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Application of Wet Milling Process for Enzymatic Hydrolysis of Egyptian and South African Peel & Flesh Green and Over-Matured Banana For Bioethanol Production

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Abstract

The purpose of this study is to find the most suitable solutions for using banana biowastes, to reduce their negative effects in terms of the environment and human health, and to increase their economic benefits, especially for producing ethanol as a green biofuel, by testing the experiment the suitability of wet fractionation and hydrolysis of banana waste from four different kinds of banana biomass (peel and flesh of over-matured banana from South Africa, peel and flesh of green banana from Egypt) for bioethanol production. The results showed that fermentation of all the tested banana biomass i.e. green banana flesh, green banana peel, over-mature banana flesh, and over-mature banana peel, were almost finished after 24 hours and verified high ethanol yield after 48 hours of fermentation were achieved (i.e. 80.9%, 90.3%, 82.0% and 84.1% from the peel of the green banana from Egypt, flesh of green banana from Egypt, peel of the over-matured banana from South Africa, and flesh of over-matured banana from South Africa, respectively). This result indicates that wet milling is a promising method to pretreat not only for low-lignin content banana flesh biomass but also for high-lignin content banana peel biomass.

Keywords: Banana, flesh, peel, wet-milling, amyloglucosidase, cellulases, β-glucanase, fermentation, bioethanol

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INTRODUCTION

During the last decade, the world has been generating a high quantity of tangerine peel waste, pomegranate peel waste, and banana peel waste. These peels have several economic benefits but there is mismanagement or inappropriate valorization that could present risks to the environment and public health (Azeddin et al., 2021). It is estimated that roughly one-third of all food produced for human consumption is lost or wasted globally (Mkhize and Sithole, 2013). This amounts to about

1.3 billion tons of biowaste per year. South Africa's contribution is estimated at between 9 and 10 million tons per annum (Nahman et al., 2012). Locked inside this material is the potential to convert biomass into a variety of food, feed, biomaterials, energy, and fertilizer products, maximizing the value of the biomass and minimizing the waste. Biomass as a renewable energy resource (Fernandes and Costa, 2010), is considered currently as a very important source of energy and it is predicted that its usage can help in the reduction of greenhouse gas emissions compared to fossil fuels if produced sustainably (IEA Bioenergy, 2008; IEA, 2012). In developing countries, biomass is exploited as a major source of energy mostly inefficiently as firewood and charcoal for cooking and heating (Kemausuor et al., 2014).

Production of fuel ethanol has become necessary for many countries to reduce their oil imports, create enabling economic opportunities in rural settings, and improve air quality. Ethanol production in the world is estimated at 51,000 million liters (RFA, 2007), and among the top world producers are USA and Brazil. Since fossil fuels are non-renewable, it is believed that in the next few decades, it will be in short supply therefore much attention has currently been given to the conversion of biomass into fuel ethanol (Arumugam and Manikandan, 2011). In Brazil, the main feedstock for bioethanol production is sugarcane while corn grains are prominently used in the USA, and many other agricultural raw materials are also utilized worldwide. Ethanol production from sugar and starch-based materials is easier as compared to lignocellulosic materials since it requires additional technical challenges such as pretreatment (Petersson et al., 2007). In addition, the deployment of sophisticated technology coupled with complicated instrumentation methods with high operating costs may invariably limit their commercialization and industrial application in developing countries (Isarankura-Na-Ayudhya, 2007).

In Sub-Saharan Africa (SSA), the level of bioethanol development is very low, compared to other countries with a fluctuated production increase of about 1% from 2011 with the largest increases in Sudan and South Africa (RFA, 2013) but it is envisaged that by the year 2050 the potential for bioenergy in SSA will increase from a current production of 347 exajoule to approximately 1548 exajoule (Smeets et al., 2007).

Ethanol can be produced from any biological feedstock that has the typical formula of $(CH_2O)N$, with appreciable amounts of sugar, or materials that can be converted into sugar. Ethanol can be produced from three main types of biomass raw materials: (a) sugar-bearing biomass; (b) starchy biomass; and (c) lignocelluloses (Rutz and Janssen 2008; Sarkar et al. 2012).

