei Yu, *Characterization of Biochar Derived from Pineapple Peel Waste and Its Application for Sorption of Oxytetracycline from Aqueous Solution.* bioresources, 2016. **11**(1): p. 9017-9035.
35. Ho, M.P. and A.K.T. Lau, *12 - Amorphous carbon nanocomposites*, in *Fillers and Reinforcements for Advanced Nanocomposites*, Y. Dong, R. Umer, and A.K.-T. Lau, Editors. 2015, Woodhead Publishing. p. 309-328.
36. Zhang, K., et al., *Characterization of biochar derived from rice husks and its potential in chlorobenzene degradation.* Carbon, 2018. **130**: p. 730-740.
37. Chamseddine Guizani, K.H., Lionel Limousy, Mejdi Jeguirim, *New insights on the structural evolution of biomass char upon pyrolysis as revealed by the Raman spectroscopy and elemental analysis.* Carbon letters, 2017. **119**: p. 519-521.
38. Smith, M.W., Pecha, B., Helms, G., Scudiero, L., Garcia-Perez, M, *Chemical and morphological evaluation of chars produced from primary biomass constituents: cellulose, xylan, and lignin.* Biomass and Bioenergy, 2017. **104**: p. 17-35.
39. Aup-Ngoen, K. and M. Noipitak, *Effect of carbon-rich biochar on mechanical properties of PLA-biochar composites.* Sustainable Chemistry and Pharmacy, 2020. **15**.

40. Rojas-Mayorga, C.K., Mendoza-Castillo,D.I.,Bonilla-Petriciolet,A.,Silvestre Albero, J.,, *Tailoring the adsorption behavior of bonechar for heavy metal removal from aqueous solution.* Adsoption Science Technol, 2016. **34**: p. 368-387.
41. Patel, S., Han,J.,Qiu,W.,Gao,W.,, *Synthesis and characterisation of mesoporous bone char obtained by pyrolysis of animal bones, for environmental application* J. Environ.Chem.Eng., 2015. **3** p. 2368-2377.
42. Person, A., Bocherens,H.,Sali ege, J.-F.,Paris,F.,Zeitoun,V.,Gerard,M.,. *Early diagenetic evolution of bone phosphate : an X-raydiffractometry analysis.* J. Archaeol.Sci, 1995. **22**: p. 211-221
43. Kang, D.-S., Lee,S.-M.,Lee,S.-H.,Roh,J.-S.,, *X-raydiffraction analysis of the crystallinity of phenolic resin-derived carbon as a function of the heating rate during the carbonization process.* Carbon letters, 2018. **27**: p. 108-111.
44. Fan, J., et al., *Enhanced As (V) Removal from Aqueous Solution by Biochar Prepared from Iron-Impregnated Corn Straw.* Journal of Chemistry, 2018. **2018**: p. 5137694.
45. Ma, X., et al., *Study of Biochar Properties by Scanning Electron Microscope – Energy Dispersive X-Ray Spectroscopy (SEM-EDX).* Communications in Soil Science and Plant Analysis, 2016. **47**(5): p. 593-601.
46. Susan S.A. Alkurdi, R.A.A.-J., JochenBundschuh, Les Bowtell, Stafford McKnight, *Effect of pyrolysis conditions on bone char characterization and its ability for arsenic and fluoride removal.* Environmental Pollution, 2020. **262**: p. 114221
47. Oni, B.A., O. Oziegbe, and O.O. Olawole, *Significance of biochar application to the environment and economy.* Annals of Agricultural Sciences, 2019. **64**(2): p. 222-236.
48. Luo, Y., et al., *Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH.* Soil Biology and Biochemistry, 2011. **43**(11): p. 2304-2314.
49. Awad, Y.M., et al., *Carbon and nitrogen mineralization and enzyme activities in soil aggregate-size classes: Effects of biochar, oyster shells, and polymers.* Chemosphere, 2018. **198**: p. 40-48.

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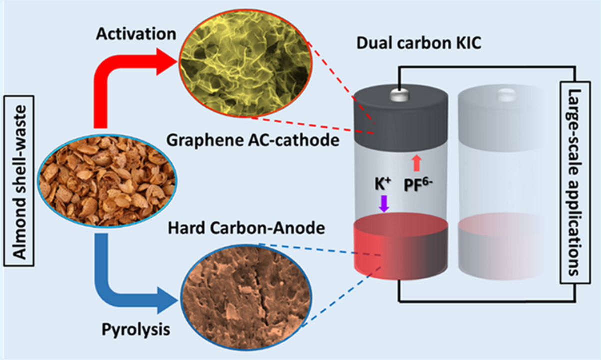

ABSTRACT: Potassium-ion storage devices are attracting tremendous attention for wide-ranging applications on account of their low cost, fast charge transport in electrolytes, and large working voltage. However, developing cost-effective, high-energy electrodes with excellent structural stability to ensure long-term cycling performance is a major challenge. In this contribution, we have derived two different forms of carbon materials from almond shells using different chemical treatments. For instance, hard carbon (HC) and graphene-like activated carbon (AC) nanosheets are developed by employing simple carbonization and chemical activation routes, respectively. The resultant hard carbon (AS-HC) and activated carbon (AS-AC) exhibit outstanding electrochemical performance as negative and positive electrodes in a potassium-ion battery (KIB), respectively, through their tailor-made surface properties. These promising benefits pave a way to construct a biomass-derived carbon potassium-ion capacitor (KIC) by employing AS-HC as the negative electrode and AS-AC as the positive electrode in a K-based electrolyte. The as-fabricated KIC delivers a reasonable specific energy of 105 Wh/kg and excellent cycling life with negligible capacitance fading over 10 000 cycles. This "waste-to-wealth" approach can promote the development of sustainable KICs at low cost and inspire their use for fast-rate K-based energy storage applications.

KEYWORDS: *biomass waste, hard carbon, graphene-like carbon, potassium-ion capacitor, high energy, high power*

■ INTRODUCTION

Currently, lithium-ion batteries (LIBs) and supercapacitors are effectively fulfilling the need of commercial appliances, such as portable devices and electric vehicles, and overriding other energy storage technologies with their abilities to offer high energy and high power, separately.[1−4] However, emergent applications including electric vehicles require a device that can efficiently deliver high power and high energy at the same time for a large number of cycles. Such critical requirements pose new challenges on existing technologies and prompt researchers to find an alternative device. A hybrid-ion capacitor (HIC) is a fast developing system, which combines the best characteristics of batteries and supercapacitors[5−8] and has the capability to deliver large energy densities at high rates for a reasonable number of cycles.[9] Previous research revealed that these HICs can provide an energy between 200 and 60 Wh/kg (considering the mass of active materials), which is superior to that of a conventional supercapacitor,[10,11] while their specific power is ranged between 200 and 20 000W/kg, which is significantly higher than those of batteries.[12,13] Nevertheless, the availability of lithium resources for the production of LIBs is restricted and the cost of raw materials shows an increasing trend, which foster the need for alternative cost-effective and high-performance energy systems.[14,15]

