TALLINN UNIVERSITY OF TECHNOLOGY

SCHOOL OF ENGINEERING

Department of Materials and Environmental Technology

THE POTENTIAL OF SEQUENTIAL BIOETHANOL AND BIOGAS PRODUCTION FROM NIGERIAN NAPIER GRASS: AN OPTIMIZED PROCESS FLOW

NIGEERIA PURPUR-HIIDHIRSIST BIOETANOOLI JA BIOGAASI JÄRJESTIKTOOTMISE POTENTSIAAL: OPTIMISEERITUD PROTSESSI VOOG

MASTER THESIS

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Tallinn, 2020

AUTHOR'S DECLARATION

Hereby I declare, that I have written this thesis independently.

No academic degree has been applied for based on this material. All works, major viewpoints and data of the other authors used in this thesis have been referenced.

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School of Engineering

THESIS TASK

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Study programme: KAYM18/19, Materials and Processes for Sustainable Energetics

main speciality: Processes for sustainable energetics

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Thesis topic: "the potential of sequential bioethanol and biogas production from Nigerian Napier grass: an optimized process flow" (in English); "Nigeeria purpur-hiidhirsist bioetanooli ja biogaasi järjestiktootmise potentsiaal: optimiseeritud protsessi voog" (in Estonian).

Thesis main objectives:

1. Examine the biogas yields for all stages of bioethanol production;
2. Detect the most efficient process route/ path
3. Suggest ideas for large scale production.

Thesis tasks and time schedule:

No	Task description	Deadline
1.	Experiment and processing of data	30/05/2019
2.	Introduction, aim of thesis and Literature review	31/03.2020
3.	Methodology, result, discussion, conclusion and editing of text	11/05/2020

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PREFACE

The topic was initiated by me, and the process flow was perfected by my supervisor Prof. Timo Kikas and co-supervisor Lisandra Meneses (Phd student). The experiments were carried out in the Institute of Technology (Laboratory of Biofuels) and the Institute of Agriculture and Environmental Sciences (Laboratory of Bio- and Environmental Chemistry) of Estonian University of Life Sciences. Main assistance in the data collection and analysis was done by my co-supervisor (Lisandra Meneses).

First and foremost, I am grateful to God Almighty for life and health. My profound gratitude also goes to the Estonian University of Life Sciences as for the support and all the facilities that were utilized for this experiment, as well as to Prof. Kikas for accepting me and my project proposal in his amazing team. Words cannot describe how deeply thankful I am to Lisandra Meneses for her inspiration, support and encouragement. I appreciate the prayers of my immediate family.

I gratefully acknowledge the financial support of the European Regional Development Fund via the Mobilitas Pluss (project MOBERA1) of the Estonian Research Council and base financed project of EULS P170025 TIPT.'

The thesis goal is to analyse the most efficient pathways of producing bioethanol and biogas from Elephant grass using nitrogen explosive decompression pretreatment method under four different temperatures: 150 °C, 170 °C, 190 °C and 200 °C. The usual path for producing bioethanol from lignocellulosic biomass was followed: pretreatment, hydrolysis, fermentation and distillation. However, for biogas production, the solid and the liquid fractions after pretreatment and hydrolysis as well as the distillation side stream and untreated biomass were analysed.

Upon comparing the results, it was discovered the solid fraction after pretreatment at 150 °C produced the highest biogas while the highest concentration of bioethanol was discovered in the liquid fraction after hydrolysis of samples pretreated at 170 °C. The liquid fraction after pretreatment can be discarded since they hold little or no potential for biofuel production.

Key words: anaerobic digestion, bioethanol, biofuel, master thesis.

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations	
S/N	Symbol/Number
TS	Total solids
VS	Volatile solids

Symbols	
$[CH_{4\ F}]$	Final amount of methane in the test bottle, mol
$[CH_{4\ F}]_{t-1}$	Final amount of methane in the bottle headspace following GC analysis, in the previous time interval, mol
$[CH_{4\ I}]_t$	Initial amount of methane in the bottle headspace at time t, mol
$[CH_{4\ I}]$	Initial amount of methane in the test bottle, mol
$[CH_{4\ C}]_{t-1}$	Cumulative methane produced in the previous time interval, mol
$[CH_{4\ C}]_t$	Cumulative methane produced in the current interval time, mol
B	Cumulative methane yield at time (t), mol CH_4/100 g
B_{max}	Maximum methane yield, mol CH_4/100 g
K	First order rate constant, d^{-1}
MF	Percentage of methane detected by the GC, %
P_I	Pressure in the bottle headspace prior to GC analysis, Pa
P_F	Pressure in the headspace of the bottle after the GC analysis
R	Ideal gas constant (8.314 $Jmol^{-1}K^{-1}$)
R^2	Goodness of fit for the fitting curves
T	Temperature in the incubator, °C
T	Time interval, days
V_{HS}	Volume of the headspace in the plasma bottle, m^3

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INTRODUCTION

Nigeria is one of the largest oil producers in Africa because of its crude oil reserve. The country's transportation system utilizes 100% of refined fossil fuel. For a long time, and as a result of huge governmental subsidy on petroleum, the energy mix of Nigeria has been dominated by petroleum with 33% of the nation's power generation coming from oil [1]. Nigeria is rich in energy resources; renewable and otherwise, which could makes the nation a viable participant in the fight against CO_2 emission and global warming [2]. The country has great potential for biomass cultivation because of the available land area. According to a report by Ben-Iwo et al, there is approximately 35,000,000ha of arable land for this purpose [3]. In 2007, the Nigerian government made a policy concerning the production of biofuels which made the nation one of the producers of green and sustainable energy thereby reducing CO_2 emissions [4][5].

In the past few years, biomass has been harnessed and in fact, it contributes 10% of the world's energy demand. Lignocellulosic biomass has demonstrated great potential in the production of sustainable biomethane, which has had positive impact on the transportation, energy and economic sector of developed nations worldwide [6]–[10]. Utilization of biofuels is seen by many countries as one important way to deal with climate change, global warming and to develop rural communities. Advancement in biofuel research enables developing countries to improve their immediate environment and better their economies[11].

Amongst one of the most researched biomasses in Nigeria is the Napier grass popularly known as the elephant grass. Napier grass has a high cellulose content of about 40% which makes it quite promising and attractive as a feedstock for biofuels production. It has a good water use efficiency ratio, amazing tolerance to drought, heating value of 16.58MJ/kg and high yield per unit area. It mostly grows in the wild uncultivated and in some cases can be used as feed for herbivores [12][13]–[22]. The potential of lignocellulosic biomass has been widely studied including that of the Napier grass. Despite this fact, more research is needed to increase its efficiency.

The essence of this thesis is to investigate the potential of the African elephant grass for integrated bioethanol and biomethane production using nitrogen explosive decompression as a pretreatment method. Different production pathways were experimented to ascertain the most effective and efficient means of production [23][24].

AIMS AND OBJECTIVES OF THIS STUDY

It is already a known fact that carbon derived fossil fuels are a major contributor to global warming and climate change. Biofuels are one of the many solutions that have been adopted to combat this problem and improve the standards of living.

This research aims at discovering the possibilities of producing higher percentages of biogas and bioethanol by pretreating the Napier grass with nitrogen explosive decompression pretreatment method and experimenting different routes for more efficient production. The solid and liquid states of the sample was analyzed for all stages and at different pretreatment temperature; 150 °C, 170 °C, 190 °C and 200 °C respectively. The results were compared for total solids (TS), Volatile solids (VS), sugar yields, ethanol yields and biogas yields. Figure 1 below shows the plan chosen for this experiment.

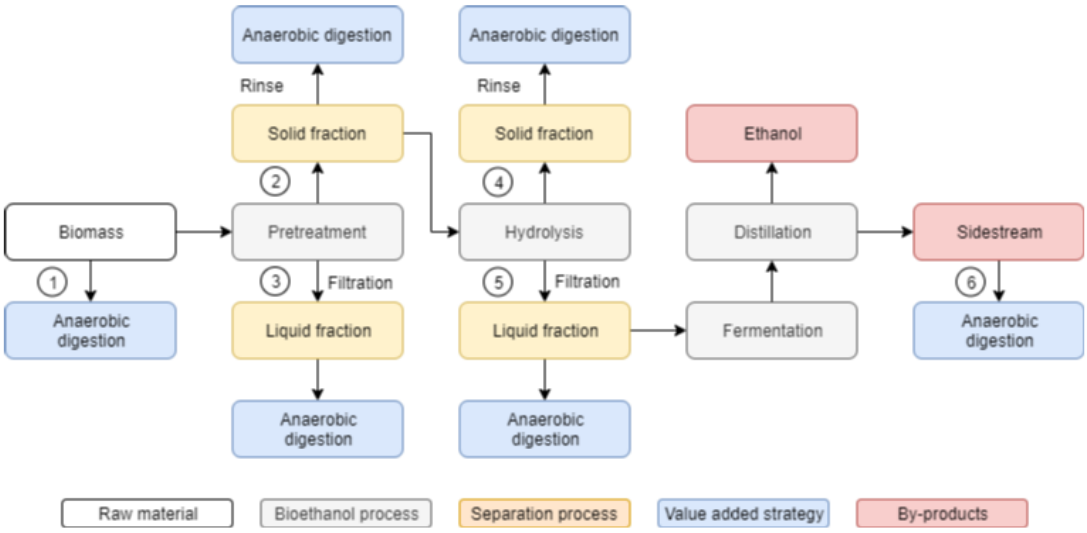

Figure 1. The production pathways utilized for this study to evaluate the potential of Napier grass (*Pennisetum purpureum/ elephant grass)* for biogas and bioethanol production by means of solid–liquid separation. 1—untreated Napier grass; 2—solid fraction of post-pretreatment broth; 3—liquid fraction of post-pretreatment broth; 4—solid fraction of post-hydrolysis broth; 5—liquid fraction of post-hydrolysis broth; 6— liquid samples of the post-distillation broth [23]

1. LITERATURE REVIEW

1.1. Classification of biofuels

Biofuels can broadly be classified either as primary or secondary biofuels depending on their mode of utilization.

The primary biofuels are unprocessed and are used in their natural forms, directly combusted to meet energy needs. The secondary biofuels are processed solids (e.g. charcoal), liquids (e.g. waste/ spent vegetable oil) or gases (e.g. biogas). Processed biofuels have a wider application range and higher efficiency than their counterparts [25]. Processing biofuels aims at providing fuels with well-defined characteristics.

Secondary biofuels are further classified into first, second, third and fourth generation biofuels. The table 1 illustrates the biofuels classification by generation.

Table 1. classification of biofuels based on biomass feedstock

S/N	BIOFUELS	FEEDSTOCK/ BIOMASS SOURCE
1	First generation biofuels	Sugars and vegetable oil
2	Second generation biofuels	Lignocellulosic biomass
3	Third generation biofuels	Algae and seaweed
4	Fourth generation biofuels	High solar efficiency cultivation

As can be observed from the table, first generation biofuels are derived from plants or cereal crops containing sugars [26]. First generation feedstock makes use of low-cost conversion technologies and brings greenhouse gas savings. However, it was necessary to evolve from the first to the second generation biofuels because, their biomass source was in competition with food crops.

Second generation biofuels are produced from nonedible agricultural, fishery and forestry biomass [27][6]. Also included in this category is waste cooking oil. Although, the cost of pretreatment of lignocellulosic biomass is high because of the use of advance technologies, lignocellulosic biomass is very abundant in nature and many at times does not require additional land for its cultivation, and as such is not in competition with the production of fiber and food crops [26].

Third generation biofuels are obtained from algae and seaweed feedstocks. These feedstocks have a number of advantages such as fast growth rate, easy to cultivate, no competition with food crops and versatility such that they can be cultured using seawater or wastewater. As with most things that have advantages there are some disadvantages associate with the use

of algae and seaweeds. These includes low lipid content, possibility of contamination in open pond system and the fact that the process of cultivating algae consumes a lot of energy [27].

The fourth generation biofuels are similar to the third generation biofuels except for the fact that they are mostly carbon negative or carbon neutral fuels. These generation of feedstock was borne in a bid to genetically modify and metabolically engineer third generation feedstock to increase their lipid content and biomass yield [28]. Even though research of fourth generation feedstock is still at its primary stage and the cost of initial investment is high, this generation of feedstock is quite promising as it proffers solutions to the pros of the aforementioned feedstocks (carbon capture and high lipid contents for algae) [27].

1.2. Lignocellulosic biomass

Lignocellulosic biomass has since been proven to be the most bio-renewable, economical and abundant feedstock available worldwide [29]. For example, in the European Union alone, the total agricultural biomass produced is estimated at 956 Million tonnes of dry matter per year from 2006- 2015 [30]. Lignocellulosic biomass generally comprises of agricultural and plant residues like wheat straw, corn stover etc., materials which serve as attractive feedstock for biofuel production because their use as feedstock for bioenergy does not interfere nor exhaust sources of food or animal feed [31].

Lignocellulosic biomass is generally composed of three types of biopolymers found only in the secondary cell wall of matured plant cells. These polymers are cellulose, hemicellulose and lignin. Cellulose and hemicellulose are the two components that make biomass a valuable feedstock for bioenergy production because, of their high polysaccharide contents. On the other hand, lignin comprises of polyphenols which are connected by a very complex network of monomeric phenyl propanoic units with different inter-unit bonds [32].

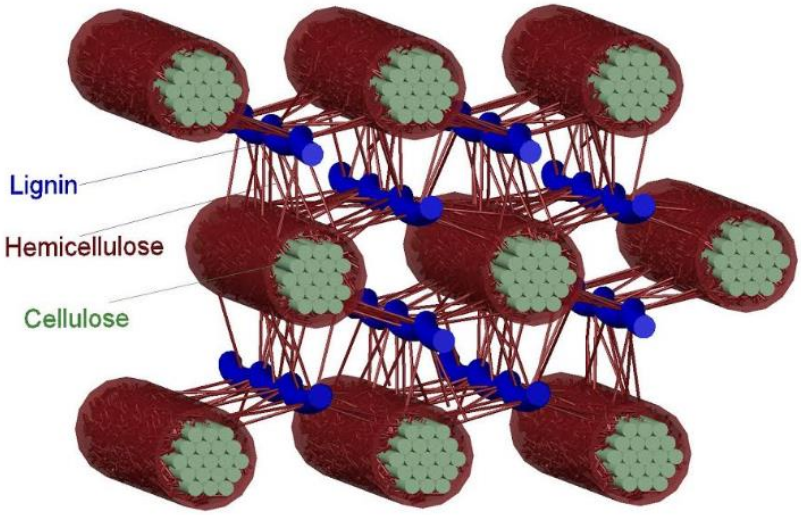

Figure 2. schematics of lignocellulosic biomass cells [33]

The percentages in which these polymers occur in different substrates varies from biomass to biomass depending on the source and type of biomass shown in the table below (table 2) [34].

Table 2. Contents of cellulose, hemicellulose, and lignin for different feedstocks [35][36]

Feedstock	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Cotton, Flax	80-95	5-20	-
Grasses	25-40	25-50	10-30
Hardwoods	45±2	30±5	20±4
Hardwood barks	22-40	20-38	30-55
Softwood	42±2	27±2	28±3
Softwood barks	18-38	15-33	30-60
Corn stalks	39-47	26-31	3-5
Corn stover	38-40	28	7-21
Sorghum stalks	27	25	11
Sorghum straw	32	24	13
Rice straw	28-36	23-28	12-14
Wheat straw	33-38	26-32	17-19
Barley straw	31-45	27-38	9-14
Bagasse	32-48	19-24	23-32

The generic chemical formula for cellulose is $(C_6H_{10}O_5)_n$. It is a linear (unbranched) homopolysaccharide and it is the principal constituent of the lignocellulosic biomass [37]. It is

insoluble both in water and in most organic solvents owing to the kind of bonds associated with its molecules. Cellulose is composed of a 10,000-15,000 D-glucose linked by β-(1,4) covalent glycosidic bond. Its strains are connected together to form cellulose fibrils which are in turn linked by intra and intermolecular hydrogen bonds [38].

At the molecular level, the hemicellulose, $(C_5H_8O_4)_n$, is the second most abundant polymer of a plant cell wall and is a highly branched heteropolysaccharide consisting of C_5 and C_6 sugars. These sugars include pentoses (β-D-xylose, α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, α-D galactose) and/or urgonic acids (α-D-glucuronic, α-D-4-O-methylgalacturonic and a-D-galacturonic acids). Unlike the cellulose, the side groups prevent the polymers from forming crystalline structures [39][37]. Their amorphous, branched short lateral chain structure, and their low molecular weight makes them relatively easy to hydrolyze [38]. The hemicellulose is tightly wrapped around the cellulose fibrils and must be detached to enhance digestibility of the cellulose, rate of hydrolysis and cellulose accessibility [40]. Hemicellulose has a small crystalline region and a small degree of polymerization. It also contains a small chemical bond with lignin [41].

The third most common polymer constituent of the lignocellulosic biomass is lignin, and it is distinctively different in structure from all the carbohydrate-based polymers. Lignin is a highly heterogenous polymer with aromatic residues rather than carbohydrate residues [42]. Lignin's dense, amorphous, complex and hydrophobic nature makes it difficult for enzymes to attack. It is crossed linked to the hemicellulose via a cell wall protein known as extension [43][44].