The main attraction of sugar-bearing raw materials for alcohol production is that their carbohydrate content is already in the fermentable, simple sugar form. Starches contain carbohydrates of greater molecular complexity, which have to be broken down into simpler sugars by a saccharification process, which adds another process step and increases the capital and operating costs. In addition, corn requires an outside source of fuel for the ethanol production process; thus, the corn-based ethanol program is inherently less efficient than sugar-based ethanol programs. Carbohydrates in cellulosic materials have an even greater molecular complexity and have to be converted to fermentable sugars by a more complex acid hydrolysis or enzymatic hydrolysis process (Thomas and Kwong, 2001).

In Egypt, bioethanol is produced through the fermentation of glucose and fructose obtained from the sucrose present in sugar cane (Abo-State et al., 2013). Raw sugarcane is about 7% sugar, but

dried it is about 50% sugar, the remainder, which is bagasse, can be burned to provide heat for drying, ethanol production, and electricity. However, the limited availability of such biomass, as an ethanol feedstock, requires the search for alternatives as well as obtaining new technology for energy generation using various types of biomass as sources of producing bioethanol.

Banana (*Musa sapientum*, and similar), the most important fruit in the world, has a global annual yield of 95 million tons, and it is a major nutritional base for many populations. Only a small proportion of the world's bananas are preserved for storage, most of the fruit production is consumed raw or cooked and forms an important source of carbohydrate throughout the tropical world (NBRP 2010). For optimal conditions, bananas require a warm, humid, and frost-free climate, with temperatures ranging between 22 ^{o}C and 31 ^{o}C. As such, production is restricted to the provinces of Mpumalanga, Limpopo, and coastal areas of Kwa-Zulu Natal, which account for 58%, 20%, and 22% of production respectively. The latest figures from the Department of Agriculture, Forestry and Fisheries (DAFF, 2010) put total production at just over 397,000 tons, slightly higher than the average for the proceeding 5 years of 368,000 tons.

Despite its semi-arid climate and high latitude, Egypt is also a significant banana producer. According to FAOSTAT (2013), the annual production factuality has varied during the period from 2000 to 2011 to attain about one million tonnes, as a result of about 23,495 hectares of harvesting areas planted with bananas that are distributed over twenty-four Egyptian governorates, especially in the Delta and Nile valley.

As banana fruits are consumed in green, averagely ripped, and ripped stages (Happi Emaga et al., 2008), the amount of fruit waste from the peels is expected to increase with the development of processing industries that utilize green and ripe bananas. Like its pulp flour counterpart, banana peel flour can potentially offer new products with standardized compositions for various industrial and domestic uses (Happi Emaga et al., 2007). Various studies have been conducted to investigate banana peel, and this includes the production of banana peel flour (Ranzani et al., 1996); the effects of the ripeness stage on the dietary fiber components and pectin of banana peels (Happi Emaga et al., 2008) and the chemical composition of banana peel, as influenced by the maturation stage and varieties of banana (Happi Emaga et al., 2008).

The chemical composition of green bananas changes dramatically during ripening. Starch is the main component of unripe bananas, corresponding to 60–80 g/100 g (dry weight) of the fruit, a percentage range similar to that of corn or potatoes (Zhang et al., 2005). It can be classified as digestible (when susceptible to the action of amylase) or resistant (when amylase-resistant). According to Juarez-Garcia et al. (2006), unripe banana fruit produced under specific conditions composed of 73.4 g/100 g total starch and 17.5 g/100 g reducing sugar.

Banana is a good feedstock for ethanol production because it contains high non-structural carbohydrate (NSC) and low fiber contents. By allowing the bananas to ripen before fermentation, enzymatic hydrolysis could be eliminated, leading to a reduction in energy and input costs. The NSC content of the bananas suggests that the fruit could be fermented without adding dilution water. In this concern, Hammond et al. (1996) found that the highest ethanol yield, in terms of degree of ripeness, resulted from using green bananas as the feedstock. This is most likely due to the approximately 12% loss of dry matter and the 15% reduction of fresh weight during a 10-day

ripening period. The ethanol yields were 0.09, 0.082, and 0.69 L/kg from green, normal ripe, and overripe green whole bananas. Furthermore, the enzymatic hydrolysis was necessary for maximum yields. Dilution of water was not essential for effective fermentation.

Banana peels can also serve as a supplementary source for the production of commercially important products like ethanol, enzymes, organic acids, vitamins, and biogas. Previous studies on the production of ethanol from banana peels alone or in combination with the other fruit residues have reported low product yield and low ethanol volumetric productivity. Thus, it is important to develop a process that results in significant product yield in less time so that it can be scaled up.