Sodium (Na, 2.6%) and potassium (K, 2.1%) are abundantly available in the earth's crust compared to Li (0.0017%), making them promising alternatives to promote in batteries.[13,16] Moreover, in the periodic table, both K and Na belong to the same group after Li, thereby exhibiting identical physicochemical characteristics. Hence, research on Na-ion/K-ion storage technologies is emerging remarkably to commercialize effective alternative energy storage systems.[17−21] Among them, K-ion storage devices are particularly emphasized due to their relatively higher working voltage and better ionic conductivity in electrolytes than those of Na.[22−27] For instance, the K/K^+ redox couple holds a potential of −2.93 V (vs standard hydrogen electrode (SHE)), which is lower than that of Na/Na^+ (−2.71 V vs SHE) and very near to the

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Li/Li$^+$ redox potential of −3.04 V vs SHE, ensuring the practical viability of high energy density and high operating potential of K-ion based energy storage devices.[13,25] Additionally, K$^+$ ions facilitate fast transportation in the propylene carbonate electrolyte because of the weaker Lewis acidity that causes a smaller Stokes radius for K$^+$ (3.6 Å), whereas Li$^+$ and Na$^+$ have values of 4.8 and 4.6 Å, respectively, in the same electrolyte.[28] Despite these fascinating advantages, developing high-energy electrode materials (both anode and cathode), which guarantees large energy values along with good structural stability to ensure long cycling performance, is a great challenge.[29,30]

Several anode and cathode materials have been explored for potassium-ion capacitor (KIC) devices.[23,31−35] Among them, carbon-based materials are encouraged as promising candidates for KICs due to their tailor-made surface properties, high electrical conductivity, and outstanding electrolyte accessibility. Generally, waste-derived pure carbons are perceived as highly competent electrode materials for various applications due to their low cost and rich abundant sources. The development of full KICs from biowaste-derived materials will not only significantly reduce the cost of raw materials, leading to low production cost, but also provide a sustainable and clean environment.

Herein, we have developed a novel dual carbon KIC by designing two different forms of carbon electrodes using a single source of biomass (almond shells), which could function as both anode and cathode materials. Briefly, using different chemical treatments, the surface properties of the carbon materials, such as surface area and pore-size distribution, were tuned to form a hard carbon (AS-HC) anode and a graphene-like activated carbon (AS-AC) cathode using almond shells as a single source of carbon-rich biowaste. Although almond shell-derived carbon is explored in other applications such as lithium−sulfur batteries[36] and microbial fuel cells,[37] they have never been investigated as anodes and cathodes for KICs. The individual electrochemical properties of the HC anode and the AC cathode in a K-ion battery (KIB) with a half-cell configuration were examined to investigate their working potential window and respective capacities. Finally, a high-performance full KIC was assembled using the HC anode and the AC cathode, which delivered a superior energy density of 105 Wh/kg and ultralong cycling life of over 10 000 cycles with a very small capacitance loss. Thus, the present investigation will open an opportunity to develop dual carbon batteries using low-cost biowaste materials, thereby providing a "waste-to-wealth" strategy.

■ EXPERIMENTAL SECTION

Hard Carbon-Derived from Almond Shells (HC). Almond shells were collected from the local market and dried in an electric oven at 80 °C for 24 h. The shells were crushed using a conventional kitchen grinder to make a fine powder. A suitable amount of almond shell powder was then washed thoroughly with water and 2 M HCl to remove all of the dirt and foreign particles and then dried in an oven for the next 24 h to achieve constant weight before carbonization. The dried biomass powder was calcined at different temperatures of 900, 1000, and 1100 °C for 2 h in nitrogen gas. The heating rate was adjusted to 3 °C/min. The product was washed repeatedly with water and ethanol and the resultant black product was then dried in air. The samples were thereafter called HC-900, HC-1000, and HC-1100, respectively.

Graphene-like Activated Carbon from Almond Shells (AC). To prepare activated carbon, potassium hydroxide (KOH) was used as an activating agent. Cleaned and dried almond shell powder was crushed with KOH in a 1:2 wt % ratio in a grinder and subsequently heated at three different temperatures of 700, 800, and 900 °C under a N_2 atmosphere for 2 h. The obtained biomass products were then repetitively washed with 10% HCl, water, and ethanol. The final product was dried at 80 °C in a vacuum oven overnight. The products prepared at 700, 800, and 900 °C were labeled AC-700, AC-800, and AC-900, respectively.

Materials Characterization. The structural and compositional information of hard carbon (HC) and activated carbon (AC) samples prepared from almond shells was extracted using X-ray diffraction (XRD, Rigaku SmartLab diffractometer), Raman (Renishaw inVia Raman microscope), and X-ray photoelectron spectroscopy (XPS, Kratos AXIS Supra spectrometer) techniques. The detailed investigation of surface morphologies and structures was performed by a field emission scanning electron microscope (FE-SEM, Tescan Mira) and a transmission electron microscope (TEM, JEOL-1400). N_2 adsorption−desorption experiments were performed to determine the surface area of the biomass-derived carbons using a Micromeritics 3 Flex instrument.

Electrode Fabrication and Testing. The electrochemical assessment of AS-HC (hard carbon) and AS-AC (activated carbon) in the K-ion battery was performed using coin-cell design. The electrodes (anode and cathode) were prepared by mixing the active material (HC or AC), Super P, and poly(vinylidene fluoride) (PVDF) binder in a weight ratio of 85:5:10 and making a paste with *N*-methyl-2-pyrrolidone (NMP). Subsequently, the obtained material was pasted on an Al foil substrate and further dried in an oven. K-ion batteries in a half-cell configuration were assembled using active materials as working electrodes, K-foil as the counter electrode, and 1 M KPF_6 in ethylene carbonate/dimethyl carbonate (EC/DMC) (1:1 v/v) as the electrolyte with a glass microfiber separator. The AS-AC cathode was cycled between 1.5 and 4.5 V (vs K/K$^+$), while the AS-HC anode was tested between 0.01 and 2.5 V (vs K/K$^+$). The average mass loaded on Al foil was 1.1 and 1.9 mg/cm^2 for HC and AC electrodes, respectively. Prior to the fabrication of the full KIC cell, the AS-HC anode was prepotassiated using the half-cell configuration at 0.1 A/g. The AS-HC//AS-AC KIC cell was cycled within 1−4 V, and the total mass loading of both electrodes was maintained to be 3.8 mg/cm^2. All of the electrochemical experiments were conducted using a Biologic VMP-300 potentiostat. Power (P) and energy (E) were calculated using the following equations

$$P = \frac{\Delta V \times i}{m} \tag{1}$$

$$E = \frac{P \times t}{3600} \tag{2}$$

$$\Delta V = \frac{(E_{\max} + E_{\min})}{2} \tag{3}$$

Here, $E_{\max}$ and $E_{\min}$ are the max and min voltages at the discharge (V), i is the discharge current (A), t is the discharge time (s), and m is the mass loading in both anode and cathode (g).