1.3. Bioethanol production process

As already noted, the complex composition of lignocellulosic biomass makes it difficult to biodegrade. To achieve ultimate performance and efficiency during utilization of the cellulose, the biomass must go through several stages such as, pretreatment, hydrolysis, fermentation and distillation [18]. In other to obtain high cellulose enzymatic digestibility yield, it is necessary to recognize the main structural limiting factor. These factors are (1) the specific surface area, (2) cellulose crystallinity index, (3) degree of polymerization (4) cellulose sheathing by hemicellulose (4) lignin content, and (6) acetyl content [45][46].

1.3.1. Pretreatment

The pretreatment is a necessary upstream process for the conversion of lignocellulosic material to ethanol because of the complex structure of the cellulose, hemicellulose and lignin

which prevents its decomposition [47]. The pretreatment step exposes the cellulose and hemicellulose for subsequent hydrolysis [6]. Pretreatment is the separation and solubilization of one or more of the biomass components by either physical, chemical, physio-chemical and biological pretreatments. This process breaks down the lignocellulosic matrix to reduce the degree of crystallinity of the cellulose and increase the amorphous cellulose part [48]. Worthy of note however, is the fact that many lignocellulosic biomasses have different physical and chemical properties, and it is therefore important to use pretreatment technology that suits their characteristics [49].

The physical pretreatment method is aimed at mechanical decrystallization, size reduction, particle density and surface area increase. This pretreatment process includes grinding, chipping, milling, freeze/ thaw cycles (pyrolysis) and radiation.

Grinding, chipping and milling is a very energy intensive process which mostly results in higher energy consumption than available theoretical energy in the biomass. The biomass sample can be reduced to particle sizes as low as 10-30mm or 0.2-2mm depending on whether it was chipped or milled/grinded respectively. While size reduction is very beneficial in reducing cellulose crystallinity and thus yielding good results, very fine particle sizes can negatively affect the enzymatic hydrolysis process. Finely milled biomass can form clumps in succeeding processes [50][51].

Pyrolysis requires less energy than milling. This process requires that the material be treated to a temperature greater than $300^{O}C$, to form gaseous products such as hydrogen, carbon monoxide and residual char [52].

Irradiating the biomass materials greatly increases the surface area and reduces the crystallinity. Although, this method is very expensive, the presence of phenolics in the irradiated samples proves its effectiveness in breaking the lignin-cellulose complex [49].

The chemical pretreatment method as the name implies, requires a chemical agent to break the complex covalent bonds of the cellulose. This process includes treatment with alkali, dilute/concentrated acids, ozonolysis and Organosolv [40]. The principle of the acid pretreatment method involves the use of an acid (usually sulphuric acid) to improve cellulose hydrolysis as the acid medium attacks the polysaccharides [53].

Although acid pretreatment is widely used, it can result in the formation of several inhibitors such as acetic acid, furfural and 5-hydroxymethylfurfural, which inhibits the growth of microorganisms. Moreover, the use of concentrated acids requires a reactor that is resistant

to corrosion. These reactors are expensive and can lead to an increase in the cost of production [49].

Unlike the acid pretreatment method, the alkali pretreatment uses hydroxides of sodium, potassium, ammonium and calcium. The alkali mostly dissolves the lignin. It removes the non-productive absorption sites of the lignin and eliminates the acetyl groups of the hemicellulose, thus making the cellulose available for enzymatic degradation. This process has been proven to be very effective depending on the material's lignin content. Compared with other pretreatment technologies, this process utilizes low temperature and pressure [27].

The ozone treatment can be operated at room temperatures and pressures. In ozonolysis, no toxic residues are produced since ozone can be decomposed by simply increasing the temperature or using a catalytic bed. Although, the process is expensive because of the use of large quantity of ozone, the degradation of the lignocellulosic biomass is limited to the lignin component [49].

The organosolvation process utilizes organic solvents (methanol, ethanol, acetic acid etc.) to break the lignin and hemicellulose bond [54].

The physico-chemical pretreatment method includes steam explosion, ammonia fiber explosion, nitrogen explosive decompression and carbon dioxide explosion. Treatment of lignocellulose biomass by steam requires that the material be heated at high pressures for a few minutes, and the reaction suddenly stopped by decompression in atmospheric pressure [55][56]. The fibres are separated once the steam expands within the matrix of the biomass [57]. This process effectively degrades the hemicellulose, but only partially degrades the lignin. This process does not require excessive amount of energy especially when compared with the mechanical grinding and there is no formation of toxic compounds. However, some inhibitors are formed during the degradation process, which would mean that the pretreated material must be washed with water to get rid of these inhibitors.

The ammonia fiber explosion (AFEX) pretreatment process mimics the steam explosion pretreatment process. The only exception is that it employs the use of liquid ammonia and there is no formation of inhibitor materials [57]. The effectiveness of this process is based on the lignin content i.e. for biomass like softwood newspaper which has a high lignin content (recall table 2), the AFEX pretreatment method is not very effective [58].

The nitrogen explosive decompression method makes use of high temperatures of up to 175°C and highly pressurized nitrogen of about 6MPa together with explosive decompression to open the biomass structure. The high pressure allows the cells of the biomass to become filled with

water saturated with nitrogen, and the sudden change in volume due to rapid pressure decrease to normal pressure causes the cells to rupture, thus exposing them to better enzymatic actions [59][60].

The carbon dioxide explosion pretreatment method is a similar operation technique to the steam and ammonia explosion methods. It, however, does not produce inhibitors and has a higher conversion yield than the steam explosion pretreatment [61].

Biological pretreatment of lignocellulose biomass relies on the use of specific enzymes such as white rot fungi containing oxidoreductases, for biodegradation of the cellulose and hemicellulose. These enzymes specifically degrade the lignin components of the matrix and increases the hemicellulose accessibility for hemi cellulase [62]. This process as compared with the physical, chemical and physicochemical pretreatments is inexpensive, low energy consuming, less harmful to the environment; but, very slow and time consuming as the bio-delignification requires extended time to run to completion [49].

To improve the pretreatment efficiency, it is possible to combine any of the above described methods [63]. Although, acid, alkaline and hydrothermal treatments are commonly used today [64], there exists other efficient, chemical free, cheaper and environmentally attractive pretreatment methods like the nitrogen decompression pretreatment method [59], [65]–[67].

1.3.2. Hydrolysis

After the sample has been pretreated, it is then subjected to hydrolysis/ saccharification; either using acids or enzymes. Hydrolysis aims at converting the pretreated cellulose and hemicellulose content to fermentable monomeric sugars [26][41]. Enzymatic hydrolysis is affected by four major parameters of the material: (1) the pretreatment conditions, (2) amount of lignin present in the material, (3) the concentration of the substrates and (4) the activity of the cellulase [6][68][69].

The most common and oldest method used in the saccharification of lignocellulosic biomass is acid hydrolysis using sulphuric acid. Although, other dilute or concentrated mineral acids such as nitric acid, hydrochloric and fluoric acids can be used, the most widely utilized acid for this process is the sulphuric acid. It is important to note that if concentrated acids are used, the operating temperature must be lowered and vice versa for dilute acids [49]. This hydrolysis method is expensive because of the further treatment the hydrolyzed material must undergo

prior to fermentation and the need to neutralize the yeast, due to the formation of inhibiting compounds [70].

Enzymatic hydrolysis on the other hand, has proven to be more advantageous than the acid hydrolysis because it does not form compounds that harm the environment, and they are easily biodegradable. Enzyme based technology is used for large scale manufacturing of ethanol. Since enzymes are specific to the reaction they catalyze, it is important that the right enzymes are chosen for great yield. Cellulase enzymes catalyze the breakdown of cellulose into glucose [49].

1.3.3. Fermentation

Simply put, and as related to bioethanol production, fermentation is the biological method of converting lignocellulosic biomass to ethanol, using yeasts or bacteria which feed on simple sugars [49]. Fermentation can be carried out by four different processes (1) separate hydrolysis and fermentation (SHF), (2) simultaneous saccharification and fermentation (SSF), (3) Simultaneous Saccharification and co-fermentation (SSCF) and (4) consolidated bioprocessing (CBP).

Separate hydrolysis and fermentation are a two-step process of hydrolysis action and fermentation actions which is performed separately in the aforementioned order, using specific enzymes. It is however, a long process which is both expensive and leads to the formation of inhibitors and contaminants [71][72].

Simultaneous saccharification and fermentation is a single step process which combines the hydrolysis and fermentation processes to convert cellulose to D-glucose and subsequently to ethanol using specific enzymes and microorganisms [73]. The downside of SSF is that the cellulases and fermenting microorganisms have different optimal operating temperatures, which must be compromised, thus, neither the saccharification nor fermentation is performed under optimal conditions [49].

The simultaneous saccharification and co-fermentation process is similar to the SHF except for the fact that it has higher yields and saccharification rates, since both processes are carried out in the same bioreactor, thus leading to lower investment cost [74]–[76].

The consolidated bio-processing is still in the development stage as research is still ongoing to determine a suitable microorganism that can efficiently degrade the lignocellulosic biomass

whilst feeding on the sugars to produce ethanol, since the aim is to combine three processes *viz* production of cellulase, hydrolysis and fermentation into one single step [77].

1.4. Distillation

Distillation of fermented ethanol is carried out either by ordinary distillation, azeotropic distillation, liquid extraction fermentation hybrid, absorption or membrane separation [64]. However, in order to be able to achieve up to 99.9% recovery of ethanol, it is necessary to dehydrate it further [40]. Worthy of note however, is the fact that factors such as cost, energy requirements and separation capacity can affect the choice off distillation used. Although, these factors also depend on the enzymatic hydrolysis and fermentation [78][36][79].

1.4.1. Ordinary distillation

Ordinary distillation utilizes the differences in the boiling points or relative volatilities of the ethanol-water azeotrope. This process can recover only 95% of ethanol. This purification procedure is not economical because, the further process of achieving anhydrous ethanol is an energy intensive process and is limited in its capability to separate volatile compounds [80].

1.4.2. Liquid extraction fermentation hybrid

Liquid extraction or fermentation hybrid or extractive fermentation is a combination of liquid-liquid extraction and fermentation which uses highly efficient solvent such as oleyl alcohol to extract anhydrous ethanol and inhibitory compounds from the fermentation broth. This in situ fermentation process has the advantage of high ethanol yield and low freshwater consumption [80].

1.4.3. Adsorption distillation

Adsorption distillation uses molecular sieves of 3Å to separate the water from the ethanol-water mixture by utilizing the differences in their molecular size (the diameter of ethanol molecules being 4Å and water molecules being 2.5Å). This process requires a minimum of two bed absorbents which could either be organic or inorganic. However, the use of inorganic absorbent beds is highly favored because they are regenerative, cheap and environmentally friendly [27].

1.4.4. Azeotropic distillation

Azeotropic distillation can be used when the ethanol-water mixture disobeys Raoult's law of thermodynamics, then a third chemical (entrainer) is added to amend the relative volatilities of the azeotropes. This entrainer can be recovered by any recovery method that ensures its reusability. Some common entrainers include benzene, cyclohexane, hexane, acetone, diethyl ether, iso octane, n-heptane, n-pentane and polymers. The high principal cost, energy input and the use of harmful substances such as benzene draws a lot of concern to this form of distillation [27].

1.4.5. Absorption or membrane separation

Membrane distillation uses a semipermeable membrane to separate the azeotropic mixture by mass transfer. Only the volatile component is transferred through the membrane. Typical organic matters can be used as membranes. One advantage of membrane separation is that it can be used at a low temperature, however there are still some issues concerning proper selection of membrane material for use and the high cost involved in its fabrication [27].

1.5. Anaerobic digestion

Anaerobic digestion (AD) is a promising method for producing environmentally friendly and socio-economically beneficial renewables like biogas [81]. AD is a mature technology which uses microorganisms to biodegrade organic materials in the presence of oxygen to produce biogas as the main product [82]. There are four stages involved in the anaerobic digestion of organic biodegradables: fermentation (hydrolysis), acidogenesis, dehydrogenation/ acetogenesis and methanogenesis (methanation) [83][84]. Each stage uses a different microorganism.

In hydrolysis, the microorganisms (exoenzymes) secretes hydrolase to convert the large complex polysaccharides, proteins and lipids into their equivalent monomers [85]. It is the key and rate limiting step of the entire AD process [86].

The acidogenesis stage uses fermentative bacteria such as *Peptoccus, Clostridium, Lactobacillus, Geobacter* etc to convert the hydrolyzed polymers to volatile fatty acids monomers, ammonia, hydrogen and carbon dioxide [85], [87]–[89].

Acetogenic bacteria such as *Acetobacterium woodii* and *Clostridium aceticum* converts organic acids and alcohol into acetates, hydrogen and carbon dioxide [83].

In methanogenesis, methane and carbon dioxide are produced when acetotrophic and hydrogenotrophic methanogens degrade acetate and hydrogen respectively. Methanosarcina and Methanosaeta are examples of typical methanogens used for this AD process [90][91]. A diagrammatic representation of the process is shown below [92][83].

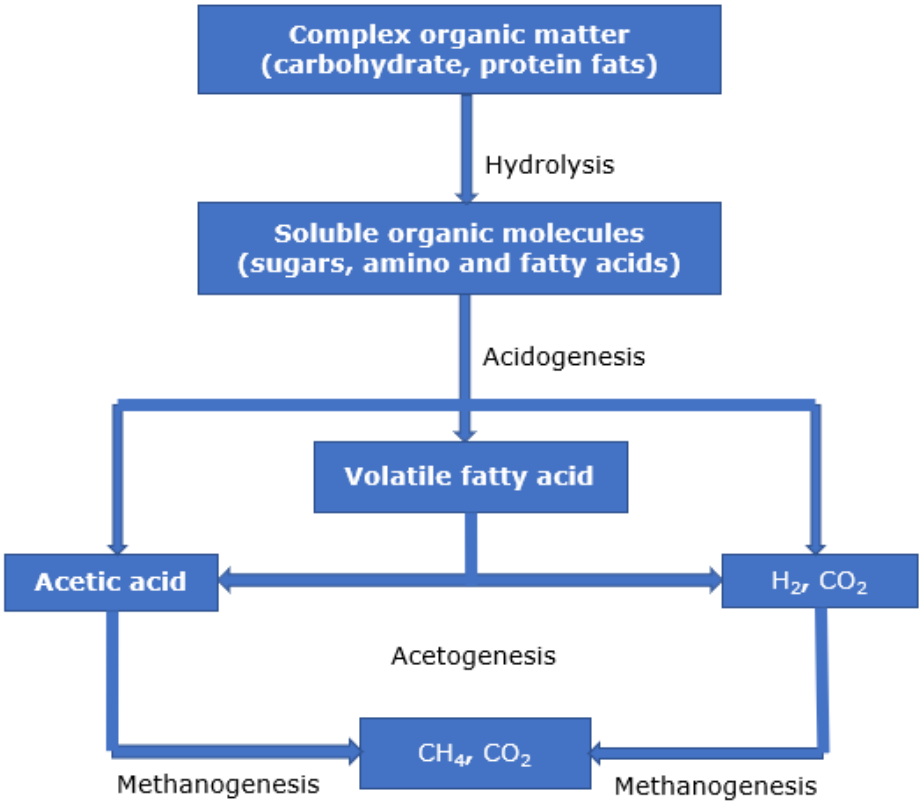

Figure 3. The three anaerobic digestion stages [93]

As a result of the action of bacteria in the AD process, the overall process is sensitive to and depends on a number of factors such as, the pH, biochemical oxygen demand (BOD), temperature, concentration of volatile fatty acid, particle size, C/N ratio, substrate mixing, inoculum, organic loading rate, hydraulic retention time, and solid concentration of the mix [94]. Some of these important parameters have been described below.

1.5.1. Temperature

Few experiments show that high temperatures support the formation of methane by the actions of thermophilic bacteria more than those of mesophilic ones. In fact, temperatures reaching 55°C have been recorded. Worthy of note however, is that the operating

temperatures must be stable and closely monitored as too high temperatures enhance the hydrolytic process rather than the methanogenic process. More so, the thermophilic process generates inhibitor compounds [94][95]. Temperatures less than 20°C enhances psychrophilic activities while those of 20-40°C and greater than 40°C favor mesophilic and thermophilic methanogenesis respectively [96][97]. Studies have also demonstrated that there can be deviations from these operating temperatures as a result of some factors such as organic loading rate (OLR) and hydraulic retention time (HRT) [98][99].

1.5.2. Particle size

Substrate with smaller and finer particle size are more easily digested than bigger and more coarse ones. Particulate matter should be such that they are not too small as very small particle size can lead to accumulation of acids and too large particle size will not allow for efficient biogas production [94][100].

1.5.3. pH effect

6.0 to 7.6 is the ideal range for methanogenesis to occur with the optimum at 7.0 to 7.2. At pH below this range, non-ionized sulphides are formed and at pH greater than the ideal, inhibitor compounds such as, non-ionized ammonia are formed in excess of their ionized counterpart. Therefore, pH fluctuations should be avoided as much as possible to prevent formation of toxic compounds, encourage microbial activity and an overall efficient anaerobic digestion process [101][97].