A study by Sharma et al. (2007) established the effective utilization of banana peels for bioethanol production using optimized fermentation parameters. Oberoi et al. (2011) demonstrated that banana peels could serve as an ideal substrate for the production of ethanol through Simultaneous Saccharification and Fermentation (SSF). The study also showed that the use of a single vessel for pre-treatment and SSF not only helped in increased ethanol production but might also help in economising the whole process by reducing the number of unit operations leading to significant savings in energy and operating costs (Hammond et al. 1996, Sharma et al. 2007).

Arumugam and Manikandan (2011) used the banana fruit wastes as potential raw material for bioethanol production and the results showed that mixed ripened fruit biomass of banana and mango can yield 36% ethanol and similarly the banana fruit peels treated with dilute acid and microbial enzymes showed a potential production of 14% ethanol. The high non-structural carbohydrates, reserve starch content, and low fiber contents revealed bananas as a potential good feedstock for ethanol production. They added, that even though the ethanol obtained was comparably lower than other starchy, sugary, and lignocellulosic feedstocks, the production of ethanol from these cheap low-cost materials can be improved by using suitable technologies that are capable of converting multiple sugars into ethanol. Increasing the available sugars of the feedstock at a fixed pretreatment level might result in reduced capital and operating costs for higher ethanol yield.

Research efforts are directed at designing and improving processes, which would transform low-cost feedstocks into sustainable transportation fuels. Wet-milling is a pretreatment process developed for the separation of kernel components, in which feed material is steeped in the water while milling, with or without sulfur dioxide. Enzymatic wet milling is a developed process from conventional wet milling for the recovery and purification of starch and co-products using enzymes to eliminate the need for sulfites and decrease the steeping time (Ramírez et al., 2009).

Therefore this study is designed to explore the suitability of the enzymatic wet-milling process for the pretreatment of four different banana feedstocks (flesh of over-matured banana, peel of over-matured banana, flesh of green banana, and peel of green banana). Chemical characterization was undertaken as well as the evaluation of these biomass streams as potential substrates for bioethanol production

Material and Methods

Feedstocks

The over-matured banana samples used in this study were collected from a Banana processing farm, Zedpro (Pty) Ltd in Levubu, Limpopo Province of South Africa (SA). The banana samples were processed in two categories, namely, the peel of over-matured banana and the flesh of over-matured banana. These samples were then stored overnight at 4 °C before drying.

Green banana samples *Cavendish* (*Musa spp.*) at the second stage of maturation were purchased from a local fruit market in Menofia Governorate in Egypt. The bananas were washed and separated into peel and flesh and finally dried and milled.

Drying

The two categories of over-matured bananas (flesh and peel) were cut into small slices and dried in an oven at 60 °C for 48 h with an occasional inversion of the samples to allow efficient drying. The respective dried banana samples, flesh (906.44 g), and peel (541.07 g) were milled using homogenized with a hand blender resulting in a powder. The particle sizes of the resulting banana waste powder were measured and the samples were marked as SABF (flesh of over-matured banana) and SABP (peel of over-matured banana), and stored at room temperature before and after shipment.

The flesh of a green banana was dipped in 0.5 % (w/v) citric acid solution for 10 minutes to reduce enzymatic browning (according to Tribess et al. 2009) and drained. Afterward, the two categories of green bananas (flesh and peel) were dried in an oven (hot air oven DIN 12880-KI. Memmert, Germany) at 60 ^{o}C for 48-72 hours. The samples were occasionally inverted to allow efficient drying. After that, the dried flesh or peels were ground in a laboratory mill (RETSCH: Cross Beater Mill SK 100) and were passed through 60 mesh screens to obtain flesh of green banana (EGBF) flour or peel of green banana(EGBP) powder, respectively. The samples were stored in airtight plastic packs in cold storage at 2±15°C for further analysis.

Chemical analysis

Dry Matter content determination

Dry Matter content (DM) of samples before composition analysis and before wet-milling processing were measured according to protocol A0001 from Enzyme Lab of DTI (Denmark), in principle by weighing the samples before and after overnight drying at 105 °C in the oven.

Ash content determination

Ash contents of samples were measured according to the protocol A0002 from Enzyme Lab of DTI (Denmark), in principle by weighing the samples before and after ashing at 550 °C for two hours in Muffle Furnace.