■ RESULTS AND DISCUSSION

Hard Carbon (HC) Anode Derived from Almond Shells. The structural features of the carbonized AS-HC samples were revealed by XRD analyses of the HC samples. As seen from Figure 1a, two broad diffraction peaks corresponding to planes (002) and (101) around 23.3 and 43.5° are observed, authorizing the presence of the graphitic domain in the biomass-derived hard carbons,[38] which is typical of the hard carbon. The interlayer $d_{(002)}$-spacing for the hard carbon was determined to be 0.39 nm using Bragg's formula. Such a large interlayer spacing is beneficial for the de/intercalation of potassium ions between the layers. It should also be noted that the intensity of diffraction peaks increases with temperature.

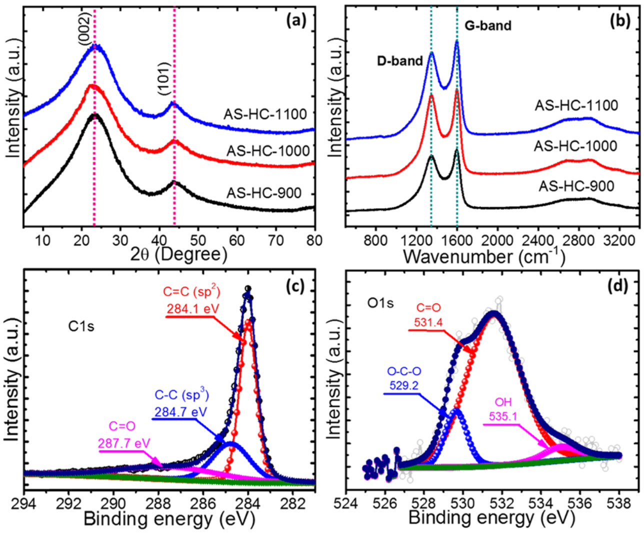

Figure 1. (a, b) XRD patterns and Raman spectra of hard carbons prepared at 900, 1000, and 1100 °C from almond shells. (c, d) Core-level XPS spectra of C 1s and O 1s of the HC-1000 sample.

The extent of graphitization was further investigated using Raman spectroscopy (Figure 1b). The strong peaks observed at ~1354 and 1591 cm^{-1} can be attributed to the D band and G band, corresponding to the disordered carbon structures and stretching vibrations of sp^2 carbon C═C sites, respectively.[39] The intensity ratio of the D to G band (I_D/I_G) reflects the degree of graphitic ordering in the carbon materials. The I_D/I_G ratio of hard carbon is around 0.92 for all three samples, suggesting the existence of fewer defects and smaller structural deformation with temperature. In addition, a small hump with maximum at 2782 cm^{-1} is a characteristic peak of two-dimensional (2D) materials, demonstrating the presence of more stacked graphite-like layers in HC as well as signifying the high degree of ordering of the graphitic layers evidently. The elemental composition in the HC samples was studied by XPS analysis. The survey spectrum of AS-HC-1000 (Figure S1) divulged only two characteristic peaks at 284 and 532 eV, which can be associated with the C 1s and O 1s signals, respectively, confirming the absence of inorganic impurities normally found in biomass-derived materials. The relative contents of C and O were found to be 95.37 and 4.63%, respectively. The deconvoluted peaks in C 1s spectra (see Figure 1c) can be attributed to C5C (284.1), C−C (284.7), and C5O groups (287.7 eV), respectively, while the O 1s spectra of hard carbon exhibit three different oxygen-containing groups such as OH^-, C5O, and O−C−O with binding energies of 535.1, 531.4, and 529.2 eV, respectively, as shown in Figure 1d.

The surface morphological features of the resultant AS-HC were revealed by FE-SEM and TEM analyses that confirm the formation of a sheetlike structure with a smooth surface (Figure 2a−c). No significant difference in the microstructure was observed for AS-HC samples prepared at three different temperatures (Figure S2). The TEM and high-resolution TEM (HRTEM) images (Figure 2d,e) further validated the formation of sheetlike nanostructures that matches with the SEM image of HC-1000 (Figure 2b). In the HRTEM images, two layers of carbon sheets can be clearly observed, which might provide an additional surface for the storage of potassium ions in addition to nanolevel graphitic layers.

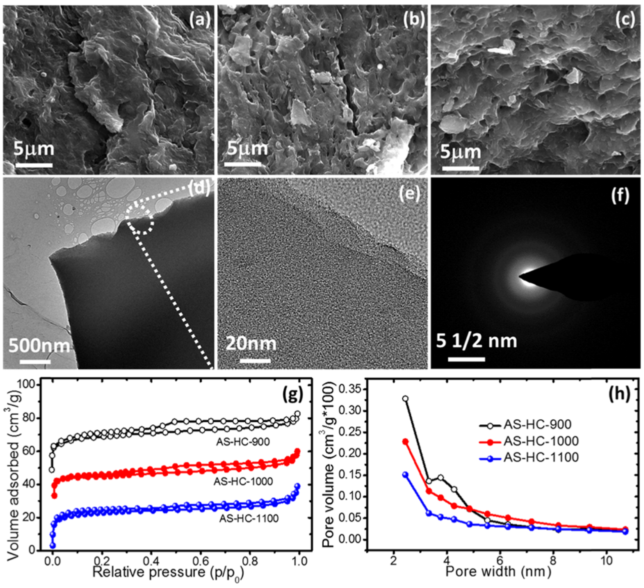

Figure 2. (a−c) SEM images of HC-900, HC-1000, and HC-1100, respectively. (d−f) TEM image, HRTEM image, and SAED pattern of the HC-1000 sample, respectively. (g, h) Nitrogen adsorption/desorption isotherm of HC samples prepared at three different temperatures with corresponding pore-size distribution curves.

Besides, the selected area electron diffraction (SAED) pattern (Figure 2f) offers two bright diffused rings, confirming the formation of graphitic carbon materials. The surface area analysis of HC samples is displayed in Figure 2g,f. All adsorption−desorption profiles of biomass-derived samples present typical characteristics of type IV isotherms, indicating their mesoporous nature. The apparent increase of the N_2-adsorption branch at high relative pressures ($P/P_0 > 0.9$) suggests the existence of numerous slitlike pores and multilayer sorption. As can be seen from Figure 2g, the Brunaeur−Emmett−Teller (BET) surface areas were 127, 84, and 42 m^2/g for AS-HC-900, AS-HC-1000, and AS-HC-1100 °C samples, respectively. The decreasing trend of the BET surface area with pyrolysis temperature might be attributed to the micropore closure during carbonization at high temperatures. The existence of mesopores with the size of ~3−4 nm was also observed for all AS-HCs (Figure 2h). Thus, the unique surface features of HC (such as rich pores and good surface area) may offer well-organized paths for the rapid transport of electrolyte ions for energy storage applications.