1.5.3. Hydraulic retention time (HRT) and organic loading rate (OLR)

The time needed for the microbes to maintain contact with the organic matter is known as the hydraulic retention time while the mass of organic matter per unit reactor volume per unit time is the organic loading rate [102]. To prevent quick microbial washout, and for optimal results, a short HRT should be avoided and one which encourages at least 75% of substrate degradation should be embraced [94].

1.5.4. C/N ratio of substrate

Microbes require carbon, nitrogen, phosphorus as well as other macronutrients such as cobalt, copper, zinc and calcium to properly function [103]. In some case studies, it has been shown that nitrogen deficit prevents the effective carbon utilization by the bacteria whereas excess nitrogen enhances formation of inhibitor compounds like non ionized ammonia [103][102].

The optimal carbon/ nitrogen ratio for easy biodegradation is in the range from 16:1 to 25:1 [104].

1.5.5. Oxidation reduction potential (ORP) and substrate mixing

ORP is defined as the amount of available oxidants and reductants that are able to gain or lose electrons. ORP higher than 50mv suggest an oxidizing environment while a ORP lower than 50mv suggests a reducing environment [105][96].

1.6. Challenges and future prospective of anaerobic digestion of biomass

Major challenge of the anaerobic digestion process is the presence of lignin because it is an inhibitor to microbial activity. Therefore, proper pretreatment is needed to combat the lignin effect and increase the quantity and quality of produced biofuel. Through the use of advanced technology such as, the solid-state AD process, environmentally friendly biofuels can be produced. However, commercially, these processes are not viable as a result of their instability and low methane yields [28], [37], [106], [107].

2. MATERIALS AND METHOD

2.1. Bioethanol production process

2.1.1. Biomass

Elephant grass (*Pennisetum Purpureum* also called Napier grass) was the selected biomass for these experiments. This feedstock was harvested towards the end of the harmattan period in January 2019 from Effrune town, Delta state, Nigeria. It was harvested and sun dried to a moisture content of 10% or 100g kg^{-1} before being transported to Tartu, Estonia for further processing.

Using a cutting mill SM 100 (Retsch GmBh), the biomass particle size was reduced to 3mm or less and sieved in preparation for a pretreatment.

2.1.2. Pretreatment

The milled biomass was treated with nitrogen explosive decompression (NED) method in order to access the cellulose and hemi cellulose that are otherwise strongly intertwined. For this experiment, NED was carried out at four different temperatures: 150 °C, 170 °C, 190 °C, and 200 °C respectively.

100g of the sample was weighed using the Mettler Toledo scale and added into a 2L non-stirred high-pressure reactor vessel. 800g of distilled water was added into this vessel and stirred to achieve a uniform slurry-like consistency. The reactor lid was tightly shut to prevent any leakage of nitrogen gas. The reactor was heated from room temperature to the temperature to be studied, under pressure of 30 bar with one-minute retention time. As soon as the desired conditions was achieved, the reactor was allowed to cool down to 80 ^{o}C (this cooling process took about an hour and it was done in other to allow the material cool to a point where it was considered safe to handle). Using a pressure valve located at the top of the lid, the pressure was abruptly released, resulting in an explosive sound (hence the name nitrogen explosive decompression). The sample was taken out of the reactor vessel right after the explosion and put in glass flasks to allow it cool to at least 50 ^{o}C. Figure 3 below illustrates the vessel schematics for the nitrogen explosive decompression method.

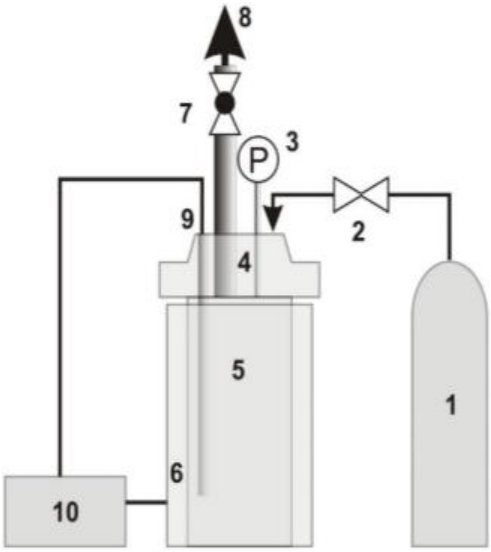

Figure 4. Diagram of Nitrogen explosive decompression pretreatment system. 1- N_2 tank, 2- pressure control valve; 3- manometer; 4- modified pressure vessel lid; 5- Parr instruments pressure vessel; 6- ceramic contact heater; 7- pressure release valve; 8- ventilation system; 9- thermocouple; 10- temperature controller unit [66]

2.1.3. Hydrolysis

After the sample had cooled to 50 °C, it was taken out of the reactor and put into a 1000 mL Erlenmeyer flask, 30 FPU per g of cellulose of the cellulase complex Accelerase 1500 © (DuPontde Nemours) was added to the mixture and topped up with distilled water to the 1000 mL mark. The flask was sealed with aluminum foil and secured tightly in an orbital shaker (IKA®-Werke GmbH & Co. KG, Staufen im Breisgau, Germany) (KS 4000 I control) for a period of 24 hours, at temperature of 50 °C and rotation speed of 250 rpm.

2.1.4. Fermentation

The glucose formed in the hydrolysis step was converted into ethanol in the fermentation stage using yeast. Firstly, the liquid and the solid hydrolysate were separated. Then the hydrolyzed liquid was placed in a 1000 mL glass bottle and 2.5 g of commercial yeast; *Saccharomyces cerevisiae* (Turbo yeast T3) was added. The bottle was tightly corked using special water-caps and left to ferment at room temperature for seven days.

2.1.5. Distillation

This process separates the liquids based on the differences in their boiling temperatures and vapor pressures. The sample to be distilled was placed in the Buchi R-210 Rotavapor System from BÜCHI Labortechnik (Flavil, Switzerland) at 175 mbar. After distillation, the stillage was analyzed in the biomethane potential assay (BMP) to ascertain its potential for biomethane production.

2.2. Anaerobic digestion

The solid and the liquid fractions were separated from the pretreatment and hydrolysis stages. Anaerobic digestion was conducted for the untreated elephant grass biomass, each separated fraction (solid and liquid) and for the stillage.

2.2.1. Inoculum

The inoculum employed for this study was procured from the Tartu municipality wastewater treatment plant in Estonia. Before its utilization, it was stabilized for four days at 36 ^{o}C in an incubator. This temperature and time were enough to allow the residual organic matter to be consumed, and removal of dissolved gasses by degasification.

2.2.2. Biomethane potential assay (BMP)

Using a modified version of Owen et al. and Angelidaki et al. guidelines [108] [109], the BMP assay was carried out until the gas pressure of the substrates and inoculum reached a constant value. Usually, it took 42 days to achieve constant results. The assays were performed in triplicates and at mesophilic conditions (37 °C±1 °C) in 575 mL plasma bottles. The glass bottles were filled with a substrate to inoculum ratio of 0.25 and total volume of 200 mL, which was estimated from analyzing the total and volatile solids. In accordance with the method 1684 (U.S. Environmental Protection Agency- EPA), the total solids (TS) was obtained by heating the samples for 24 hours and 105 ^{o}C, while the volatile solids (VS) were analyzed by incinerating the residue from the total solid (TS) for 2 hours and 550^{o}C. Before the bottles were sealed with rubber stoppers and aluminum caps, nitrogen gas was purged into the 375 mL headspace of the bottles for 3 mins, to get rid of the oxygen and to ensure anaerobic conditions. After flushing and sealing, the bottles were mixed and incubated at 36 ^{o}C until the methane produced was less than 1% of the total amount produced.

The bottles were shaken to mix the samples within, and the methane production was measured every day for the first week of the experiment. In the second week it was measured

every other day, in the third and fourth week, measurement was taken twice a week, and by the fifth and sixth week, measurement was taken only once per week. The amount of biogas produced was analyzed by measuring the pressure increase in the bottles before and after the gas chromatograph (GC) analysis using a pressure meter WAL BMP-Testsystem (from WAL Mess-und Regelsysteme GmbH, Germany). The gas chromatograph had a thermal conductivity detector, 2 Molsieve 5A Backflush heated column and a PoraPLOT U heated column. Columns 1 and 2 had argon and helium, respectively, as carrier gases. For column 1, the injection temperature was 110 ^{o}C, while the column temperature was 120 ^{o}C and the column pressure was 50 Psi. For column 2, the injection temperature remained the same, while the column temperature and pressure were set to 150 ^{o}C and 22 Psi. A SevenMulti™ S47-dual pH/conductivity meter was used at the end of each experiment to measure the pH of the samples, to ensure that they stayed within acceptable anaerobic digestion range of (6.8- 7.2) [110].

Accompanying each set of experiments was a blank test made up of only inoculum sludge. The biomethane produced from the blanks was also measured and recorded, and its value subtracted from that of the substrate samples to obtain the final biogas and methane production of the substrates. The results were expressed in moles of methane per 100 g of initial dry biomass at standard condition.

2.3. Analytical methods

An ANKOM 2000 analyzer (ANKOM Technology, Macedon, NY, USA) was used to determine the fiber content (i.e. the proportion of cellulose, hemicellulose and lignin) of the biomass, while the Kern MLS-50-3D moisture analyser from Kern & Sohn GmbH was used to measure the moisture content.

The solid and the liquid fractions analysed in the experiments were separated after the pretreatment and hydrolysis stages and were analysed for biomethane production. The broth obtained after pretreatment and hydrolysis were separated using a centrifuge (Thermoscientific Heraeus Megafuge) at a rotational speed of 10,000 rpm for 20 min. Before rinsing the solid fraction with distilled water to get rid of residual solubles, a vacuum filtration pump was used to separate the liquid fractions from the solid fractions. To finalize the drying process, the solid fraction was placed in an oven for 24 hours at 40 ^{o}C, to reduce the moisture content to 4.5 % or less.

Liquid fractions from post hydrolysis and post fermentation were quantified for glucose, xylose, galactose, arabinose, mannose, glycerol, acetic acid, and ethanol using a HPLC

(Prominence-i LC-2030C 3D Plus, Shimadzu, Japan) with a RID detector at 60 °C (20A, Shimadzu, Japan), a mobile phase Milli-Q water with flow rate of 0.6mL/min, the Rezex RPM-monosaccharide Pb2+ column (Phenomenex, Torrance,USA) operating at 85 °C was used to quantify sugar concentrations while the HPX-87H (Biorad, Hercules, USA) at 50 °C and mobile phase of 5 mM sulphuric acid was used to measure acid content [111].

2.4. Calculations

The initial quantity of methane gas produced in the plasma bottle [CH_4 I] (mol CH_4) was calculated using equation (1):

$$[\mathrm{CH_4\ I}] = \mathrm{MF}\ \frac{\mathrm{P}_I \mathrm{V}_{HS}}{\mathrm{R}(273.15+\mathrm{T})} \quad (1)$$

Where

PI (Pa) is the total pressure at the headspace determined prior to the GC analysis

V_{HS} (m^3) is the volume of the headspace of the bottle

MF is the methane fraction determined by the GC in the current period

R is the ideal gas constant (8.314 $Jmol^{-1}\,K^{-1}$)

T is the temperature in the incubator (°C).

The final quantity of methane gas in the plasma bottle headspace [CH_4 F] is calculated using equation (2):

$$[\mathrm{CH_4\ F}] = \mathrm{MF}\frac{\mathrm{P}_F \mathrm{V}_{HS}}{\mathrm{R}(273.15+\mathrm{T})} \quad (2)$$

where P_F (Pa) is the total pressure at the headspace determined after the GC analysis.

The cumulative molar concentration in the current time period is determined using equation (3):

$$[CH_{4C}]_t = ([CH_{4I}]_t - [CH_{4F}]_{t-1}) + [CH_{4C}]_{t-1} \quad (3)$$

where

$[CH_{4C}]_t$ is the initial quantity of methane in the head space of the test bottle in the current time period

$[CH_{4I}]_t$ is the final concentration of methane in the test bottle head space in the prior period of time

$[CH_{4F}]_{t-1}$ is the cumulative molar concentration of methane gas produced in the previous time period

$[CH_{4C}]_{t-1}$ is the cumulative amount of methane produced in the previous time interval.

The results of the methane gas produced were modelled using a nonlinear regression model in the statistics software GraphPad Prism 5.0 that was further inserted in an exponential first-order association model (Equation (4).

$$B = B_{max}\,(1 - e^{-kt}) \qquad (4)$$

Where

B is the cumulative methane produced (mol CH_4/100g) at time interval(t)

B_{max} is the maximum methane yield (mol CH_4/100 g)

k is the kinetics rate constant (d^{-1}) [19][20].

2.5. Statistical analysis

The software GraphPad Prism 5. Was used to analyze the statistics while the normal variables distribution was determined using the Shapiro-Wilk's normality test. The differences between the variables was determined using the Kruskal–Wallis test and the post hoc test Dunn's multiple comparison test. These results are represented by their standard errors (±SE) and when *p*-value was $p \leq 0.05$, the results was considered different.

3. RESULTS AND DISCUSSION

This study used the NED pretreatment method to analyze the potential of the Napier grass for biomethane and bioethanol production. An extended version of the results is available in annex 1.

3.1. Chemical composition

In table 3, the proportions of cellulose, hemicellulose and lignin of the Napier grass are presented. The Napier grass contains 35.7 of cellulose, 26.9% of hemicellulose, 5.2% of lignin, 6.1% of moisture and 9.6% of ash. Comparing the cellulose values for this study to the cellulose values obtained by Mohammed et al. and Nascimento and Rezende, the proportion of cellulose in this study is slightly higher. It is 1% higher than the NGT (Napier grass total) cellulose values that were described by Mohammed , et al in literature [112], and 6% lower than the cellulose values described by Nascimento and Rezende [113]. Similar comparisons was also made for the hemicellulose, lignin, ash and moisture content. In relation to the aforementioned studies, the proportions of hemicellulose are also slightly higher than the hemicellulose proportion in the said literature; 2% to 6%, while the lignin is significantly lower (23%) in comparison with lignin values in literature. The ash and moisture content were 3% to 7% higher and 68% lower, than the respective ash and moisture content literature values [112][113]. The differences in the percentage composition of cellulose and hemicellulose may be due the analytical choices. In fiber analysis lignin is not really directly measure, but calculated. The Napier grass used in this study grew in the wild, it was harvested in early January, the leaves and soft part of the stem was harvested and used for the experiment and dried naturally in the sun. There is also the probability that Mohammed et al. may have included the very hard part of the stem for his analysis, and this part is found in a more matured form of the plant. Moreso, in the studies conducted by Mohammed et al. and Nascimento & Rezende the growing, harvesting, and drying conditions were closely monitored in order to ensure maximum growth of the Napier grass and that optimal conditions of the substrate were met [112][113]. Worthy of note is that the low percentages of lignin present implies that the pretreatment method required to break the cell wall is less intensive, thus making the bioethanol production attractive since the energy input would be lower than for example biomass with a lignin content of 27% [23].

Table 3. Composition of untreated Napier grass in comparison with literature

Component	Composition from experiment (%)	Composition from literature (%)	
	This study	Mohammed et al. (NGT) [112]	Nascimento & Rezende [113]
Cellulose	35.7 ± 0.3	34.2 ± 2.2	41.8 ± 0.2
Hemicellulose	26.9 ± 1.2	20.4 ± 1.7	24.7 ± 1.0
Lignin	5.2 ± 0.5	24.3 ± 1.3	28.0 ± 1.5
Ash	9.6 ± 0.0	6.3 ± 0.0	2.0 ± 0.0
Moisture	6.1 ± 0.3	74.2 ± 0.2	-

The total solids (TS) and volatile solids (VS) content for untreated Napier grass, samples from the solid and liquid fraction from different steps of bioethanol production chain (pretreatment, hydrolysis, fermentation and distillation), pretreated with NED at different temperatures is presented in table 4. The TS content of untreated material was 956 g/kg. For samples of the solid fraction the TS content was higher, it varied between 966 g/kg and 987 g/kg and was lower for samples from the liquid fraction; between 13.4 g/kg and 37.4 g/kg. Statistically significant differences were found between TS content of samples from the solid and liquid fractions pretreated at different temperatures ($p < 0.05$). These results show that samples from the solid fraction had higher TS content than those from the liquid fraction. High TS content indicates there is more substrate available for the anaerobic digestion process, leading to higher methane and biogas yields. The TS content in the raw material is ten times higher than each step of the liquid fraction, which means that dry matter was partially decomposed during the pretreatment and that the NED was effective in the reduction of the TS content. The decrease in the TS in the post distillation broth is as a result of the loss of sugars to the production of ethanol.