Carbohydrate characterization

The Carbohydrate composition of samples and dried solid fraction after hydrolysis were determined following the protocol A0003 from Enzyme Lab of DTI (Denmark), in principle of releasing the

monomer sugars by two steps of acid hydrolysis and quantifying the released sugars by HPLC analysis. Samples were first made soluble in 72% (w/w) H_2SO_4 at 30°C for 60 minutes and then hydrolyzed in 4% (w/w) H_2SO_4 at 121°C for 60 minutes. Klason lignin is determined as the ash-free residue after hydrolysis. The released monosaccharides were then quantified by a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) using a refractive index detector equipped with an Aminex HPX-87H column (Bio-Rad Laboratories Ltd., USA) running at 63°C with 4 mM H_2SO_4 as eluent with a flow rate of 0.6 ml/min.

Pretreatment

Wet-milling

In this study, four kinds of banana biomass, i.e. peel of the green banana from Egypt (EGBP), the flesh of green banana from Egypt (EGBF), the peel of the over-matured banana from South Africa (SABP), the flesh of over-matured banana from South Africa (SABF), were pre-treated by wet milling as following described.

Due to the different liquefaction status after mixing, the dried milled samples were mixed with water at different ratios. That is, 135 g EGBP was mixed with 2.8 L water, 300 g EGBF was mixed with 2.4 L water, 300 g SABP was mixed with 2.8 L water, and 300 g SABF was mixed with 2.4 L water. The mixed materials were then pretreated by the same procedures as described below.

The mixed slurry was pre-heated for 15 minutes at 95°C in a water bath with mechanical stirring. After that, 1 ml Termamyl ® Classic was added to the slurry and further incubated at 95°C with continuous stirring. During 60 60-minute period of incubation at 95°C, the slurry was intermittently wet milled (using a “Fryma” colloid mill) at 15-minute intervals to increase available surface area and disperse soluble components. The final pretreated material was collected for the fermentation trials.

Enzymatic hydrolysis

The wet-milled materials were checked for pH (all were in the range of 5.4-6.0) and poured into 100 ml blue cap flasks with 60 ml working volume. 2 µl/g-biomass of amylo-glucosidase NS22180 (Novozymes A/S, Denmark) were added into the wet-milled materials and the materials were incubated at 60 °C with magnetic stirrer speed of 350 rpm for two hours. After that, pH of the hydrolysate was checked again and adjusted to 5.1 by 10 M HCl. 25 µl/g-biomass cellulases NS81016 (Novozymes A/S, Denmark) and 8.3 µl/g-biomass β-glucanase NS81223 (Novozymes A/S, Denmark) were added into the materials which were then afterward transferred to 15 ml Falcon tubes to continue the hydrolysis in Enviro-Genie mixer (Scientific Industries, Inc) at vertical rocking speed of 30 cycles per minute under 45 °C for 48 hours. The hydrolysates were collected back into sterilized 100 ml Blue caps for the following ethanol fermentation.

Samples were taken at 0, 24, and 48 h and the monomer sugar concentrations were measured by a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) using a refractive index detector equipped with an Aminex HPX-87H column (Bio-Rad Laboratories Ltd., USA) running at 63°C with 4 mM H_2SO_4 as eluent with a flow rate of 0.6 ml/min.

Fermentation

After the enzymatic hydrolysis, 2 g/L dry commercial yeast *Saccharomyces cerevisiae* (Quick Yeast, Doves Farm Foods Ltd.) were inoculated into the hydrolysates and the fermentation flasks were incubated under 32 °C for 48 hours in an orbital shaking incubator (Grant Instruments Ltd.) at 120 rpm. The estimated ethanol concentrations were calculated according to the measured weight loss, following the formula equation of $C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$, and the assumption of all the weight loss coming from the CO_2 production.

$M_{ethanol}$ (g/L) = M_{CO2} * 46 (g/mol ethanol)/44 (g/mol CO2) (equation 1)

Samples were taken at 0, 24, and 48 h. The monomer sugar concentrations were determined by a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) using a refractive index detector equipped with an Aminex HPX-87H column (Bio-Rad Laboratories Ltd., USA) running at 63°C with 4 mM H2SO4 as eluent with a flow rate of 0.6 ml/min. Ethanol concentrations were determined by GC-MS, using a CP-Sil 8 CB/MS column (Varian Inc.) with Split Injection. The injector temperature was 260 °C, and the oven temperature was 120 °C. The pressure of the carrier gas is 15 psi.