The resultant AS-HC samples were tested as anode materials in the potassium-ion battery using the half-cell configuration. Figure 3a displays the cyclic voltammetry (CV) curves measured at 0.2 mV/s between 0.01 and 2.5 V (vs K/K^+). At the first cycle, a broad reduction peak (potassiation) at around ~1.0 V and a large reduction current are commonly ascribed to the decomposition of the electrolyte and the solid-electrolyte interface (SEI) layer. The peak observed at around 0.58 V corresponds to the oxidation process during the depotassiation. It is also noticeable that the AS-HC electrode presents a small capacity above 0.3 V, whereas a large capacity is exhibited close to 0 V and a plateau is observed in the discharge curve. Thus, this kind of profile eliminates the risks related to metal plating. From the second cycle, most of the CV curves overlap, which signifies the high reversibility of insertion/deinsertion of potassium ions in the AS-HC-1000 electrode. Further, galvanostatic discharge−charge experiments

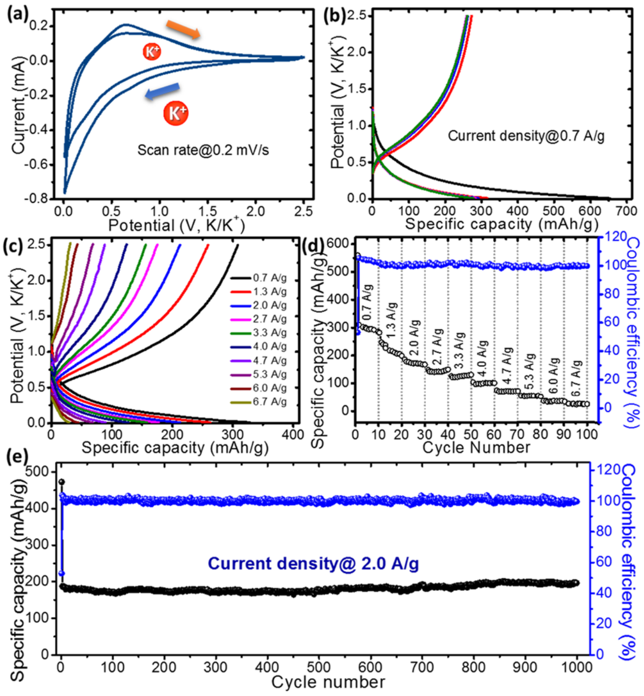

Figure 3. Electrochemical performances of the HC-1000 anode material in the K-ion half cell: (a) first two cyclic voltammetry curves at a scan rate of 0.2 mV/s. (b) Initial galvanostatic charge/discharge (GCD) curves recorded at 0.7 A/g. (c) GCD curves measured at different current densities. (d) Rate performance of the HC electrode recorded at different current densities. (e) Long-term cycling stability with corresponding Coulombic efficiency over 1000 cycles at 2 A/g.

reveal that the AS-HC-1000 sample demonstrates a relatively high capacity with good Coulombic efficiency (Figure S3), which might be assigned to the controlled surface area and uniform distribution of pores. The initial charge/discharge curves (shown in Figure 3b) of the HC-1000 sample exhibit a similar trend as CV curves, suggesting highly reversible K-ion storage. The voltage profiles for the initial cycles at 0.7 A/g reveal that the charge and discharge capacities for the AS-HC-1000 electrode were 272.8 and 653 mAh/g, respectively, with 41.7% of irreversible capacity. The initial capacity fading can be due to the SEI layer formed during the first discharge because of the electrolyte reduction and/or due to irreversible potassium insertion into the carbon material where special positions are available. Nevertheless, the curves become stable after a few cycles and show a reversible capacity of 294 mAh/g, indicating large storage of K$^+$ ions in the hard carbons.

The GCD curves for the AS-HC-1000 electrode were recorded at different rates and are presented in Figure 3c. The reversible specific capacities were determined to be 292 and 104 mAh/g at 0.7 and 4 A/g, respectively, which suggests that the HC electrode exhibits excellent structural stability and can sustain cycling at high current densities. The superior rate capability of the HC-1000 sample may originate from the adequate graphitic structure and surface characteristics of the carbon materials (Figure 3d). The stability of the HC-1000 electrode was recorded over 1000 cycles at 2.0 A/g. As illustrated in Figure 3e, no significant decay in capacity was recorded over 1000 cycles except for a small decrease in the initial cycles, ensuring high structural stability. The initial increase in capacity upon cycling can be ascribed to the activation process of the AS-HC-1000 electrode. It is important to note that the HC-1000 sample displays good electrochemical cycling performances at higher rates even though the K$^+$-ion has a bigger ionic size than the Na$^+$-ion.[40] Researchers have reported a similar phenomenon for hard carbons.[23,32] For instance, relatively higher K$^+$ diffusion coefficients in hard carbons were observed than that of Na$^+$, which can be ascribed to the low activation energy of the K-ion instigated by the different binding energies between K/C and Na/C.[32] Furthermore, the *b*-value calculated from a log

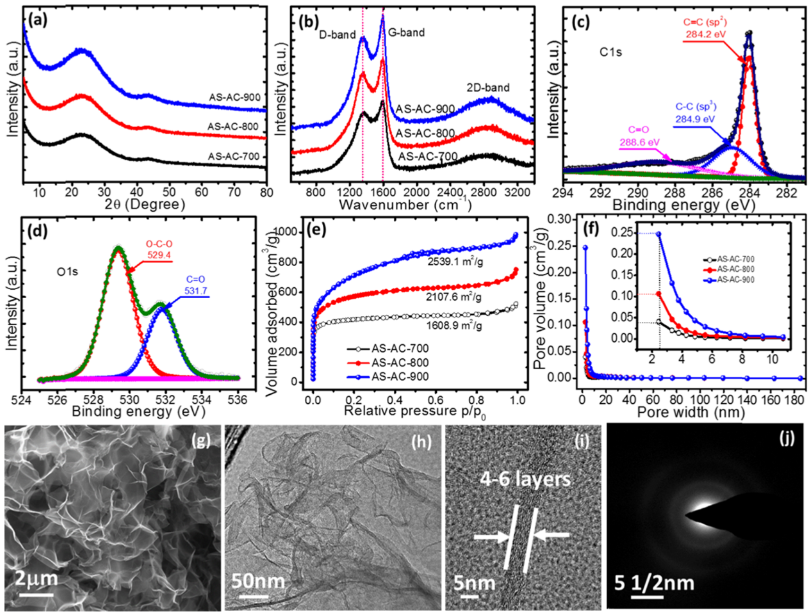

Figure 4. (a, b) XRD patterns and Raman spectra of activated carbon nanosheets prepared at 700, 800, and 900 °C from almond shells. (c, d) Core-level XPS spectra of C 1s and O 1s of the AC-800 sample. (e, f) Nitrogen adsorption/desorption isotherm of AC samples prepared at three different temperatures with corresponding pore-size distribution curves. (g−j) SEM, TEM, and HRTEM images and SAED patterns of the AC-800 sample, respectively, showing carbon nanosheets with a few graphitic layers.