The VS content of untreated Napier grass was 889 g/kg. For samples of the solid fraction the VS content varied between 929 g/kg and 975 g/kg, and for samples from the liquid fraction between 994 g/kg and 1000 g/kg. The highest VS values were recorded post hydrolysis and post distillation of the liquid fraction. This is because the separation of the liquid phase after pretreatment leaves the liquid phase with mainly the hemicellulose part of the biomaterial, which in turn is converted into glucose by enzymes after hydrolysis, thus making the potential for biomethane production quite high as compared with the other fractions. This potential reduces after distillation because much of the sugars have been fermented and converted to ethanol, and as a result, the quantity bioconvertible material is reduced. Statistically significant differences were found between VS content of samples from the solid and liquid fractions pretreated at different temperatures ($p < 0.05$). The VS content is an indicator of the biodegradability of the samples and represents the portion of substrate that can be

converted into biogas and biomethane. Research has shown that high VS content is a desirable condition in the anaerobic digestion process since it leads to higher biogas and biomethane yields [114][115][116][23].

Table 4. Total solids (TS) and volatile solids (VS) content of untreated material, samples from solid and liquid fractions and stages pretreated at 150 °C, 170 °C, 190 °C, and 200 °C

Fraction	Stage	Temperature	TS g/kg	VS g/kgTS
Untreated	-	-	956.0 ± 1.7	898.0 ± 0.7
Solid fraction	**After pretreatment**	**150 °C**	973.0 ± 2.3	938.0 ± 4.4
		170 °C	972.0 ± 2.4	931.0 ± 3.1
		190 °C	986.0 ± 2.9	930.0 ± 4.2
		200 °C	987.0 ± 2.2	929.0 ± 3.8
	After hydrolysis	**150 °C**	966.0 ± 2.1	930.0 ± 3.5
		170 °C	973.0 ± 5.9	972.0 ± 4.8
		190 °C	971.0 ± 3.1	970.0 ± 1.9
		200 °C	976.0 ± 3.6	975.0 ± 2.9
Liquid fraction	**After pretreatment**	**150 °C**	15.7.0 ± 2.2	995.0 ± 0.8
		170 °C	20.9.0 ± 0.8	994.0 ± 0.2
		190 °C	22.6 ± 0.8	994.0 ± 0.2
		200 °C	15.7 ± 1.7	996.0 ± 0.5
	After hydrolysis	**150 °C**	22.2 ± 2.4	999.0 ± 0.2
		170 °C	32.3 ± 0.6	1000.0 ± 0.4
		190 °C	34.9 ± 0.19	1000.0 ± 0.2
		200 °C	37.4 ± 3.1	1000.0 ± 0.17
	After distillation	**150 °C**	15.4 ± 0.5	998.0 ± 0.3
		170 °C	16.8 ± 0.5	999.0 ± 0.0
		190 °C	13.4 ± 1.0	999.0 ± 0.19
		200 °C	13.9 ± 2.2	999.0 ± 0.16

3.2. Sugar composition and bioethanol yields

The liquid fractions after pretreatment and hydrolysis were further analyzed in terms of cellobiose, glucose, xylose, galactose, arabinose and mannose concentrations (table 5). The composition of the sugars in the post pretreatment broth were low and below 1.0 g/L because this stage is mainly to break down the lignin and expose the plants cellulose and hemicellulose for enzymatic hydrolysis.

For the post hydrolysis broth, the composition of the convertible sugars (glucose) for samples pretreated at 150 °C was 14.9 g/L and 23.4 g/L for samples pretreated at 170 °C. While the samples pretreated at 190 °C and 200 °C, had glucose composition of 25.1 g/L and 31.6 g/L respectively. It was noticed that as the temperature increased so did the glucose yield. Hence,

the samples pretreated at 200 °C had the highest enzymatic conversion of cellulose and hemicellulose to sugars.

Table 5. Sugars yields (g/L) after pretreatment and hydrolysis at 150 °C, 170 °C, 190 °C and 200 °C

Fraction	Stage	Temperature	Cellobiose g/L	Glucose g/L	Xylose g/L	Galactose g/L	Arabinose g/L	Mannose g/L
Liquid fraction	**After pretreatment**	**150°C**	0.3±0.0	0.8±0.0	0.0±0.0	0.3±0.0	0.12±0.0	0.8±0.0
		170°C	0.16±0.0	0.4±0.0	0.0±0.0	0.17±0.0	0.2±0.0	0.5±0.0
		190°C	0.13±0.0	0.3±0.0	0.4±0.0	0.0±0.0	0.17±0.0	0.3±0.0
		200°C	0.11±0.0	0.3±0.0	0.3±0.0	0.0±0.0	0.13±0.0	0.2±0.0
	After hydrolysis	**150°C**	0.5±0.0	14.9±1.8	2.1±0.2	0.0±0.0	0.3±0.0	0.0±0.0
		170°C	0.5±0.0	23.4±0.6	3.0±0.19	0.0±0.0	0.3±0.0	0.0±0.0
		190°C	0.8±0.12	25.1±0.9	2.2±0.13	0.0±0.0	0.2±0.1	0.2±0.0
		200°C	0.5±0.15	31.6±1.4	2.5±0.2	0.0±0.0	0.14±0.12	0.3±0.0

Table 6 shows the bioethanol yield of the samples after hydrolysis. For the post fermentation broth, over 98% conversion of glucose to bioethanol occurred in all examined samples. After the fermentation process, samples pretreated at 150 °C had ethanol yield of 8.4 g/L while those pretreated and hydrolyzed at 170 °C yielded 10.3 g/L bioethanol. The samples pretreated at 190 °C and 200 °C had ethanol yields of 8.4 g/L and 9.4 g/L respectively. Hydrolysates pretreated at 170 °C had the highest bioethanol theoretical yield with ethanol concentrations of 10.3 g/L, closely followed by substrates pretreated at 150 °C with ethanol concentrations of 8.4 g/L which represents a 72% increase as compared to the expected yield from the glucose present. Samples pretreated above 170 °C resulted in a decrease in ethanol yields, most likely caused by the formation of inhibitors as a result of pretreatment at a high temperature and the degradation of hemicellulose. At 190 °C, the ethanol yield was 8.4 g/L (a 78% decrease from the expected yield) and at 200 °C, the ethanol titer was 5.6 g/L which amounts to a 42% decrease from the expected ethanol yield with respect to the 31.6 g/L glucose that was present in the post hydrolysis broth [23].

Table 6. Concentration of monosaccharides, glycerol, acetic acid and ethanol yields from hydrolysates pretreated at 150 °C, 170 °C, 190 °C and 200 °C

Fraction	**Stage**	**Temperature**	**Glucose g/L**	**Xylose g/L**	**Xylitol g/L**	**Glycerol g/L**	**Acetic acid g/L**	**Ethanol g/L**
Liquid fraction	**After fermentation**	**150°C**	0.0±0.0	2.1±0.17	0.0±0.0	0.5±0.11	1.6±0.9	8.4±1.3
		170°C	0.0±0.0	2.4±0.4	0.0±0.0	0.8±0.16	0.7±0.11	10.3±0.8
		190°C	0.0±0.0	2.2±0.2	0.0±0.0	0.9±0.0	0.4±0.2	8.4±1.2
		200°C	0.0±0.0	2.0±0.5	0.0±0.0	0.9±0.5	0.6±0.0	9.4±0.2

3.3. Biogas recovery

The biogas produced in the anaerobic digestion process of the samples of solid and liquid fractions from different stages of bioethanol production process is presented in figures 5, 6, 7, 8 and 9. Figure 10 represents the maximum biogas yield for samples from all the stages of bioethanol production process. The biogas yield was 2.04 mol CH_4/100 g for the untreated biomass material. At the end of the experiment, the samples pretreated at 150 °C had the highest biogas yield of 2.00 mol CH_4/100 g, closely followed by the samples that were pretreated at 170 °C with a biogas recovery of 1.86 mol CH_4/100 g. For samples pretreated at 190 °C and 200 °C, the biogas produced was 1.78 mol CH_4/100 g and 1.63 mol CH_4/100 g respectively. It could be observed that as the biomass pretreatment temperature increased, the quantity of biogas produced decreased. This decrease in biogas yield as temperature increased might have been as a result of reduction in the amount of volatile solids present in the solid material due to their more efficient degradation at high temperatures as well as formation of inhibitors like furfurals which hinder the activities of microbes. [117][118][119]. Same trend was observed in the solid samples after hydrolysis; with samples pretreated at 150 °C and 200 °C having the highest and lowest methane yield of 1.81 mol CH_4/100 g and 1.17 mol CH_4/100 g respectively.

Samples from the liquid fraction of post pretreatment broth had the highest biogas production of 0.42 mol CH_4/100 g at 190 °C and the lowest biogas production was at 150 °C (0.26 mol CH_4/100 g). At 170 °C, the biogas recovery was 0.38 mol CH_4/100 g and at 200 °C, the biogas recovery was 0.29 mol CH_4/100 g. For the post hydrolysis broth, the highest biogas yield was at 200 °C (0.99 mol CH_4/100 g) while the lowest biogas yield was still at 150 °C (0.69 mol CH_4/100 g). Samples from the liquid fraction after the distillation that were pretreated at 170 °C and 190 °C had the same composition of biogas (0.59 mol CH_4/100 g), while samples pretreated at 200 °C had the lowest biogas yield (0.26 mol CH_4/100 g). In general, the hydrolysates had the highest biogas yield because of the formation of glucose from the cellulose. The post treatment broth has the lowest biogas yield because there has not any conversion of cellulose to sugars, only loosening of the structures of the complex biomass structure has occurred and the separation of the fractions (solid and liquid fractions). The biogas yield in the distillate side stream was between 0.26 mol CH_4/100 g and 0.59 mol CH_4/100 g because part of the sugars after the fermentation stage have been converted to ethanol.

Overall, the solid fractions had higher biogas yields than the liquid fractions mainly due to the fact that the solid fraction has high total solids; high composition of cellulose and lignin which

means more sugar release for conversion (because of the presence of cellulose) and slower reactions (because of the lignin). Also, the difference between the biogas yield of the solid and the liquid fractions were statistically significant ($p < 0.05$).

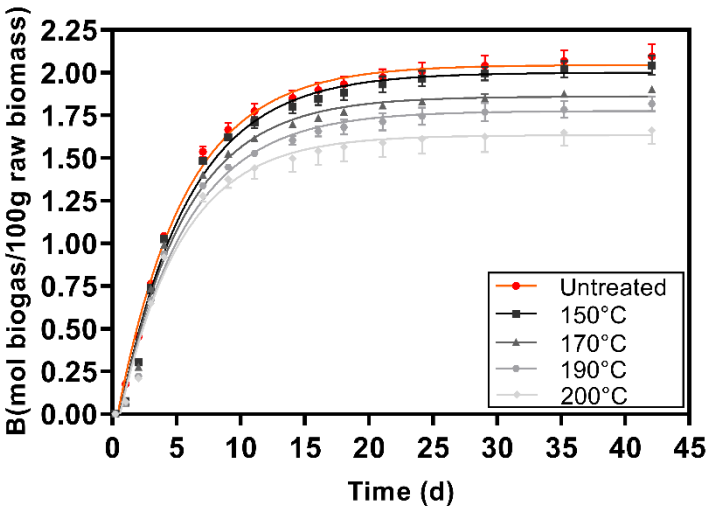

Figure 5. Solid fraction after pretreatment at 150 °C, 170 °C, 190 °C, and 200 °C in mol CH4/100 g raw biomass

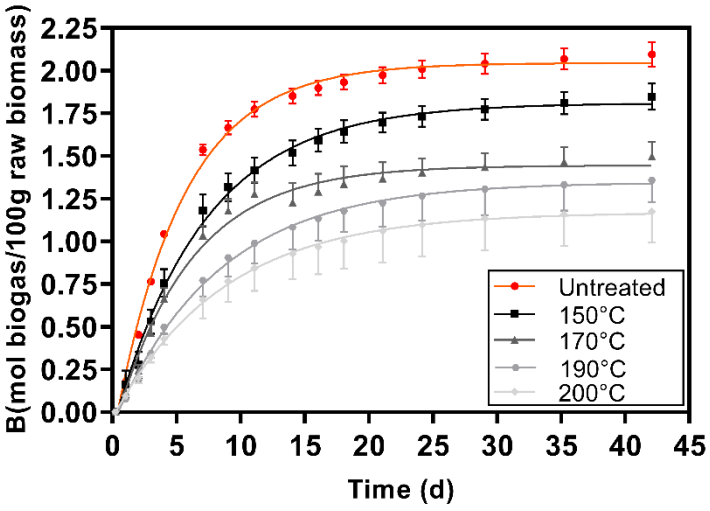

Figure 6. Solid fraction after hydrolysis at 150 °C, 170 °C, 190 °C, and 200 °C in mol CH4/100 g raw biomass

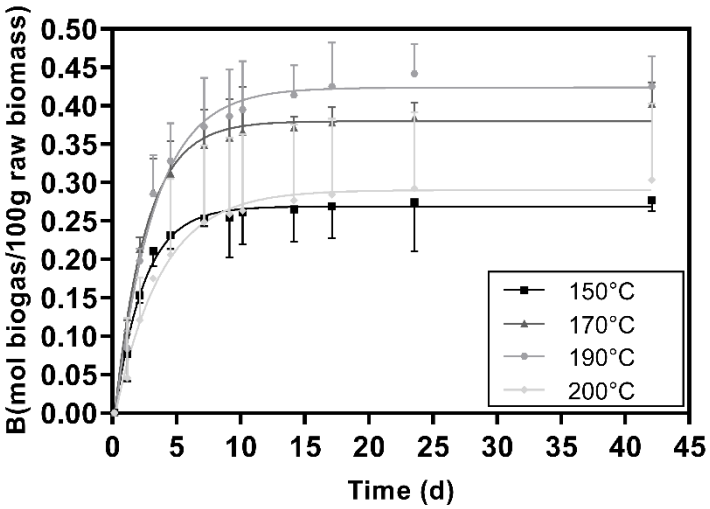

Figure 7. Liquid fraction after pretreatment at 150 °C, 170 °C, 190 °C, and 200 °C in mol CH4/100 g raw biomass

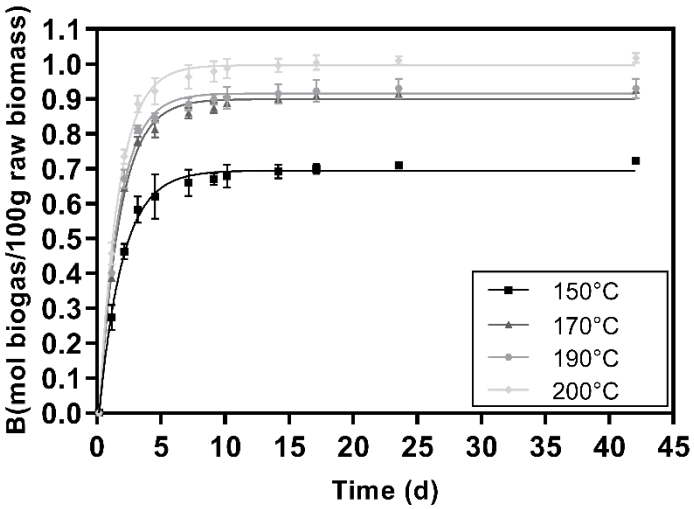

Figure 8. Liquid fraction after hydrolysis at 150 °C, 170 °C, 190 °C, and 200 °C in mol CH4/100 g raw biomass

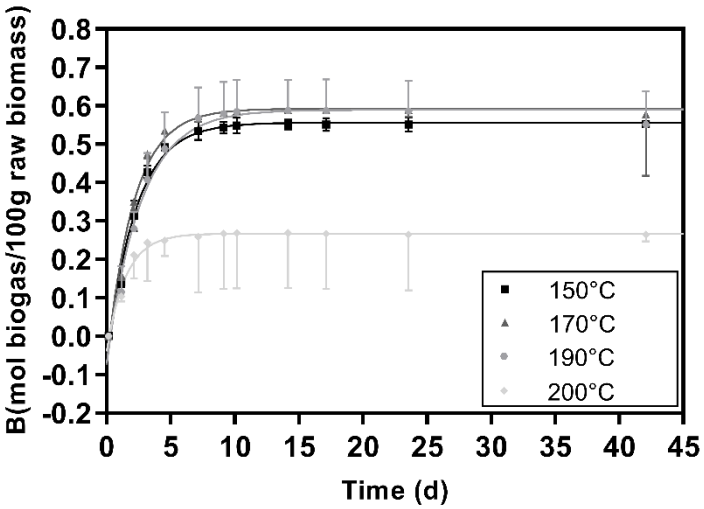

Figure 9. Liquid fraction after distillation at 150 °C, 170 °C, 190 °C, and 200 °C in mol CH4/100 g raw biomass

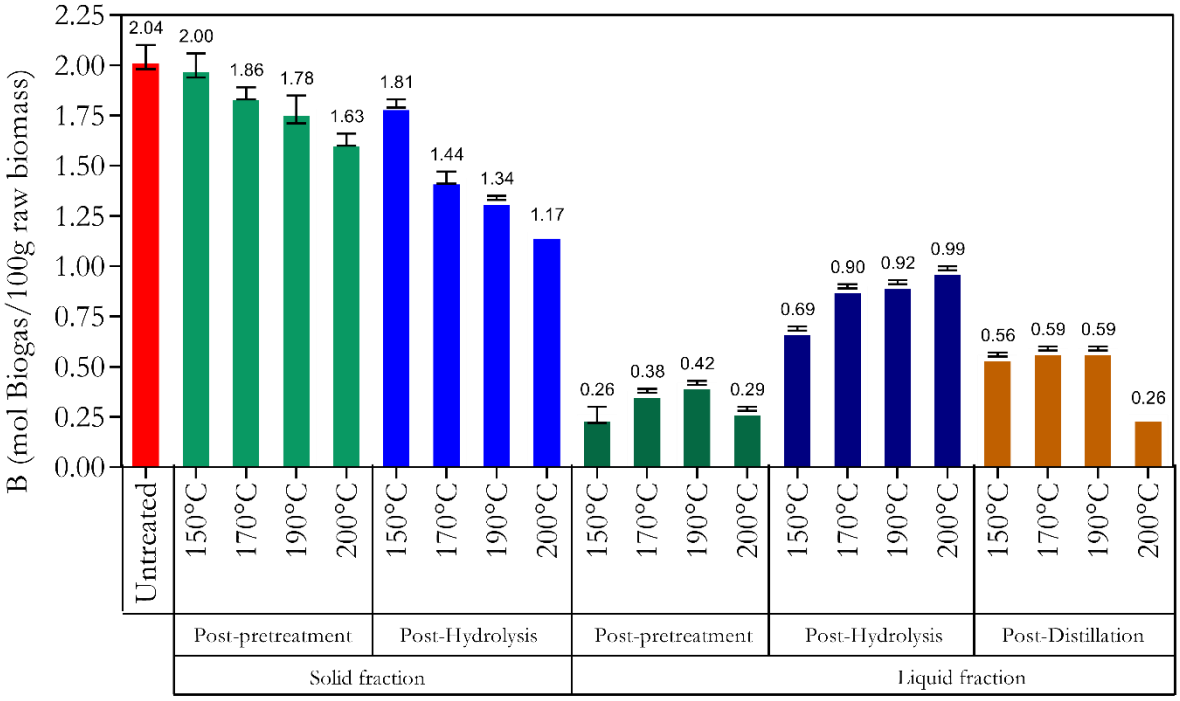

Figure 10. Maximum biogas yield

3.4. Kinetic evaluation of biomass bioconversion

Figure 11 represents the kinetic rate constant (k) for all the samples from the solid and liquid fractions that were pretreated at 150 °C, 170 °C, 190 °C, and 200 °C. The solid pretreatment broth had slower kinetic rates ranging from 0.1167 to 0.204 for both the post pretreatment and post hydrolysis stages over the four different temperatures, while for the liquid fraction had faster/higher kinetic rates ranging from 0.278 to 0.6732 for all three stages of analysis at different temperature. The overall highest kinetic rate was at 200 °C (0.6732±0.06) from the liquid fraction post distillation broth, while the solid fraction pretreated at 200 °C had the overall lowest kinetic rate of 0.1167. The overall correlation coefficient (R^2) varied between 0.9991 and 0.9849 for the entire anaerobic digestion process. The kinetic rates for the solid fractions were slower because of the presence of lignin in the broth, which was recalcitrant to the microorganisms resulting in slower reactions. However, the liquid fractions only contained hemicellulose, hence the microorganisms were able to acclimatize quickly to the substrate and biodegrade faster.

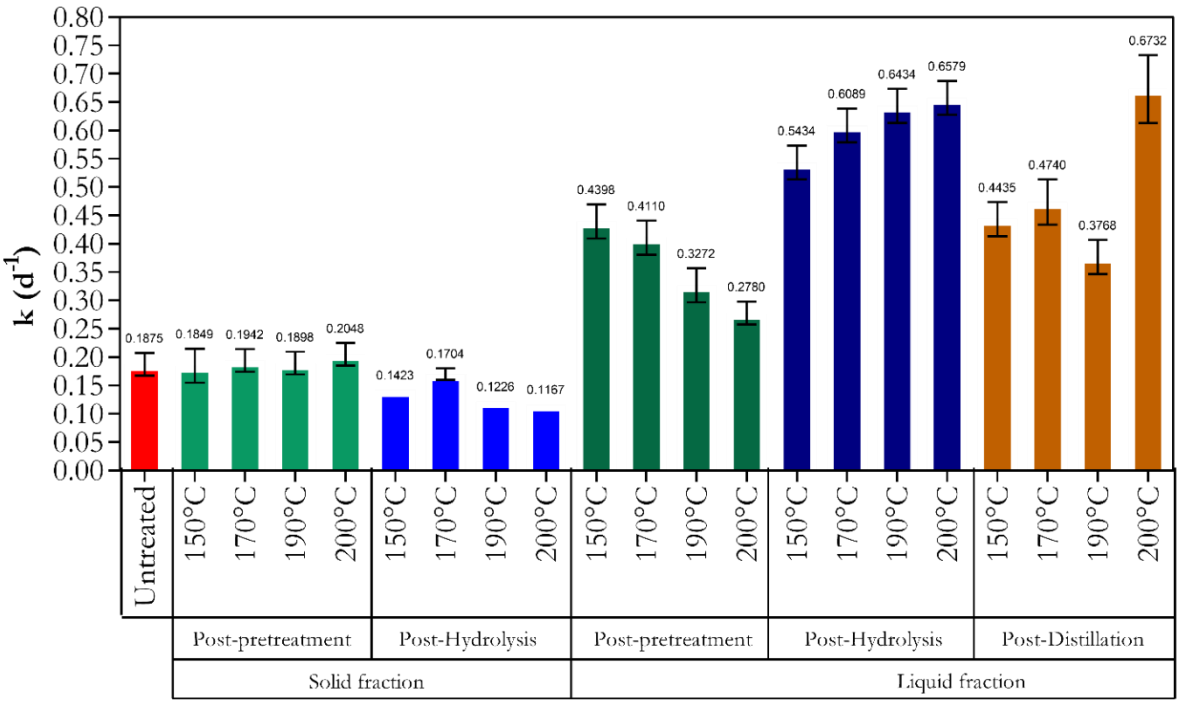

Figure 11. Kinetic rate constant of all the analyzed samples

Table 7 contains values which indicates the time it took for the microorganisms to achieve 85% B_{max} and 95% $B_{max.}$ in general, the time taken for all biomaterial to reach 85% B_{max} varied

from 3 days to ~16 days, and to reach 95% B_{max}, it took ~5 to ~26 days. It took the untreated biomass ~10 days to reach 85% B_{max} (1.74 mol CH_4/100 g) and ~16 days to attain 95% B_{max} (1.94 mol CH_4/100 g). The solid fraction samples had the longest anaerobic digestion time as compared with both the untreated Napier grass and the liquid fractions. The digestion time of this lot varied from t = 9.9. to t = 16.4 for the 85% B_{max} and t = 15.2 to t = 25.5. For the post treatment solid fraction, as the pretreatment temperature increased, the digestion time and 85% B_{max} and 95% B_{max} decreased. Samples pretreated at 150 °C had 85% B_{max} of 1.70 mol CH_4/100 g at t = 10.5 days and 95% B_{max} of 1.90 mol CH_4/100 g at t = 16.8 days, which declined until t = 9.9 days (0.99 mol CH_4/100 g), 85% B_{max} and t = 15.2 days (1.55 mol CH_4/100 g), 95% B_{max}. The digestion time (85% B_{max} and 95% B_{max}) of samples from the solid fraction after hydrolysis were the longest (t = 11.6 days to t = 16.4 days and t = 18.1 days to t = 25.5 days respectively), with the 200 °C hydrolyzed broth having the highest overall digestion time of t = 16.4 days, 85% B_{max} and t = 25.5 days, 95% $B_{max.}$ on the other hand, the 170 °C hydrolyzed broth had the lowest digestion time of the solid samples analyzed after hydrolysis: t = 11.6 days, 85% B_{max} and t = 18.1 days, 95% $B_{max.}$

For the liquid fraction, the opposite was the case. The lowest post pretreatment digestion time was at the 150 °C, t = 4.5 days (85% B_{max}) and t = 7.0 days, 95% $B_{max,}$ while the highest was at the 200 °C, t = 7.1 days, 85% B_{max} and t = 11.0 days, 95% $B_{max.}$ After hydrolysis, the digestion times reduced. The liquid broth with the lowest digestion time was pretreated at 190 °C and 200 °C with the same digestion times of t = 3.1 days for 85% B_{max} and t = ~4.7 days, 95% $B_{max.}$ The post distillation broth pretreated at 200 °C had the overall shortest anaerobic digestion time of t = 3.0 days to reach 85% B_{max} and t = 4.7 days to reach 95% B_{max}.

Data in from table 8 was represented in a bar chart in figure 10.

Table 7. Digestion time (85% B_{max} and 95% B_{max}) for the untreated Napier grass and NED treated fractions of biogas production process pretreated at 150 °C, 170 °C, 190 °C, and 200 °C

Fraction	Stage	Temperature	85% B_{max} mol CH_4/100g	Days	95% B_{max} mol CH_4/100g	Days
Untreated	**-**	**-**	1.74	10.5	1.94	16.4
Solid fraction	**After pretreatment**	**150°C**	1.70	10.8	1.90	16.8
		170°C	1.58	10.3	1.77	16.0
		190°C	1.51	10.6	1.69	16.4
		200°C	1.39	9.9	1.55	15.2
	After hydrolysis	**150°C**	1.54	13.7	1.72	21.4
		170°C	1.23	11.6	1.37	18.1
		190°C	1.14	15.8	1.28	24.5
		200°C	0.99	16.4	1.11	25.5
Liquid Fraction	**After pretreatment**	**150°C**	0.23	4.5	0.26	7.0
		170°C	0.32	4.8	0.36	7.5
		190°C	0.36	6.0	0.40	9.4
		200°C	0.25	7.1	0.28	11.0
	After hydrolysis	**150°C**	0.59	3.7	0.66	5.7
		170°C	0.76	3.3	0.85	5.1
		190°C	0.78	3.1	0.87	4.8
		200°C	0.85	3.1	0.95	4.7
	After distillation	**150°C**	0.47	4.5	0.53	7.0
		170°C	0.50	4.3	0.56	6.6
		190°C	0.50	5.3	0.56	8.3
		200°C	0.23	3.0	0.25	4.7

SUMMARY

In the wake of discovering more clean, affordable and sustainable alternatives to energy generation in Nigeria, this study evaluated the possibility of the utilization of the Nigerian Napier grass for efficient production of bioethanol and biogas. By pretreating this inedible lignocellulosic biomass with the novel nitrogen explosive decompression (NED) method at four temperatures (150 °C, 170 °C, 190 °C, and 200 °C), and separating the phases (solid and liquid fractions) for each stage of analysis (pretreatment, hydrolysis and distillation sidestreams), the following results were obtained.

After the individual fractions of samples were hydrolyzed, the lowest glucose and ethanol yield was gotten from samples pretreated at 150 °C; 14.9 g/L and 8.45 g/L respectively. On the other hand, the highest glucose and ethanol yield was found in samples pretreated at 200 °C (31.6 g/L) while the highest bioethanol yield was discovered in samples pretreated at 170 °C (10.3 g/L) respectively. These results demonstrate that an increase in temperature provides more energy to the NED process of breaking down the complex plant structure, thus exposing more cellulose and hemicellulose for enzymatic conversion to glucose in the hydrolysis step. However, after fermentation, the sample with the highest glucose composition produced less bioethanol, because of the formation of inhibitors when pretreating at high temperature. Therefore, for more efficient production of bioethanol using Napier grass as feedstock, NED should be carried out at 170 °C.

The biogas yield was higher in the solid fractions than in the liquid ones. The highest biogas yield was the solid fraction after pretreatment at 150 °C; 2.00 mol CH4/100 g while the lowest biogas yield was the liquid fraction after pretreatment at 200 °C; 0.26 mol CH4/100 g. It was also observed that the rate of kinetics was much lower for the solid fraction samples than for the liquid fraction samples because the solid fraction is made up of cellulose and lignin; a structure which hampers the anaerobic digestion process, whereas the liquid fraction is composed of hemicellulose with no lignin and as such, no inhibition of the digestion process. However, the composition of hemicellulose (26.9%) is little as compared to the cellulose value (35.7%) and coupled with the fact that part of the sugars have been used up by the fermentation process the distillation sidestream sample pretreated at 200 °C yielded the least biogas.

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ANNEX 1

sustainability

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Bioenergy Yields from Sequential Bioethanol and Biomethane Production: An Optimized Process Flow

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Abstract: This study investigates the potential of different stages of the bioethanol production process (pretreatment, hydrolysis, and distillation) for bioethanol and biomethane production, and studies the critical steps for the liquid and the solid fractions to be separated and discarded to improve the efficiency of the production chain. For this, Napier grass (a fast-growing grass) from Effurun town of Delta State in Nigeria was used and the novel pretreatment method, nitrogen explosive decompression (NED), was applied at different temperatures. The results show that the lowest glucose (13.7 g/L) and ethanol titers (8.4 g/L) were gained at 150 °C. The highest glucose recovery (31.3 g/L) was obtained at 200 °C and the maximum ethanol production (10.3 g/L) at 170 °C. Methane yields are higher in samples pretreated at lower temperatures. The maximum methane yields were reported in samples from the solid fraction of post-pretreatment (pretreated at 150 °C, 1.13 mol CH4/100 g) and solid fraction of the post-hydrolysis stage (pretreated at 150 °C, 1.00 mol CH4/100 g). The lowest biomethane production was noted in samples from the liquid fraction of post-pretreatment broth (between 0.14 mol CH4/100 g and 0.24 mol CH4/100 g). From the process point of view, samples from liquid fraction of post-pretreatment broth should be separated and discarded from the bioethanol production process, since they do not add value to the production chain. The results suggest that bioethanol and biomethane concentrations are influenced by the pretreatment temperature. Napier grass has potential for bioethanol and further biomethane production and it can be used as an alternative source of energy for the transportation sector in Nigeria and other countries rich in grasses and provide energy security to their population.

Keywords: anaerobic digestion; biofuel; lignocellulose; sidestreams; zero-waste

1. Introduction

The worldwide economic advancements and population growth have been contributing to the increased demand for the electricity generation capacity. About 82% of the gross inland energy consumption in the world still derives from petroleum (32%), coal (28%), and natural gas (22%) [1]. The same trend is evident in different continents and countries around the world, including Nigeria which is one of the major economies in Sub-Saharan Africa and the largest oil producer in Africa. Its total primary energy consumption comes from traditional solid biomass and waste (80%) [2]. The fossil fuels utilised in Nigeria are mainly being consumed in the transportation sector (100% oil), productive (17% gas and 16% oil), and residential uses (3% oil). Nigerian power generation comes mainly from gas (62%), oil (33%), and hydro (5%) [3]. Therefore, there is a need for affordable

alternative sources of energy that will decrease the share of fossil fuels utilised in the transportation sector and productive uses, reduce environmental concerns caused by the extensive utilization of these resources (e.g., climate change and global warming), increase energy security, and improve the access of the population to electricity. Renewable energy sources from lignocellulosic biomass have been reported as promising solutions to these problems [4]. Nowadays, biomass itself is responsible for 10% primary energy consumption worldwide [5] and it can be used as a promising feedstock for biofuel production. The sustainability of wastes and by-products as a biofuel for the transportation sector in a circular economy has been studied in the literature, in order to make the biogas–biomethane chain more sustainable [6–8]. These studies show the positive impact of biofuels in the transportation section in Europe. However, further research needs to be done in order to apply circular economy models to emerging economies.

Sub-Saharan Africa has great potential to develop renewable energy sources, such as wind, biomass, solar, and hydro. Just in Nigeria, the biomass potential is about 144 million tonnes per year and the potential of its lignocellulosic agricultural waste varies between 0.4 and 2.3 t/ha, as reported in previous publications [9]. However, the utilisation of biomass resources for electricity, biofuel, or biogas generation has not been extensively utilised or studied in most African countries [9–11]. From the different lignocellulosic materials currently available in Nigeria, Napier grass has been reported to be a particularly attractive feedstock for production of biofuels and bio-based products mainly due to its high cellulose content (34.2–40%), high yields per unit area, tolerance to drought, and a good water use efficiency (ratio of water used by the crop to water lost by evapotranspiration) [12–17].

Napier grass is a perennial C4 plant endemic to Sub-Saharan Africa with a high heating value biomass (16.58 MJ/kg) [18]. This crop is mainly used to feed cattle, but it can also be used for grazing, silage, or hay production or fish food [15]. In moderate climates, it can be harvested up to four times per year [19,20]. However, in most of the cases, Napier grass is a neglected crop that exists in the wild and that does not need to be cultivated [21,22], making it a particularly attractive feedstock for biogas and bioethanol production. Sawasdee and Pisutpaisal [23] studied the potential of Napier grass for biogas production. At 5% total solids, the authors obtained the highest kinetics rate for biogas production and concluded that this feedstock can be grown for this purpose. Narinthorn et al. [13] also investigated the biomethane potential of Napier grass. For this, the authors applied combined alkaline and biological pretreatment methods as a strategy to enhance biomethane yields from Napier grass. The results reveal that alkaline pretreatment method increased the anaerobic digestibility from 49% (untreated grass) to 77% and improved the biomethane yields by about 34%. Janejadkarn and Chavalparit [24] quantified biogas production from Napier grass. The results indicated that with a 2% volatile solids content and an organic load rate of 0.57 kg VS/m^3, it is possible to achieve the maximum biogas yield (0.529 m^3/kg VS). Under the same conditions, the methane production was 0.242 m^3/kg VS added. All this suggests that Napier grass can be successfully converted into biogas by means of anaerobic digestion.