(current, mA) vs log (scan rate, mV/s) plot from CV curves at different scan rates suggests that HC-1000 exhibits a diffusion-controlled charge storage mechanism (Figure S4).

Graphene-like Activated Carbon (AS-AC) Cathode. Given the fact that the performance of hybrid capacitors, such as high energy and power density, depends on the electrochemical properties of cathode materials, it is crucial to find electrodes with better capacitive performance. Hence, electrodes with a high specific surface area (SSA) and homogeneous pores to withstand the rapid charge and discharge for capacitance reactions are essential. Activated carbon (AC) being a high-surface-area material is utilized as the cathode in hybrid-ion capacitors to enhance the capacity of the energy storage device. In this respect, graphene-like AC nanosheets are prepared using almond shells as a carbon source and KOH as an activating agent to realize a high-SSA and porous cathode material. Figure 4a illustrates the XRD patterns of graphene-like AC samples synthesized at different temperatures in the presence of KOH. All of these AS-AC samples display nearly identical XRD diffraction patterns with diffraction peaks centered at 23 and 43°, which can be assigned to (002) and (101), respectively. Raman analysis of samples was further carried out to scrutinize the extent of graphitization of graphene-like nanosheets after activation (Figure 4b). All graphene-like AS-AC samples possess the D band (1350 cm^{-1}) and G band (1590 cm^{-1}) corresponding to the structural defects. The I_D/I_G ratio of AC samples was found to be in the range of 0.83−0.88, indicating the presence of a large number of defects and active sites. Typically, the I_D/I_G ratio for AC is close to 0.5−0.6; however, in the present case, the ratio is close to 1, which reveals the formation of a graphene-like structure. Along with G and D bands, a broad 2D band (at 2810 cm^{-1}) can be clearly seen, which is characteristic of graphene. The compositional analysis of the AC-800 sample was performed using the XPS technique, indicating the presence of C and O (Figure S5). The core-level C 1s spectra of AC-800 can be deconvoluted into three peaks as shown in Figure 4c: a sharp C5C peak at 284.2 eV and a C−C peak at 284.9 eV endorse the presence of sp^2 carbon and another peak at 288.6 eV corresponds to the oxygen-containing C5O bond. Meanwhile, the O 1s spectrum (Figure 4d) shows an O−C−O bond peak at 529.4 eV and a C5O bond peak at 531.7 eV, suggesting the massive generation of oxygen-containing groups after KOH activation. To examine the SSA and porosity of AS-AC samples, BET measurements were performed. According to Figure 4e, all AS-AC samples exhibited typical characteristics of type IV isotherms, implying the presence of micro/mesopores. Remarkably, the BET surface areas of graphene-like AC were in the order of AS-AC-900 (2539 m^2/g) > AS-AC-800 (2107 m^2/g) > AS-AC-700 (1608 m^2/g), signifying that the increase in temperature paved a way to gain a higher surface area property. Figure 4f reveals the pore-size distribution curves of these samples, suggesting the uniform pore-size distribution with maxima centered approximately at 2.4 nm with abundant mesopores due to the KOH activation. The SEM images (Figure 4g) of the AC-800 divulge a uniform graphene sheet-like morphology with a number of folds, scrolls, and intrinsic wrinkles. The low-magnification images (Figure S6) revealed the formation of a macroscopically open sponge. Such an open porous and nanosheet-like microstructure permits the entry of the electrolyte efficiently to the active surfaces available in the carbon, subsequently diminishing high rate diffusional losses through the electrolyte when applied as a cathode in the KIB. In contrast, the commercial activated carbons (ACs) are well know to possess a tortuous pore network with the pores of micron depth and could results in the noteworthy ion diffusional losses particularly at high scan rates/current densities. Figure 4h shows the TEM image of AS-AC-800, suggesting the formation of a spongelike network of graphene-like nanosheets. A high-resolution TEM (HRTEM) micrograph of AS-AC-800 is presented in Figure 4i, indicating poor degree of graphitization with a few graphitic layers (4−6 layers) of the material as well as a rich porous structure due to the selective activation. Additionally, the SAED pattern (Figure 4j) presents typical fused-ring characteristics, confirming the poor orientation of graphitic layers.

The electrochemical capacitive performance of the AS-AC-800 sample was examined between 1.5 and 4.5 V (vs K/K^+) (Figure S7). As seen from Figure 5a, the CV curves of AC-800 display quasirectangular shapes, signifying a characteristic double electrical layer capacitance (EDLC) behavior with additional pseudocapacitive humps. The pseudocapacitive contribution might have originated from residual oxygen functional groups at the AS-AC electrode surface. Accordingly, the shape of galvanostatic charge/discharge curves is a straight line between 1.5 and 4.5 V recorded at various applied currents (Figure 5b). The specific discharge capacity for the graphene-like AS-AC-800 cathode was found to be 94 mAh/g at 0.14 A/g, and the subsequent charge and discharge capacities suggested a reversible potassiation/depotassiation process. The rate performance of the AS-AC-800-electrode at gradually increased rates was further investigated by determining the specific capacities at different current densities as a function of cycle number (Figure 5c). A capacity of 94 mAh/g was obtained at 0.14 A/g, which was maintained to be 62 mAh/g at 5.71 A/g, suggesting excellent rate capability. Figure 5d displays the cycling stability of the AC-800 electrodes over 12 000 cycles at 0.86 A/g. Notably, the AS-AC-800 cathode retains 88% of the initial capacitance with almost 100% Coulombic efficiency (Figure 5d). Based on electrochemical performances, AS-AC-800 can be a potential cathode material to assemble a hybrid potassium-ion capacitor (KIC).

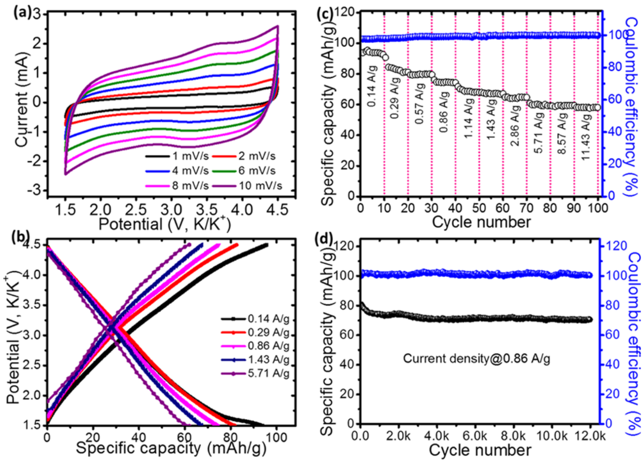

Figure 5. Electrochemical performances of the AC-800 cathode material in the K-ion half cell: (a) CV curves measured at different scan rates, (b) rate capability measurements of the AC electrode recorded at different current densities, (c) GCD curves measured at different current densities, and (d) long-term cycling stability with corresponding Coulombic efficiency over 12 000 cycles at 0.86 A/g.