Liu et al. [25] investigated the potential of Napier grass for bioethanol production by using dilute-alkali and dilute-acid pretreatment methods. The results show that, for a feeding concentration of 10 g/L, the theoretical conversion rate of this feedstock is about 12.6%, and for a feeding concentration of 15 g/L its conversion rate increased to 23%. The authors concluded that agricultural waste had potential for bioethanol production. Wongwatanapaiboon et al. [26] analysed the potential of Napier grass as feedstock for lignocellulosic bioethanol production by using alkaline peroxide as a pretreatment method. The ethanol yields from Napier grass produced by simultaneous saccharification and cofermentation (SSCF) are 1171.69 L/ha/year, indicating that Napier grass has potential for cellulosic ethanol production.

Although bioethanol production from lignocellulosic materials has been widely studied, its production still has environmental, economic, and energetic constraints. From the environmental perspective, the sidestream generated after the distillation stage has a high pollutant potential and the best handling options still need to be studied. Economically, the energy costs required in the

pretreatment stage are still high, making biofuel production less competitive compared to fossil fuels. Energetically, ethanol from biomass has a low-energy return on energy invested (ERoEI) when compared to coal, oil, and gas. Therefore, solutions to add value to the bioethanol production chain to make its production more competitive are needed. Having this in mind, anaerobic digestion (AD) has been proposed as a handling option for waste recovery from biodegradable waste and bioethanol sidestreams [4,8,27].

As the Nigerian biofuel sector is in a developing stage, this paper aims at evaluating the potential of Nigerian Napier grass for bioethanol and biogas production and at investigating its reliability as an alternative source of energy for the transportation sector in Nigeria and other African countries with high availability of this grass. For this, samples taken from different stages of bioethanol production (pretreatment, hydrolysis, and distillation) and bioethanol sidestream were used. These samples went through a separation process (solid and liquid fractions) and different production pathways in order to enhance bioenergy yields, improve the efficiency of the production chain, decrease the energy and water requirements, and reduce the sidestream volume generated at the end of process.

2. Materials and Methods

2.1. Bioethanol Production

2.1.1. Biomass

The *Penisetum purpurum* (Napier grass) grew in the wild and was harvested near Effurun town of Delta State in Nigeria. It was harvested in the Harmattan period in early January of 2019 and allowed to dry naturally in the sun. After drying, the biomass was shipped to Estonia where all the experiments were carried out. The samples were milled and sieved to the size of 3 mm or smaller in the Cutting Mill SM 100 Comfort (from Retsch GmbH).

2.1.2. Pretreatment

The Napier grass was pretreated with the nitrogen explosive decompression (NED) method. For pretreatment, 100 g of raw material were added into the 2 L non-stirred pressure vessel and soaked in 800 g of distilled water. The vessel was closed, and the samples were heated up from room temperature (23 °C) up to 150 °C, 170 °C, 190 °C, or 200 °C, under constant pressure (30 bar), for the retention time of one minute. Once the desired temperature was reached, the reactor was cooled down to approximately 80 °C and the pressure was released in an explosive manner using the pressure release valve. Figure 1 illustrates the pretreatment system utilised in these experiments. After the pretreatment process, the samples were cooled down to 50 °C for the following enzymatic hydrolysis.

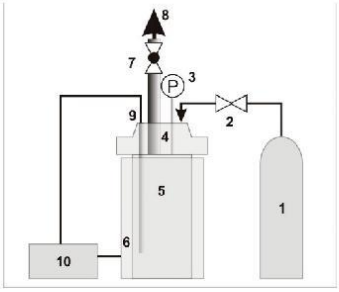

Figure 1. Schematic diagram of the 2 L pressure vessel system (series 4600) for NED pretreatment: 1—nitrogen tank; 2—pressure control valve; 3—manometer; 4—modified pressure vessel cap; 5—Parr instruments pressure vessel; 6—ceramic contact heater; 7—pressure release valve; 8—ventilation system; 9—thermocouple; 10—temperature controller unit [28].

2.1.3. Hydrolysis

The material obtained from the pretreatment process was added into a 1000 mL shake flask for enzymatic hydrolysis. For this, 30 FPU g/cellulose of the cellulase complex Accelerase 1500 (DuPont de Nemours) was added into the suspension, and the flask was filled up with distilled water to 1000 mL (working volume). The process was carried out in an orbital shaker (IKA®-Werke GmbH & Co. KG, Staufen im Breisgau, Germany) (KS 4000 I control) during a 24 h period, at temperature of 50 °C and rotation speed of 250 rpm.

2.1.4. Fermentation

Glucose in the hydrolysate was converted into ethanol in the following fermentation step. The fermentation process was performed in glass bottles with a working volume of 1000 mL, using 2.5 g of the commercial yeast *Saccharomyces cerevisiae* (Turbo yeast T3). After adding the yeast, the glass bottles were closed with an airlock and the fermentation process was carried through for seven days, at room temperature.

2.1.5. Distillation

After the fermentation, the samples went through a distillation process at 175 mbar using a rotating evaporation system designed for ethanol separation, Buchi R-210 Rotavapor System from BÜCHI Labortechnik (Flavil, Switzerland). The material obtained after the distillation process (bioethanol production sidestream) was analysed in terms of its potential for biomethane production in the biomethane potential assay (BMP).

2.2. Biomethane Potential (BMP)

Samples from the solid and liquid fractions of different stages of bioethanol production process (pretreatment, hydrolysis, and distillation) were used as a feedstock. Figure 2 illustrates the different production pathways utilised in this study. The BMP was measured in untreated Napier grass (pathway 1), samples from the solid fraction of post-pretreatment broth (pathway 2) and post-hydrolysis broth (pathway 4), and samples from the liquid fraction of post-pretreatment broth (pathway 3), post-hydrolysis broth (pathway 5) and post-distillation broth (pathway 6).

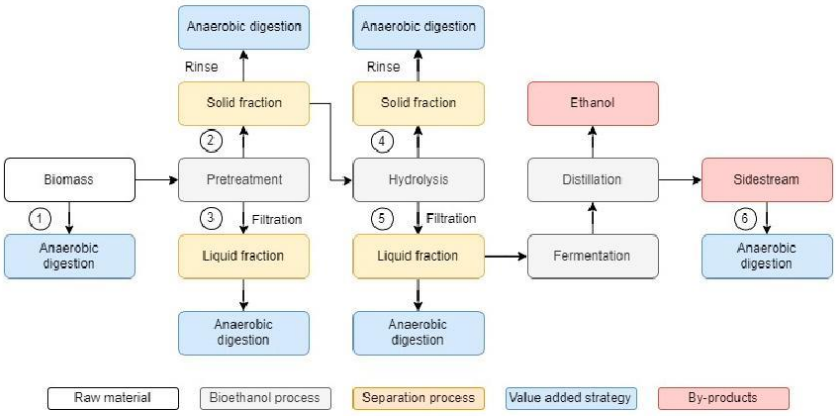

Figure 2. Different production pathways utilised in this study to evaluate the potential of *Pennisetum purpureum* for bioethanol and biogas production by means of solid–liquid separation. 1—untreated Napier grass; 2—samples from the solid fraction of post-pretreatment broth; 3—samples from the liquid fraction of post-pretreatment broth; 4—samples from the solid fraction of post-hydrolysis broth; 5—samples from the liquid fraction of post-hydrolysis broth; 6—samples from post-distillation broth.

The BMP assay utilised in these experiments is based on a modified version of the methods reported by Owen et al. [29] and Angelidaki et al. [30]. The inoculum sludge utilised in this study was obtained from the local wastewater treatment plant (Tartu, Estonia). Prior to use, the inoculum was stabilized for four days in an incubator at 36 °C, so the residual organic matter would be consumed, and the dissolved gases would be removed by a process of degasification. The assays were performed in 575 mL glass bottles, with a working volume of 200 mL, headspace volume of 375 mL and VS substrate/VS inoculum ratio of 0.25 (based on the volatile solids content that characterizes the quantity of organic material available in the solid). Before sealing the bottles, nitrogen gas was purged into the headspace of the flasks for approximately three minutes to ensure anaerobic conditions. The bottles were further sealed with rubber stoppers and aluminium caps, mixed, and incubated for 42 days, under mesophilic conditions (37 °C ± 1 °C) until the methane production was less than 1% of the total amount produced. The bottles were mixed daily by shaking. The experiments were performed in triplicates and a blank test with inoculum sludge only was also prepared in order to determine the methane production from the inoculum sludge itself, which was later utilised in the calculations of methane gas produced.

The biogas production was evaluated by measuring the increase of the total headspace pressure in the test flasks before and after the gas chromatograph (GC) analysis with a pressure meter WAL BMP-Testsystem (from WAL Mess-und Regelsysteme GmbH, Germany).

The pH of all the samples was measured at the end of the experiments with a SevenMulti™ S47-dual pH/conductivity meter to ensure that the anaerobic digestion was performed under optimum conditions (pH of 6.8–7.2) from the beginning until the end of the process [31]. The biomethane results are reported in moles of methane per 100 g of raw material using standard conditions to understand the amount of methane that can be obtained from the original raw material, and in L CH_4/kg VS.

2.3. Analytical Methods

The composition of the samples in terms of cellulose, hemicellulose, and lignin (fibre analysis) was determined using an ANKOM 2000 analyzer (ANKOM Technology, Macedon, NY, USA). The percentage of moisture in the samples was analysed in the Kern MLS-50-3D moisture analyser from Kern & Sohn GmbH.

The active volume of substrate and inoculum was determined from the analysis of the total solids (TS) and volatile solids (VS) content, which were determined according to the method 1684 from the U.S. Environmental Protection Agency (EPA). The methane content in the biogas was measured chromatographically using the GC (CP-4900 Micro-GC, Varian Inc., Palo Alto, CA, USA). The gas chromatograph was equipped with a thermal conductivity detector, a Molsieve 5A Backflush heated column (20 m × 0.53 mm) and a PoraPLOT U heated column (10 m × 0.53 mm). Argon was used as a carrier gas in column 1, and the operational conditions of this column were as follows: injection temperature 110 °C, column temperature 120 °C, and column pressure 50 Psi. In column 2, the carrier gas was helium and the injection temperature, column temperature, and column pressure were set to 110 °C, 150 °C, and 22 Psi, respectively.

The samples of solid and liquid fractions investigated in this study were obtained from different stages of the bioethanol production process (pretreatment, hydrolysis, and distillation) with a separation process using the pathway illustrated in Figure 2. For this, post-pretreatment broth and post-hydrolysis broth were collected and centrifuged using Thermo Scientific Heraeus Megafuge at a rotational speed of 10,000 rpm for 20 min until the solid and liquid fractions were fully separated. To ensure a full separation of the supernatant (liquid fraction) and the retentate (solid fraction), the samples were separated using vacuum filtration. After that, samples from the solid fraction were rinsed with distilled water to remove residual solubles and dried at 40 °C to a moisture content of 4.5% (or less). Both fractions were analysed for BMP.

Glucose, xylose, galactose, arabinose, mannose, glycerol, acetic acid, and ethanol were quantified with a high pressure liquid chromatography [32] using fractions after hydrolysis and fermentation steps.

2.4. Calculations

The quantity of methane gas (initial) produced in the test flask [$CH_{4\ I}$] (mol CH_4) is given by Equation (1):

$$[CH_{4\ I}] = MF\frac{P_I V_{HS}}{R(273.15 + T)} \quad (1)$$

where P_I (Pa) is the total pressure at the headspace determined prior to the GC analysis, V_{HS} (m^3) is the volume of the headspace of the bottle, MF is the methane fraction determined by the GC in the current period of time, R is the ideal gas constant (8314 $Jmol^{-1}\ K^{-1}$), and T is the temperature in the incubator (°C).

The quantity of methane gas (final) in the headspace of the test flask [$CH_{4\ F}$] (mol CH_4) is determined by Equation (2):

$$[CH_{4\ F}] = MF\frac{P_F V_{HS}}{R(273.15 + T)} \quad (2)$$

where P_F (Pa) is the total pressure at the headspace determined following the GC analysis.

The cumulative methane produced in the current period of time $[CH_{4\ C}]_t$ (mol CH_4) is defined by Equation (3):

$$[CH_{4\ C}]_t = ([CH_{4\ I}]_t - [CH_{4\ F}]_{t-1}) + [CH_{4\ C}]_{t-1} \quad (3)$$

where $[CH_{4\ I}]_t$ (mol CH_4) is the quantity of methane in the headspace of the flask (initial) in the current period of time, $[CH_{4\ F}]_{t-1}$ (mol CH_4) is the quantity methane in the headspace of the test bottle (final) in the prior period of time, and $[CH_{4\ C}]_{t-1}$ (mol CH_4) is the quantity of cumulative methane gas produced in the prior period of time.

The results of methane gas produced were modelled in the statistics software GraphPad Prism 5.0 using a nonlinear regression model that was further fitted in an exponential first-order association model (Equation (4) [19,20]:

$$B = B_{max}(1 - e^{-kt}) \quad (4)$$

where B is the cumulative methane produced (mol CH_4/100 g) at time interval (t), B_{max} is the maximum methane yield (mol CH_4/100 g), k is the kinetics rate constant (d^{-1}).

2.5. Statistics

The statistical analysis was performed with the software GraphPad Prism 5. The Shapiro-Wilk's normality test was utilised to determine the normal distribution of the variables. The Kruskal–Wallis test and the post hoc test Dunn's multiple comparison test were used to investigate the differences between the variables. The results are represented with the respective error bars and intervals that denote one standard deviation. The results were considered significantly different when the *p*-value was inferior to $p < 0.05$.

2.6. Napier Grass Availability, Production, and Growth

The estimated biomass yields (of all the feedstocks with exception of Napier grass) were obtained from FAO (Food and Agriculture Organisation of UN) bioenergy and food security rapid appraisal tool (Excel-based tools) and represent a ten-years average of annual production at country level [33]. The different Napier grass yields were obtained from the literature [34–36].

3. Results

3.1. Napier Grass Availability, Production and Growth

Although Nigeria has been reported as an emerging economy for biofuel production [37], a study by Rocha-Meneses et al. [38] shows that the country is only 19th out of 27 in the equatorial Africa with more potential available for bioenergy production. The study reported the utilization of agricultural

waste as barley, wheat, millet, oat, rice, rye, sorghum, and maize for bioenergy production. Besides agricultural residues, Nigeria has a large quantity of neglected feedstocks that can be further utilised for bioenergy production. Napier grass is one of these substrates.

Napier grass is a perennial crop native to Africa with low input requirements (e.g., low nutrient, fertilizer, and water requirements) and fast-growing characteristics. Its heating value is relatively high, varying between 16.21 MJ/kg (leaves) and 18.12 MJ/kg (stems). The heating value of the full plant is about 16.58 MJ/kg [39]. In addition, Napier grass has a great soil carbon sequestration potential, it can grow in marginal lands, but it is largely available in the wild, decreasing the competition with arable lands and therefore reducing the food versus fuel competition [40]. These unique characteristics make Napier grass one of the most prospective renewable energy sources for biofuel production in this region [36]. Under suitable conditions, Napier grass can grow up to 2–4.5 m tall and has a production of 40–60 t/DM/ha. In temperate climates, it can be harvested up to four times per year. Napier grass can be harvested as soon as three to four months after planting and it can continue in periods of six to eight weeks for up to five years [35,36]. From unfertilized stands, the dry matter yields of Napier grass are between 2 and 10 t/ha. Its dry matter production varies between 4.6 and 20.5 t/ha/year in Ethiopia, 12.1 and 19 t/ha/year in Kenya and 90 t/ha/year in Zimbawe [34].

Figures 3 and 4 represent the distribution of Napier grass in the different countries of Sub-Saharan Africa, and the land suitability for Napier grass production in Nigeria, respectively. As it can be seen from the figures, the majority of the Nigerian territory is highly suitable for Napier grass production. Particularly, the South East zone (SE) and the North central (NC) have the highest land suitability in the country. In the south of Nigeria, South West (SW) is the zone with more potential for Napier grass cultivation. Regarding the productivity of this grass in Nigeria, research has shown that the NC zone is the most productive zone of the country, followed by the North East (NE), and North West (NW) [41]. In terms of bioethanol production, Chukwu (2018) [41] predicted that the NC zone has the highest potential for cellulosic bioethanol production, followed by the NE zone.

Table 1 represents the energy output from Napier grass at different production rates (2 t/ha, 10 t/ha, 20 t/ha, 30 t/ha, 40 t/ha, and 60 t/ha) in comparison with other feedstocks currently available in Nigeria. The results show that even at low production yield (2 t/ha), Napier grass has higher energy yields than some well-known crops such as maize, rice, groundnut, wheat, coffee, sorghum, soybean, and millet. At high production yields, Napier grass has the highest energy yields, followed by sugarcane (bagasse and leaves), coconut (shells and husk), and oil palm (straw/pods). These results show that independent of the production yields, Napier grass is among the top 10 crops in Nigeria with higher energy yields, indicating the potential of this feedstock for further bioenergy production.

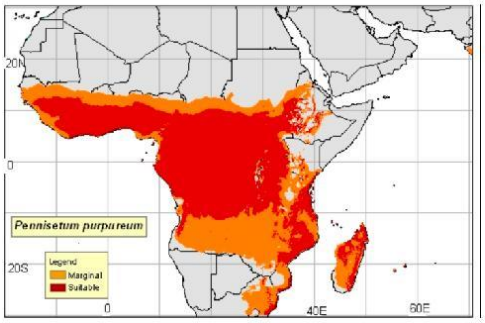

Figure 3. Napier grass distribution in Sub-Saharan Africa (inclusive Nigeria) [42].

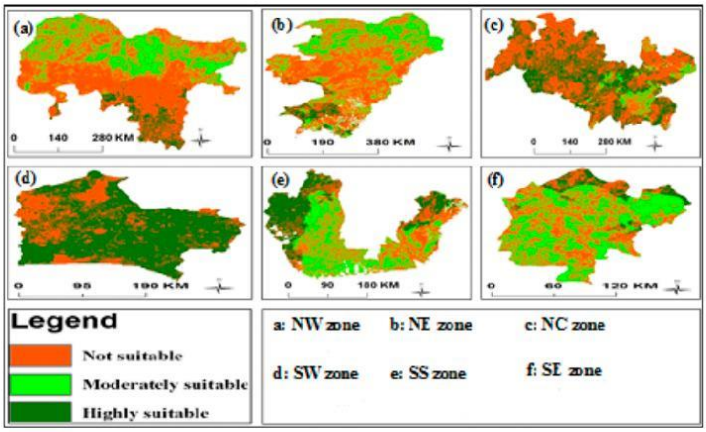

Figure 4. Land suitability for Napier grass production in Nigeria (by geopolitical zone) [41].

Table 1. Estimated energy yield from Napier grass and different agricultural residues available in Nigeria.

Feedstocks	Estimated Production Yield (t/ha) [33–36]	Calorific Value (MJ/kg)	Estimated Energy Yield (MJ/kg)/ha
Napier grass	60	16.58 [43]	994,800
Napier grass	40	16.58 [43]	663,200
Napier grass	30	16.58 [43]	497,400
Sugarcane bagasse	23.72	15.62 [44]	370,506
Napier grass	20	16.58 [43]	331,600
Sugarcane leaves	23.72	9.11 [44]	216,089
Napier grass	10	16.58 [43]	165,800
Coconut shells	6.26	17.9 [45]	112,054
Coconut husk	6.26	9.8 [45]	61,348
Oil palm (straw/ponds)	2.60	18–21 [46]	46,800–54,600
Napier grass	2	16.58 [43]	33,160
Maize cob	1.76	15.4 [46]	27,104
Rice husk/straw	1.75	14.44 [47]	25,270
Groundnut husk	1.30	18.81 [47]	24,453
Wheat straw	1.37	15.2 [45]	20,824
Coffee husk	1.28	16.00 [45]	20,480
Sorghum straw/stalk	1.21	16.00 [47]	19,360
Soybean husk	0.93	16.48 [44]	15,326
Millet straw	1.19	12.48 [47]	14,851

3.2. Chemical Composition

The structural composition of the Napier grass is presented in Table 2. The Napier grass contains 35.69% of cellulose, 26.9% of hemicellulose, 5.2% of lignin, and 9.6% of ash. The proportion of cellulose is relatively low, being 8.5% to 17% lower than the values that were described in the literature, while the hemicellulose content is 25% to 35% higher. The percentage of lignin is particularly low (81% lower) in comparison with the values reported in the available bibliography [43,48]. The ash content is 79% lower than the values reported in the literature [43,48]. These differences in the proportions of cellulose, hemicellulose, and lignin may be due to the growing, harvesting, and drying conditions of the samples. The Napier grass used in this study grew in the wild, it was harvested in early January (possibly the plant was not fully matured) and dried naturally in the sun, while in the studies of Mohammed et al. [43] and Nascimento and Rezende [48] the growing, harvesting, and drying conditions were closely monitored, ensuring the maximum growth and the optimal composition of the substrate. Although the cellulose content was lower than the values reported in the literature, the lignin percentage is also very low, making the delignification process easier. This means that less

energy input should be required to break the plant cell wall, and thus making the cellulose easily accessible, degradable, and convertible into sugars. Research has shown that high lignin content leads to low digestibility of the biomass. Therefore, low lignin content is a desirable condition for bioethanol and biomethane production [4,49].

Table 2. Composition of untreated Napier grass.

Component	Composition (%)
Cellulose	35.7 ± 0.3
Hemicellulose	26.9 ± 1.2
Lignin	5.2 ± 0.5
Ash	9.6 ± 0.0
Moisture	6.1 ± 0.3

Table 3 represents the total solids (TS) and volatile solids (VS) content for untreated Napier grass, samples from the solid and liquid fraction from different steps of bioethanol production chain, pretreated with NED at different temperatures. The TS content of untreated material was 956 g/kg. For samples of the solid fraction the TS content varied between 966 g/kg and 987 g/kg, and for samples from the liquid fraction between 13.4 g/kg and 37.4 g/kg. Statistically significant differences were found between TS content of samples from the solid and liquid fractions pretreated at different temperatures. These results show that the solid fraction had higher TS content than liquid fraction. High TS content is particularly important since it indicates there is more substrate available for the anaerobic digestion process, leading to higher methane and biogas yields [50].

Table 3. Total solids and volatile solids content of untreated material, samples from different fractions and stages pretreated at 150 °C, 170 °C, 190 °C, and 200 °C.

Fraction	Stage	Temperature	TS g/kg	VS g/kgTS
Untreated	-	-	956 ± 2	898 ± 1 ^{a,b,c}
Solid fraction	Post-pretreatment broth	150 °C	973 ± 2 ^{a,b,c,d}	938 ± 4 ^{d,e,f}
		170 °C	972 ± 2	931 ± 3 ^{g,h,i}
		190 °C	986 ± 3 ^{e,f,g,h,i,j}	930 ± 4 ^{j,k,l,m,n,o,p}
		200 °C	987 ± 2 ^{k,l,m,n,o,p,q}	929 ± 4 ^{q,r,s,t,u,v,w}
	Post-hydrolysis broth	150 °C	966 ± 2	930 ± 6 ^{x,y,z,aa,bb,cc,dd}
		170 °C	973 ± 6 ^{r,s,t,u}	972 ± 5
		190 °C	971 ± 3 ^{v,w,x,y}	970 ± 2 ^{ee,ff,gg}
		200 °C	976 ± 4 ^{z,aa,bb,cc,dd,ee}	975 ± 2.9 ^{hh,ii}
Liquid fraction	Post-pretreatment broth	150 °C	15.7 ± 2.2 ^{e,k,z}	995 ± 1
		170 °C	20.9 ± 0.7 ^{l}	994 ± 0
		190 °C	22.6 ± 0.8	994 ± 0
		200 °C	15.7 ± 1.7 ^{a,f,m,r,v,aa}	996 ± 1
	Post-hydrolysis broth	150 °C	22.2 ± 2.4	999 ± 0 ^{j,q,x}
		170 °C	32.3 ± 0.7	1000 ± 0 ^{a,d,g,k,r,y,ee}
		190 °C	34.9 ± 0.2	1000 ± 0 ^{b,e,h,l,s,z,ff,hh}
		200 °C	37.4 ± 3.1	1000 ± 0 ^{f,l,m,t,aa,gg,ii}
	Post-distillation broth	150 °C	15.4 ± 0.5 ^{b,g,n,s,w,bb}	998 ± 0
		170 °C	16.8 ± 0.5 ^{h,o,cc}	999 ± 0 ^{n,u,bb}
		190 °C	13.4 ± 0.9 ^{c,i,p,t,x,dd}	999 ± 0 ^{o,v,cc}
		200 °C	13.9 ± 2.2 ^{d,j,q,u,y,ee}	999 ± 0 ^{p,x,dd}

The superscripts designate statistically significant differences ($p < 0.05$) between the variables inside the column.

The VS content of untreated Napier grass was 889 g/kg. For samples of the solid fraction the TS content varied between 929 g/kg and 975 g/kg, and for samples from the liquid fraction between 994 g/kg and 1000 g/kg. Statistically significant differences were found between VS content of samples from the solid and liquid fractions pretreated at different temperatures. The VS content is an indicator

of the biodegradability of the samples and represents the portion of substrate that can be converted into biogas and biomethane. Research has shown that high VS content is a desirable condition in the anaerobic digestion process since it leads to higher biogas and biomethane yields [51,52].

3.3. Sugar Composition from Different Stages of the Liquid Fraction and Ethanol Production from Hydrolysates

Monosaccharide concentrations present in all the post-treatment broths were low (>1.0 g/L, Table 4) indicating that sugars were in the form of oligomers non-detected by the methodology employed. Higher temperatures during pretreatment resulted in higher concentrations of glucose after hydrolysis, reaching up to 31.56 g/L when 200 °C was used (Table 4). Regarding the fermentation step, at least 98.9% of all glucose was consumed (as glucose was detected, but the concentration was below the limit of quantification, 0.25 g/L) in all hydrolysates as substrates. The highest ethanol titer was 10.3 g/L (Table 5), 90% of theoretical yield (0.51 g ethanol/g glucose) from the fermentation using the hydrolysate from the pretreatment at 170 °C. The post fermentation broth from the pretreatment at 150 °C contained 8.45 g/L of ethanol, indicating that the cellulases continued the hydrolysis process during the fermentation process as the yield was 28% over the theoretical one (0.66 g ethanol/g glucose). Using temperatures over 170 °C for the pretreatment resulted in decrease in ethanol yields, 0.33 g ethanol/g glucose (190 °C) and 0.18 g ethanol/g glucose (200 °C), probably due to the presence of inhibitors such as acetic acid and furfurals coming from the degradation of hemicellulose.

Table 4. Concentrations of sugars (g/L) for samples from the liquid fraction post-pretreatment and post-hydrolysis broth pretreated at 150 °C, 170 °C, 190 °C, and 200 °C.

Fraction	Stage		Cellobiose	Glucose	Xylose	Galactose	Arabinose	Mannose
Liquid fraction	Post-pretreatment broth	150 °C	0.28 ± 0.05	0.78 ± 0.05	n.d	0.30 ± 0.02	0.12 ± 0.02	0.85 ± 0.01
		170 °C	>0.25	0.41 ± 0.01	n.d	>0.25	0.22 ± 0.02	0.53 ± 0.07
		190 °C	>0.25	0.31 ± 0.02	0.43 ± 0.04	>0.25	>0.25	0.31 ± 0.05
		200 °C	>0.25	0.31 ± 0.02	0.30 ± 0.03	>0.25	>0.25	>0.25
	Post-hydrolysis broth	150 °C	0.49 ± 0.01	13.7 ± 1.16	2.10 ± 0.23	>0.25	0.34 ± 0.02	n.d
		170 °C	0.46 ± 0.04	23.41 ± 0.60	3.01 ± 0.19	>0.25	0.26 ± 0.02	n.d
		190 °C	0.80 ± 0.12	25.07 ± 0.92	2.24 ± 0.13	n.d	0.27 ± 0.08	>0.25
		200 °C	0.57 ± 0.15	31.56 ± 1.37	2.57 ± 0.25	n.d	>0.25	0.26 ± 0.05

n.d: non detected.

Table 5. Concentration of glucose, xylose, glycerol, acetic acid, and ethanol (g/L) for samples from the liquid fraction of post-fermentation broth pretreated at 150 °C, 170 °C, 190 °C, and 200 °C.

Fraction	Stage	Temperature	Glucose	Xylose	Glycerol	Acetic acid	Ethanol
Liquid fraction	Post-fermentation broth	150 °C	>0.25	2.11 ± 0.17	0.46 ± 0.11	1.56 ± 0.95	8.45 ± 1.28
		170 °C	>0.25	2.43 ± 0.36	0.84 ± 0.16	0.72 ± 0.11	10.3 ± 0.89
		190 °C	>0.25	2.25 ± 0.21	0.90 ± 0.07	0.44 ± 0.22	8.49 ± 1.22
		200 °C	>0.25	2.02 ± 0.57	0.95 ± 0.50	0.60 ± 0.00	5.68 ± 0.20

3.4. Methane Recovery

Figures 5–10 represent the maximum methane yield (B_{max}) of untreated Napier grass and samples from different fractions of bioethanol production process pretreated at 150 °C, 170 °C, 190 °C, and 200 °C. The methane yield of untreated material was 1.18 mol CH_4/100 g. For samples from the solid fraction of post-pretreatment broth, the methane yields were highest at 150 °C (1.13 mol CH_4/100 g), followed by samples pretreated at 170 °C (1.04 mol CH_4/100 g), 190 °C (1.00 mol CH_4/100 g), and 200 °C (0.90 mol CH_4/100 g). A similar trend was seen in samples from the solid fraction of post-hydrolysis broth. The samples pretreated at 150 °C had the highest methane yields (1.00 mol CH_4/100 g) while samples pretreated at 200 °C had the lowest methane yields (0.70 mol CH_4/100 g). For samples from the liquid fraction of post-pretreatment broth, the methane yields were highest for samples pretreated at 190 °C (0.27 mol CH_4/100 g), followed by samples pretreated at 170 °C (0.24 mol CH_4/100 g), 150 °C (0.17 mol CH_4/100 g), and 200 °C (0.14 mol CH_4/100 g). The methane yields for samples from the liquid fraction of post-hydrolysis broth varied between 0.41 CH_4/100 g (150 °C) and 0.57 CH_4/100 g (200 °C),

and for samples from the liquid fraction of post-distillation broth between 0.25 CH_4/100 g (200 °C) and 0.41 CH_4/100 g (190 °C).

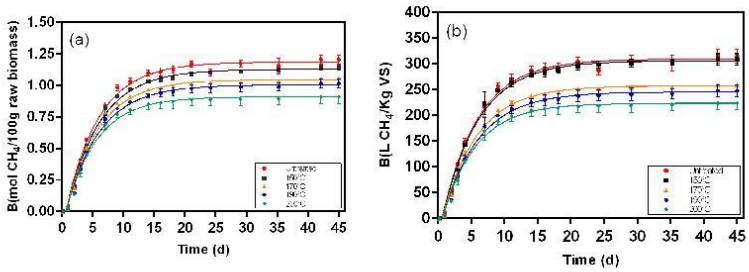

Figure 5. Biochemical methane results and respective fitting curves for samples from the solid fraction of post-pretreatment broth pretreated at 150 °C, 170 °C, 190 °C, and 200 °C. (**a**) results in mol CH4/100 g raw biomass; (**b**) results in L CH4/Kg VS.

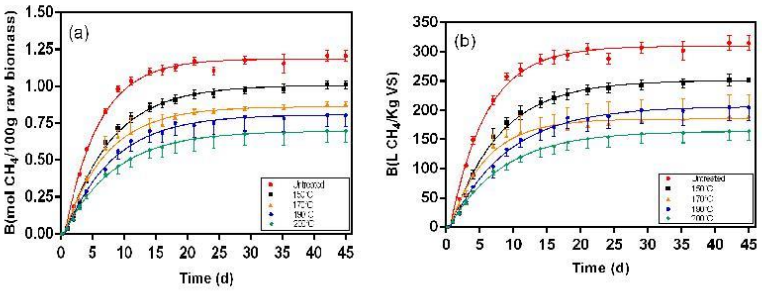

Figure 6. Biochemical methane results and respective fitting curves for samples from solid fraction of post-hydrolysis broth pretreated at 150 °C, 170 °C, 190 °C, and 200 °C. (**a**) results in mol CH4/100 g raw biomass; (**b**) results in L CH4/Kg VS.

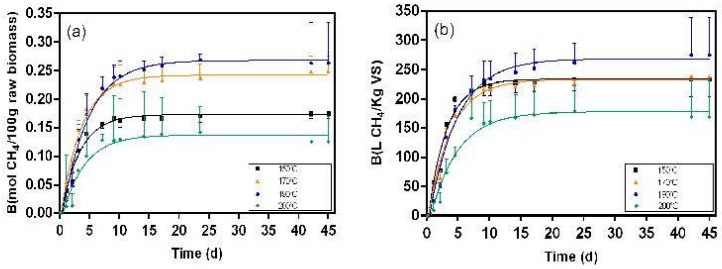

Figure 7. Biochemical methane results and respective fitting curves for samples from the liquid fraction of post-pretreatment broth pretreated at different temperatures (150 °C, 170 °C, 190 °C, and 200 °C). (**a**) results in mol CH4/100 g raw biomass; (**b**) results in L CH4/Kg VS.

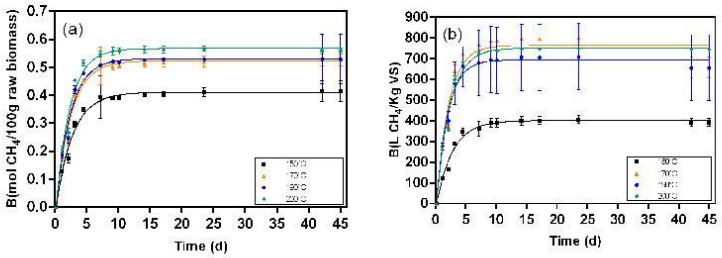

Figure 8. Biochemical methane results and respective fitting curves for samples from the liquid fraction post-hydrolysis broth pretreated at different temperatures (150 °C, 170 °C, 190 °C, and 200 °C). (**a**) results in mol CH4/100 g raw biomass; (**b**) results in L CH4/Kg VS.