Dual Carbon Potassium-Ion Capacitor (KIC). The electrochemical performance of the dual carbon potassium-ion capacitor (KIC) was examined by assembling an asymmetric cell with AS-HC-1000 as the anode and AS-AC-800 as the cathode. Before assembling the dual carbon KIC, the AS-HC-1000 electrode was cycled for 5 cycles at 0.7 A/g within 0.01−2.5 V (vs K/K$^+$) (Figure 6a). The capacity is attained by the reversible release and adsorption of K$^+$ and PF$_6^-$ ions upon polarization, i.e., EDLC of PF$_6^-$ as well as via K$^+$ interaction with oxygen residues and surface defects. The mass ratio of the active materials in HC and AC was maintained to be 1:3. The CV curves recorded for the AS-HC//AS-AC KIC at various scan rates present a quasirectangular shape, confirming the contribution from the capacitance mechanism (Figures S8 and S9). Interestingly, the CV curves retained their shapes even at high scan rates of 10 mV/s, indicating the high reversibility and good rate performance of the device. The charge/discharge performance of the dual carbon KIC was tested under various current densities (Figure 6b), which showed quasilinear GCD curves, suggesting the electric double layer capacitor behavior of the carbon materials. However, the slight diversion from its linear nature can be ascribed to the pseudocapacitance contribution by the oxygen functionalities at the AS-AC cathode. An approximately triangular-shaped CD curve confirms the rapid response and high reversibility. The rate performance of the KIC was evaluated by determining the specific capacitance at applied currents and is shown in Figure 6c. The capacitance of the AS-HC//AS-AC dual carbon KIC was 88 F/g (73 mAh/g) at 0.053 A/g that was reduced to 24 F/g (20 mAh/g) at 0.816 A/g. The decrease in the device capacitance can be assigned to the small number of electrochemical active sites accessible at high current densities.

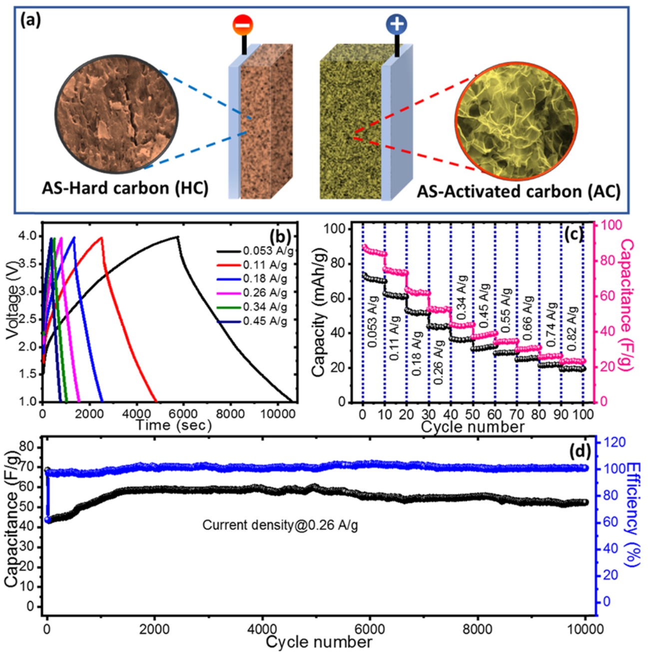

Figure 6. Electrochemical performances of the HC-1000//AC-800 full K-ion capacitor (KIC): (a) schematic illustration of fabrication of the KIC full cell, (b) GCD curves recorded at different current densities, (c) variation of specific capacity and specific capacitance with number of cycles at different current densities, and (d) cycling stability of the full cell with corresponding Coulombic efficiency over 10 000 cycles at 0.26 A/g.

The cycling stability of the AS-HC//AS-AC dual carbon KIC was tested at 0.263 A/g for 10 000 cycles. As shown in Figure 6d, there is an increase in capacitance from 43 to 59 F/g until 1500 cycles with a capacity retention of 122% of its initial capacity, which gradually decreases to 55 F/g (93%) over 6000 cycles. We hypothesize that this improvement corresponds to the oxygen functional groups and activation of the electrode material. A capacitance retention of 96% was obtained over 10 000 cycles, suggesting good structural stability of carbon electrodes. Notably, the device displayed an excellent Coulombic efficiency (close to 100%) during the 10 000 charge/discharge cycles. The Ragone plot in Figure 7 compares our AS-HC//AS-AC KIC device with other hybrid capacitors. Our constructed device based on nanocarbons derived from almond shells offer a high energy density of 105 Wh/kg. Our constructed KIC displays comparable energy and power densities to other reported devices such as N-CNTs//LCG (K),[41] NGC//K$_2$Ti$_6$O$_{13}$ (K),[42] AC//graphite (K),[43] AC//LTO (Li),[44] AC//Na-TNT (Na),[45] AC//carbon nanosheets (CNS),[46] and AC//onion-like carbon (OLC).[47] These outstanding results are attributed to the rapid ion transport, which is offered by a high specific surface area and rich porous structures of almond shell-derived carbons through a potential channel pathway.

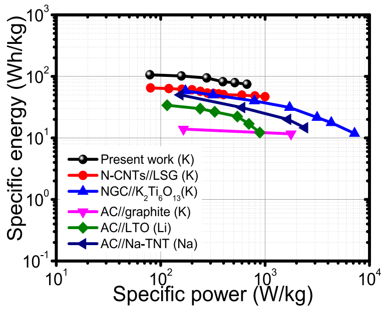

Figure 7. Ragone plot with specific energy and power values calculated by the total active mass in both cathode and anode. The values are compared with those of representative Li-based, Na-based, and K-based hybrid devices.

CONCLUSIONS

A hybrid dual carbon potassium-ion capacitor (KIC) was assembled by developing two different forms of carbon nanostructures as anode and cathode materials using almond shells as biomass. Using simple surface chemical treatments, amorphous non-graphitic hard carbon and highly porous graphene-like activated carbon were successfully prepared and implemented as anode and cathode materials, respectively, in the KIC. Activation with KOH chemically optimized the graphene-like structure and porosity of AC to obtain a high specific capacity carbon cathode. The assembled dual carbon KIC offered an energy density of 105 Wh/kg and good cycle life over 10 000 cycles in the optimized voltage window. Such a "double carbon" configuration for the device and electrode design allowed gaining high energy and power density by reducing the mismatch of electrode kinetics between the HC anode and the AC cathode. Thus, the investigation paves a way to develop full hybrid capacitors with adequate performance using porous nanocarbons from biomass waste, which has great commercialization potential.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.0c12379.