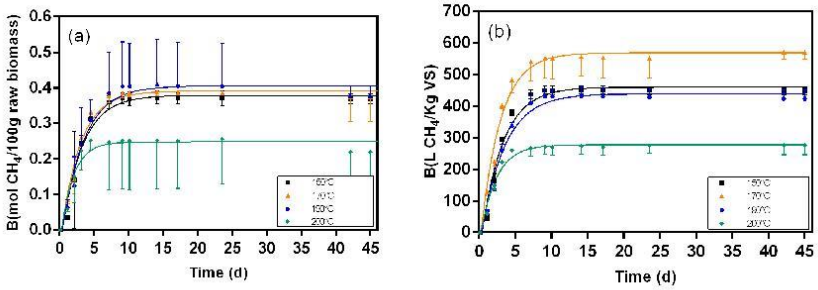

Figure 9. Biochemical methane results and respective fitting curves for samples from the liquid fraction post-distillation broth pretreated at different temperatures (150 °C, 170 °C, 190 °C, and 200 °C). (**a**) results in mol CH4/100 g raw biomass; (**b**) results in L CH4/Kg VS.

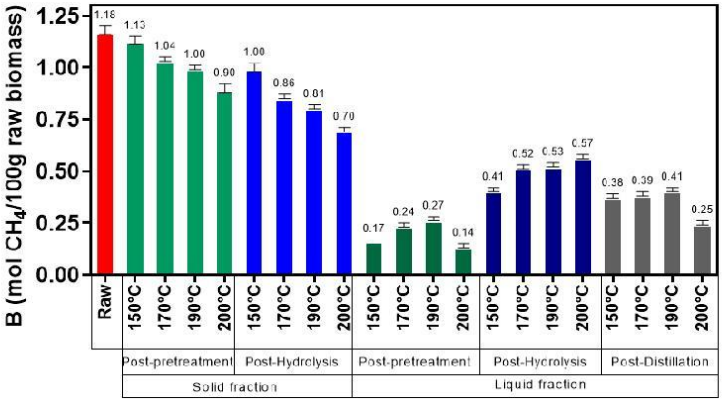

Figure 10. Maximum methane yield (B_{max}) of the fitting curves of samples from the solid and liquid fractions.

Statistically significant differences were found between methane yields of samples from the solid and liquid fractions and between samples pretreated with different temperatures (Table 6).

Table 6. Statistically significant results between the variables investigated in the methane recovery analysis.

Fraction	Stage	Temperature	B_{max} mol CH_4/100 g
Untreated	-	-	1.18 ± 0.02^{a}
Solid fraction	Post-pretreatment broth	150 °C	1.13 ± 0.02^{b}
		170 °C	1.04 ± 0.01^{c}
		190 °C	1.00 ± 0.01^{d}
		200 °C	$0.90 \pm 0.02^{a,b,e,f}$
	Post-hydrolysis broth	150 °C	$1.00 \pm 0.02^{a,g}$
		170 °C	$0.86 \pm 0.01^{a,b,c,h}$
		190 °C	$0.81 \pm 0.01^{a,b,c,d,g,i}$
		200 °C	$0.70 \pm 0.01^{a,b,c,d,g,j}$
Liquid fraction	Post-pretreatment broth	150 °C	$0.17 \pm 0.00^{a,b,c,d,f,g,h,i,j,k}$
		170 °C	$0.24 \pm 0.01^{a,b,c,d,f,g,h,i,j,l}$
		190 °C	$0.27 \pm 0.01^{a,b,c,d,f,g,h,i,j,m}$
		200 °C	$0.14 \pm 0.01^{a,b,c,d,f,g,h,i,j,m,n}$
	Post-hydrolysis broth	150 °C	$0.41 \pm 0.01^{a,b,c,d,f,g,h,i,j,k,l,m,n,o}$
		170 °C	$0.52 \pm 0.01^{a,b,c,d,f,g,h,i,k,l,m,n,p}$
		190 °C	$0.53 \pm 0.01^{a,b,c,d,f,g,h,k,l,m,n,o,q}$
		200 °C	$0.57 \pm 0.01^{a,b,c,d,e,f,g,k,l,m,n,o,r}$
	Post-distillation broth	150 °C	$0.38 \pm 0.01^{a,b,c,d,f,g,h,i,j,k,l,n,p,q,r}$
		170 °C	$0.39 \pm 0.01^{a,b,c,d,f,g,h,i,j,k,l,n,p,q,r,s}$
		190 °C	$0.41 \pm 0.01^{a,b,c,d,f,g,h,i,j,k,l,m,n,q,r,t}$
		200 °C	0.25 ± 0.01

The superscripts designate no statistically significant differences ($p < 0.05$) between the variables.

As it can be seen from Figure 10 (for samples from the solid fraction), higher pretreatment temperatures tend to decrease the biomethane yields. This may be because higher pretreatment temperatures can reduce the amount of VS available in the solid material, thus leading to lower biomethane yields [53]. Also, research has shown that higher temperatures can produce inhibitory components (such as furan derivatives) that can inhibit the microbial process and lead to lower biomethane yields [54,55].

Overall, samples from the solid and liquid fractions have distinct biomethane yields, mainly due to the composition of the biomaterial. Samples from the solid fraction have higher methane yields than samples from the liquid fraction mainly due to its composition in terms of cellulose and lignin. Samples from the liquid fraction have mainly hemicellulose in its composition, therefore the reaction speed will be faster (as there is no lignin) than in samples from the solid fraction, but the sugar release will be lower (since the amount of cellulose is negligible). From the process point of view, the performance of the solid and liquid fractions in different stages of the bioethanol production chain is also distinct. Samples from the liquid fraction from the post-pretreatment broth have the lowest methane yields, followed by samples from the post-distillation broth and post-hydrolysis broth. Due to its low potential, samples from the liquid fraction from the post-pretreatment broth that contains inhibitory component of the anaerobic digestion process and reduces the efficiency of the process, should be separated and discarded from the bioethanol production process. Sidestream from bioethanol production brings added costs to the production chain since it has a high BOD and COD and needs to be properly handled [4]. When compared with samples from the liquid fraction of post-pretreatment broth, samples from the liquid fraction of post-hydrolysis broth have higher biomethane yields. This is due to the glucose produced during the hydrolysis stage. Biomethane yields in samples from the liquid fraction of post-distillation broth tend to decrease because most of the glucose was fermented during the process or due to the presence of inhibitory compounds such as lignin degradation products generated after the hydrolysis stage [56]. However, this stage still presents

some potential for biomethane production mainly due to cellulose, hemicellulose, enzymes, and yeast left in the broth at the end of the process and that creates additional sources for biogas production.

The biomethane yields obtained in this study were improved when compared with the methane yields from samples that have not been through the optimization process [57]. However, further research needs to be done and mass balances should be performed in order to quantify gains from the optimized process.

The results suggest that samples from the liquid fraction still have potential for biomethane production, it is of interest to add further steps to the pathway proposed in this study and investigate new strategies to improve the digestibility of the samples by the anaerobic microorganisms.

3.5. Kinetic Evaluation of Biomass Bioconversion and Digestion Time

Figure 11 represents the kinetic rate constant (k) and the goodness-of-fit (R^2) for samples from different fractions of bioethanol production process pretreated at 150 °C, 170 °C, 190 °C, and 200 °C. As it can be seen from the figure, the kinetic rate of untreated material was 0.185 (d^{-1}), while for samples from the solid fraction of post-pretreatment broth it varied between 0.181 (d^{-1}) (samples pretreated at 150 °C) and 0.196 (d^{-1}) (samples pretreated at 200 °C). For samples from the solid fraction of post-hydrolysis, the kinetic rate constant of the bioconversion was higher for samples pretreated at 170 °C (0.150 d^{-1}), followed by samples pretreated at 150 °C (0.138 d^{-1}), 190 °C (0.130 d^{-1}), and 200 °C (0.123 d^{-1}). Regarding samples from the liquid fraction of post-pretreatment broth, the kinetic rate constant varied between 0.224 d^{-1} (samples pretreated at 190 °C) and 0.314 d^{-1} (samples pretreated at 150 °C). For samples from the liquid fraction of post-hydrolysis broth, the kinetic rate constant was higher for samples pretreated at 200 °C (0.444 d^{-1}), followed by samples pretreated at 190 °C (0.437 d^{-1}), 170 °C (0.422 d^{-1}), and 150 °C (0.380 d^{-1}). Concerning samples from the liquid fraction of post-distillation broth the kinetic rate constant was lower for samples pretreated at 190 °C (0.302 d^{-1}), and higher for samples pretreated at 200 °C (0.532 d^{-1}). The goodness of the fitting curves varied between 0.9517 and 0.9984.

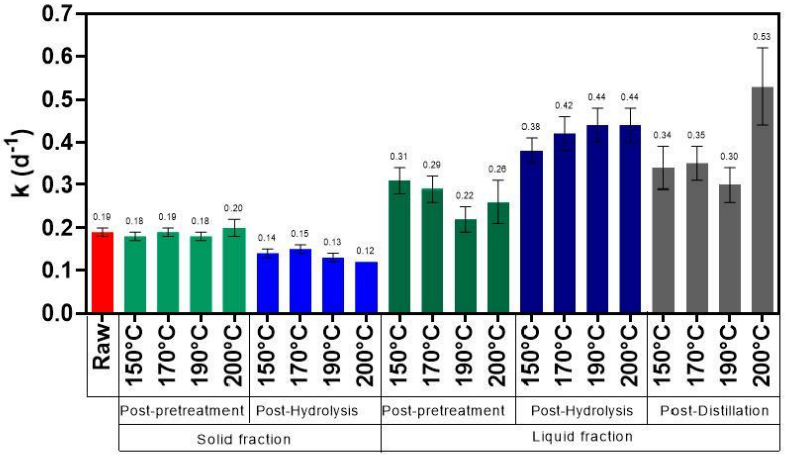

Figure 11. Kinetic constant and correlation coefficient of the fitting curves of samples from solid and liquid fraction.

As it can be seen from Figure 11, the kinetic rate is slower in samples from the solid fraction, and faster in samples from the liquid fraction. The digestion time (85% B_{max} and 95% B_{max}) of the biomaterial is represented in Table 7. The time needed for untreated Napier grass to achieve 85% B_{max} and 95% B_{max} is ~11 days (1.00 CH_4/100 g) and ~17 days (1.12 CH_4/100 g), respectively. Samples

from the solid fraction of post-pretreatment broth pretreated at 200 °C had the shortest digestion time. It achieved 85% B_{max} 7 days, and 95% B_{max} 5 days before samples from raw Napier grass. Samples from the solid fraction of post-pretreatment broth pretreated at 170 °C also achieved 85% B_{max} and 95% B_{max} before untreated samples (1 day). On the other hand, the digestion time (85% B_{max} and 95% B_{max}) of samples from the solid fraction of post-hydrolysis broth was 2.2 to 7.6 days longer than untreated Napier grass. Considering the different temperatures, it can be seen that samples pretreated at 170 °C had the shortest digestion time (t = 13.1 days), followed by samples pretreated at 150 °C (t = 14.4 days), 190 °C (t = 15.1 days) and 200 °C (t = 15.7 days). Samples from the liquid fraction of post-pretreatment broth reached 85% B_{max} and 95% B_{max} 1.9 to 7 days before untreated material. As it can be seen from Table 7, samples pretreated at 150 °C had the shortest digestion time (t = 6.3 days), followed by 170 °C (t = 6.9 days), 190 °C (t = 9.0 days), and 200 °C (t = 7.9 days). The opposite trend was noted in samples from the liquid fraction of the post-hydrolysis broth, where pretreated material at 200 °C had the shortest digestion time (t = 4.5 days), followed by samples pretreated at 190 °C (t = 4.6 days), 170 °C (t = 4.7 days), and 150 °C (t = 5.2 days). The samples from this stage had a digestion times 5.7 to 9.9 days shorter than that of untreated Napier grass. Finally, samples from the liquid fraction of post-distillation broth had digestion times 4.2 to 10.9 days shorter than those of the raw material. Samples pretreated at 200 °C had the shortest digestion time (t = 3.9 days), while samples pretreated at 190 °C had the longest digestion time (6.7 days).

Table 7. Digestion time (85% B_{max} and 95% B_{max}) for samples from different fractions of bioethanol production process pretreated at 150 °C, 170 °C, 190 °C, and 200 °C.

Fraction	Stage	Temperature	85% B_{max}		95% B_{max}	
			mol CH_4/100 g	Days	mol CH_4/100 g	Days
Untreated	-	-	1.00	10.9	1.00	10.9
Solid fraction	Post-pretreatment broth	150 °C	0.96	11.1	0.96	11.1
		170 °C	0.89	10.8	0.89	10.8
		190 °C	0.85	11.0	0.85	11.0
		200 °C	0.77	10.3	0.77	10.3
	Post-hydrolysis broth	150 °C	0.85	14.4	0.85	14.4
		170 °C	0.73	13.1	0.73	13.1
		190 °C	0.69	15.1	0.69	15.1
		200 °C	0.59	15.7	0.59	15.7
Liquid fraction	Post-pretreatment broth	150 °C	0.15	6.3	0.15	6.3
		170 °C	0.20	6.9	0.20	6.9
		190 °C	0.23	9.0	0.23	9.0
		200 °C	0.12	7.9	0.12	7.9
	Post-hydrolysis broth	150 °C	0.35	5.2	0.35	5.2
		170°C	0.44	4.7	0.44	4.7
		190 °C	0.45	4.6	0.45	4.6
		200 °C	0.48	4.5	0.48	4.5
	Post-distillation broth	150 °C	0.32	6.1	0.32	6.1
		170 °C	0.33	5.7	0.33	5.7
		190 °C	0.35	6.7	0.35	6.7
		200 °C	0.21	3.9	0.21	3.9

These results show that when compared to samples from the solid fraction, samples from the liquid fraction had a better performance in terms of time needed to degrade the biomaterial. This is due to the composition of the samples (solid vs. liquid fractions). On the other hand, samples from the solid fraction had higher methane production. Samples that were pretreated at 200 °C had shorter digestion times. This may be due to the effect of the pretreatment method. High pretreatment temperatures will be more effective in disrupting the biomass, removing lignin, and making the cellulose more accessible for the hydrolysis [58]. When the cellulose is easily accessible, the microbial degradation starts faster, and the overall efficiency of the hydrolysis and fermentation processes is improved.

Production of energy in the form of biogas–methane can contribute to the reduction of greenhouse gas emissions and be utilised as a replacement for fossil fuels, especially in the transportation sector [8]. These green gases (biogas–biomethane) can help developed societies to achieve decarbonisation from fossil fuels, and support emerging societies to achieve their energetic independence.

4. Conclusions

This study investigated the effect of NED pretreatment method (physio-chemical pretreatment) on bioethanol and biomethane yields from Nigerian Napier grass (*Pennisetum purpureum*) by means of solid–liquid separation. For this, different pretreatment temperatures were applied (150 °C, 170 °C, 190 °C, and 200 °C) and samples from different stages (pretreatment, hydrolysis, and sidestream) and fractions (solid and liquid) of the bioethanol production process were used. The results show that the lowest glucose yields (13.7 g/L) and the lowest ethanol yields (8.4 g/L) were gained at 150 °C. Samples that were pretreated at 200 °C had the highest glucose titer (31.3 g/L), while samples that were pretreated at 170 °C had the highest bioethanol concentration (10.3 g/L). The kinetic rate constant of the anaerobic digestion process was higher in samples from the liquid fractions (between 0.22 d^{-1} and 0.53 d^{-1}) and lower in samples from the solid fractions (between 0.12 d^{-1} and 0.20 d^{-1}). The maximum methane yields were reported in samples from the solid fraction of post-pretreatment broth at 150 °C (1.13 mol CH_4/100 g) and samples from the solid fraction of post-hydrolysis broth pretreated at 150 °C (1.00 mol CH_4/100 g). The lowest methane yields were reported in samples from the liquid fraction of post-pretreatment broth at 200 °C (0.14 mol CH_4/100 g). Nigerian *Pennisetum purpureum* is a promising feedstock for bioethanol and biomethane production. The bioethanol and biomethane productions are influenced by the pretreatment temperatures. From the different stages and fractions of the bioethanol production process, samples from the post-pretreatment stage (liquid fraction) have the lowest methane yields. From the process point of view, the results suggest that the liquid fraction after the pretreatment stage should be separated and discarded from the bioethanol production process, since it has inhibitory compounds in its composition and does not add value to the production chain. Further research needs to be done and additional strategies weighed in order to further optimise the mass flow and maximise the added value to the process. Further research needs to be done in order to apply circular economy models to emerging economies especially because environmentally friendly fuel sources are not on the agenda of developing economies. It is not reasonable to wait for emerging countries to fully develop and only then invest in their decarbonisation. There is an urge to accelerate the worldwide bioeconomy.

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