> Survey XPS spectra; SEM and TEM images of hard carbon and graphene-like carbons; electrochemical properties of hard carbons and activated carbon in half and full cell configurations and table showing the comparison of electrochemical properties of K-ion capacitors with earlier reports (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Armand, M.; Tarascon, J. M. Building better batteries. *Nature* **2008**, *451*, 652−657.

(2) Nitta, N.; Wu, F.; Lee, J. T.; Yushin, G. Li-ion battery materials: present and future. *Mater. Today* **2015**, *18*, 252−264.

(3) Wang, Y.; Song, Y.; Xia, Y. Electrochemical capacitors: mechanism, materials, systems, characterization and applications. *Chem. Soc. Rev.* **2016**, *45*, 5925−5950.

(4) Pazhamalai, P.; Krishnamoorthy, K.; Sahoo, S.; Mariappan, V. K.; Kim, S.-J. Carbothermal conversion of siloxene sheets into silicon-oxy-carbide lamellae for high-performance supercapacitors. *Chem. Eng. J.* **2020**, *387*, No. 123886.

(5) Thangavel, R.; Kaliyappan, K.; Kang, K.; Sun, X.; Lee, Y.-S. Going Beyond Lithium Hybrid Capacitors: Proposing a New High-Performing Sodium Hybrid Capacitor System for Next-Generation Hybrid Vehicles Made with Bio-Inspired Activated Carbon. *Adv. Energy Mater.* **2016**, *6*, No. 1502199.

(6) Zuo, W.; Li, R.; Zhou, C.; Li, Y.; Xia, J.; Liu, J. Battery-Supercapacitor Hybrid Devices: Recent Progress and Future Prospects. *Adv. Sci.* **2017**, *4*, No. 1600539.

(7) Zhang, W.; Liu, Y.; Guo, Z. Approaching high-performance potassium-ion batteries via advanced design strategies and engineering. *Sci. Adv.* **2019**, *5*, No. eaav7412.

(8) Aravindan, V.; Gnanaraj, J.; Lee, Y.-S.; Madhavi, S. Insertion-Type Electrodes for Nonaqueous Li-Ion Capacitors. *Chem. Rev.* **2014**, *114*, 11619−11635.

(9) González-Gil, A.; Palacin, R.; Batty, P. Sustainable urban rail systems: Strategies and technologies for optimal management of regenerative braking energy. *Energy Convers. Manag.* **2013**, *75*, 374−388.

(10) Dubal, D. P.; Ayyad, O.; Ruiz, V.; Gómez-Romero, P. Hybrid energy storage: the merging of battery and supercapacitor chemistries. *Chem. Soc. Rev.* **2015**, *44*, 1777−1790.

(11) Dubal, D. P.; Jayaramulu, K.; Sunil, J.; Kment, Š.; Gomez-Romero, P.; Narayana, C.; Zbořil, R.; Fischer, R. A. Metal−Organic Framework (MOF) Derived Electrodes with Robust and Fast Lithium Storage for Li-Ion Hybrid Capacitors. *Adv. Funct. Mater.* **2019**, *29*, No. 1900532.

(12) Hou, R.; Liu, B.; Sun, Y.; Liu, L.; Meng, J.; Levi, M. D.; Ji, H.; Yan, X. Recent advances in dual-carbon based electrochemical energy storage devices. *Nano Energy* **2020**, 72, No. 104728.

(13) Dubal, D. P.; Jayaramulu, K.; Fischer, R. A.; Zboril, R.; Gomez-Romero, P. Unveiling $BiVO_4$ nanorods as a novel anode material for high performance lithium ion capacitors: beyond intercalation strategies. *J. Mater. Chem. A* **2018**, *6*, 6096−6106.

(14) Jeżowski, P.; Fic, K.; Crosnier, O.; Brousse, T.; Beguin, F. Lithium rhenium(VII) oxide as a novel material for graphite pre-lithiation in high performance lithiumion capacitors. *J. Mater. Chem. A* **2016**, *4*, 12609−12615.

(15) Jayaramulu, K.; Dubal, D. P.; Schneemann, A.; Ranc, V.; Perez-Reyes, C.; Stráská, J.; Kment, S.; Otyepka, M.; Fischer, R. A.; Zboril, R. Shape-Assisted 2D MOF/Graphene Derived Hybrids as Exceptional Lithium-Ion Battery Electrodes. *Adv. Funct. Mater.* **2019**, *29*, No. 1902539.

(16) Naguib, M.; Adams, R. A.; Zhao, Y.; Zemlyanov, D.; Varma, A.; Nanda, J.; Pol, V. G. Electrochemical performance of MXenes as K-ion battery anodes. *Chem. Commun.* **2017**, *53*, 6883−6886.

(17) Eftekhari, A.; Jian, Z.; Ji, X. Potassium Secondary Batteries. *ACS Appl. Mater. Interfaces* **2017**, *9*, 4404−4419.

(18) Komaba, S.; Hasegawa, T.; Dahbi, M.; Kubota, K. Potassium intercalation into graphite to realize high-voltage/high-power potassium-ion batteries and potassium-ion capacitors. *Electrochem. Commun.* **2015**, *60*, 172−175.

(19) Fan, L.; Chen, S.; Ma, R.; Wang, J.; Wang, L.; Zhang, Q.; Zhang, E.; Liu, Z.; Lu, B. Ultrastable Potassium Storage Performance Realized by Highly Effective Solid Electrolyte Interphase Layer. *Small* **2018**, *14*, No. 1801806.

(20) Feng, Y.; Chen, S.; Wang, J.; Lu, B. Carbon foam with microporous structure for high performance symmetric potassium dual-ion capacitor. *J. Energy Chem.* **2020**, *43*, 129−138.

(21) Fan, L.; Ma, R.; Zhang, Q.; Jia, X.; Lu, B. Graphite Anode for a Potassium-Ion Battery with Unprecedented Performance. *Angew. Chem., Int. Ed.* **2019**, *58*, 10500−10505.

(22) Hwang, J.-Y.; Myung, S.-T.; Sun, Y.-K. Recent Progress in Rechargeable Potassium Batteries. *Adv. Funct. Mater.* **2018**, *28*, No. 1802938.

(23) Jian, Z.; Luo, W.; Ji, X. Carbon Electrodes for K-Ion Batteries. *J. Am. Chem. Soc.* **2015**, *137*, 11566−11569.

(24) Luo, W.; Wan, J.; Ozdemir, B.; Bao, W.; Chen, Y.; Dai, J.; Lin, H.; Xu, Y.; Gu, F.; Barone, V.; Hu, L. Potassium Ion Batteries with Graphitic Materials. *Nano Lett.* **2015**, *15*, 7671−7677.

(25) Pramudita, J. C.; Sehrawat, D.; Goonetilleke, D.; Sharma, N. An Initial Review of the Status of Electrode Materials for Potassium-Ion Batteries. *Adv. Energy Mater.* **2017**, *7*, No. 1602911.

(26) Beltrop, K.; Beuker, S.; Heckmann, A.; Winter, M.; Placke, T. Alternative electrochemical energy storage: potassium-based dual-graphite batteries. *Energy Environ. Sci.* **2017**, *10*, 2090−2094.

(27) Chen, Y.; Luo, W.; Carter, M.; Zhou, L.; Dai, J.; Fu, K.; Lacey, S.; Li, T.; Wan, J.; Han, X.; Bao, Y.; Hu, L. Organic electrode for non-aqueous potassium-ion batteries. *Nano Energy* **2015**, *18*, 205−211.

(28) Marcus, Y. Thermodynamic functions of transfer of single ions from water to nonaqueous and mixed solvents: Part 3 - Standard potentials of selected electrodes. *Pure Appl. Chem.* **1985**, *57*, 1129.

(29) Ramasamy, H. V.; Senthilkumar, B.; Barpanda, P.; Lee, Y.-S. Superior potassium-ion hybrid capacitor based on novel P3-type layered K0.45Mn0.5Co0.5O2 as high capacity cathode. *Chem. Eng. J.* **2019**, *368*, 235−243.

(30) Ming, F.; Liang, H.; Zhang, W.; Ming, J.; Lei, Y.; Emwas, A.-H.; Alshareef, H. N. Porous MXenes enable high performance potassium ion capacitors. *Nano Energy* **2019**, *62*, 853−860.

(31) Fan, L.; Lin, K.; Wang, J.; Ma, R.; Lu, B. A Nonaqueous Potassium-Based Battery−Supercapacitor Hybrid Device. *Adv. Mater.* **2018**, *30*, No. 1800804.

(32) Jian, Z.; Xing, Z.; Bommier, C.; Li, Z.; Ji, X. Hard Carbon Microspheres: Potassium-Ion Anode Versus Sodium-Ion Anode. *Adv. Energy Mater.* **2016**, *6*, No. 1501874.

(33) Lang, J.; Li, J.; Ou, X.; Zhang, F.; Shin, K.; Tang, Y. A Flexible Potassium-Ion Hybrid Capacitor with Superior Rate Performance and Long Cycling Life. *ACS Appl. Mater. Interfaces* **2020**, *12*, 2424−2431.

(34) Chang, X.; Zhou, X.; Ou, X.; Lee, C. S.; Zhou, J.; Tang, Y. Ultrahigh Nitrogen Doping of Carbon Nanosheets for High Capacity and Long Cycling Potassium Ion Storage. *Adv. Energy Mater.* **2019**, *9*, No. 1902672.

(35) Yu, A.; Pan, Q. G.; Zhang, M.; Xie, D. H.; Tang, Y. B. Fast Rate and Long Life Potassium-Ion Based Dual-Ion Battery through 3D Porous Organic Negative Electrode. *Adv. Funct. Mater.* **2020**, *30*, No. 2001440.

(36) Benítez, A.; González-Tejero, M.; Caballero, Á.; Morales, J. Almond Shell as a Microporous Carbon Source for Sustainable Cathodes in Lithium¯Sulfur Batteries. *Materials* **2018**, *11*, No. 1428.

(37) Li, M.; Ci, S.; Ding, Y.; Wen, Z. Almond shell derived porous carbon for a high-performance anode of microbial fuel cells. *Sustainable Energy Fuels* **2019**, *3*, 3415−3421.

(38) Nanjundan, A. K.; Gaddam, R. R.; Niaei, A. H. F.; Annamalai, P. K.; Dubal, D. P.; Martin, D. J.; Yamauchi, Y.; Searles, D. J.; Zhao, X. S. Potassium-Ion Storage in Cellulose-Derived Hard Carbon: The Role of Functional Groups. *Batteries Supercaps* **2020**, *3*, 953−960.

(39) Dubal, D. P.; Gomez-Romero, P. All nanocarbon Li-Ion capacitor with high energy and high power density. *Mater. Today Energy* **2018**, *8*, 109−117.

(40) Liu, Y.; Fan, F.; Wang, J.; Liu, Y.; Chen, H.; Jungjohann, K. L.; Xu, Y.; Zhu, Y.; Bigio, D.; Zhu, T.; Wang, C. In Situ Transmission Electron Microscopy Study of Electrochemical Sodiation and Potassiation of Carbon Nanofibers. *Nano Lett.* **2014**, *14*, 3445−3452.

(41) Moussa, M.; Al-Bataineh, S. A.; Losic, D.; Dubal, D. P. Engineering of high-performance potassium-ion capacitors using polyaniline-derived N-doped carbon nanotubes anode and laser scribed graphene oxide cathode. *Appl. Mater. Today* **2019**, *16*, 425−434.

(42) Dong, S.; Li, Z.; Xing, Z.; Wu, X.; Ji, X.; Zhang, X. Novel Potassium-Ion Hybrid Capacitor Based on an Anode of K2Ti6O13 Microscaffolds. *ACS Appl. Mater. Interfaces* **2018**, *10*, 15542−15547.

(43) Le Comte, A.; Reynier, Y.; Vincens, C.; Leys, C.; Azaïs, P. First prototypes of hybrid potassium-ion capacitor (KIC): An innovative, cost-effective energy storage technology for transportation applications. *J. Power Sources* **2017**, *363*, 34−43.

(44) Kim, H.; Park, K.-Y.; Cho, M.-Y.; Kim, M.-H.; Hong, J.; Jung, S.-K.; Roh, K. C.; Kang, K. High-Performance Hybrid Supercapacitor Based on Graphene-Wrapped Li4Ti5O12 and Activated. *ChemElectroChem* **2014**, *1*, 125−130.

(45) Yin, J.; Qi, L.; Wang, H. Sodium Titanate Nanotubes as Negative Electrode Materials for Sodium-Ion Capacitors. *ACS Appl. Mater. Interfaces* **2012**, *4*, 2762−2768.

(46) Chen, J.; Yang, B.; Hou, H.; Li, H.; Liu, L.; Zhang, L.; Yan, X. Potassium-Ion Batteries: Disordered, Large Interlayer Spacing, and Oxygen-Rich Carbon Nanosheets for Potassium Ion Hybrid Capacitor (Adv. Energy Mater. 19/2019). *Adv. Energy Mater.* **2019**, *9*, No. 1970069.

(47) Chen, J.; Yang, B.; Li, H.; Ma, P.; Lang, J.; Yan, X. Candle soot: onion-like carbon, an advanced anode material for a potassium-ion hybrid capacitor. *J. Mater. Chem. A* **2019**, *7*, 9247−9252.