Calculation

The measured ethanol yield Y ethanol (g/g) was calculated from the total ethanol produced divided by the total glucose that existed in the raw biomass (equation 2).

$$Y\ ethanol\ (g/g) = \frac{Ethanol\ concentration\ (g/l) * Substrate\ Volume\ (l)}{Glucose\ concentration\ (\%DM) * Sample\ for\ hydrolysis\ (g) * DM\ of\ Sample}$$

(Equation 2)

This value was further divided by the theoretical ethanol yield (0.51 g/g) and the result was expressed as % theoretical ethanol yield (equation 3).

$Y_{ethanol,\ theoretical}$ (%) =$Y_{ethanol}$/0.51 (equation 3)

Results and Discussion

Biomass Composition Characterization

In this study, the carbohydrate composition was analyzed by the method as described in protocol A0003 from Enzyme Lab of DTI (Denmark) and the results are listed in (Table 1). It showed that the flesh of green bananas from Egypt (EGBF) contained the highest content of glucose (i.e. 82.2%), while banana flesh from over-matured bananas (SABF) only contained about half of the amount. Glucose content in banana peels from over-matured bananas (SAPB) is the lowest. This indicated the possibility of sugar degradation during the over-matured (rotten) process. Moreover, higher contents of lignin from banana peels than from flesh parts were discovered (i.e. around 4 times higher in peels than in flesh parts, from both green and over-matured banana feedstock). Since lignin is a very difficult structure to break down, it implies that the banana peel may require harsher pretreatment conditions than banana flesh, to release the monomer sugars for further biochemical conversion.

Furthermore, it is worth mentioning here that the flesh of an over-matured banana from South Africa (SABF) very easily absorbs water to become a viscous, sticky sludge, when stored in the

normal sample bucket under room temperature. This makes it difficult to take dried powder from the sample for carbohydrate content analysis. Since the carbohydrate analysis only uses small amounts of mass (i.e. ~0.16 g/analysis) for analysis and the absorbance of water makes the distribution of flesh content uneven, it is recommended that each time before carbohydrate content analysis of brown banana flesh samples, the samples should be redried and milled (ø 1 mm) to facilitate a more accurate result.

Table 1 Biomass composition of the feedstocks, dry matter (DM) where DM1 is the dry matter of the samples received after shipping and DM2 is the dry matter of the samples before wet-milling processing or direct enzymatic hydrolysis, in which SABF was re-dried at 50°C for 2 days. Ash, lignin, glucose, xylose, and arabinose are determined after strong acid hydrolysis and calculated in percent of a dried sample.

Sample	DM 1 (%)	DM 2 (%)	Ash (% DM)	Lignin (% DM)	Glucose (% DM)	Xylose (% DM)	Arabinose (% DM)
EGBP	90.7	90.51	20.5	15.0	34.5	9.9	4.0
EGBF	90.7	88.71	5.4	3.6	82.2	7.2	0.5
SABP	89.5	88.74	19.9	23.3	21.1	14.9	3.8
SABF	77.9	85.94	11.3	5.9	44.3	9.7	1.1

EGBP – Egypt Unmatured Banana Peel; EGBF – Egypt Unmatured Banana Flesh

SABP - South Africa Over-matured Bana Peel; SABF - South Africa Over-matured Banana Flesh

Wet-milling pre-treatment and ethanol production

Wet milling, as a physicochemical pretreatment method, has been proven to have high economic potential for separating/extracting value-added products such as starch, proteins, oils, and arabinoxylan from substrates like corn grains (Moreau et al., 2009). In this study, a modified enzymatic wet-milling process was developed to make the bioconversion process more economically feasible, i.e. extract value-added products and improve the hydrolysis efficiency with lower enzyme loadings and recovering higher amounts of sugars for the following fermentation. Figure 1 illustrates the primary design of the wet-milling process for pretreating banana biomass. In the reported experiment, the slurries after three times wet-milling were not separated into fibers and liquid parts but were applied directly to the continued hydrolysis and fermentation to produce ethanol. The rationale behind this is to verify the technical feasibility of wet-milling on different banana biomass and a demonstration of the fermentability of the different banana biomass.

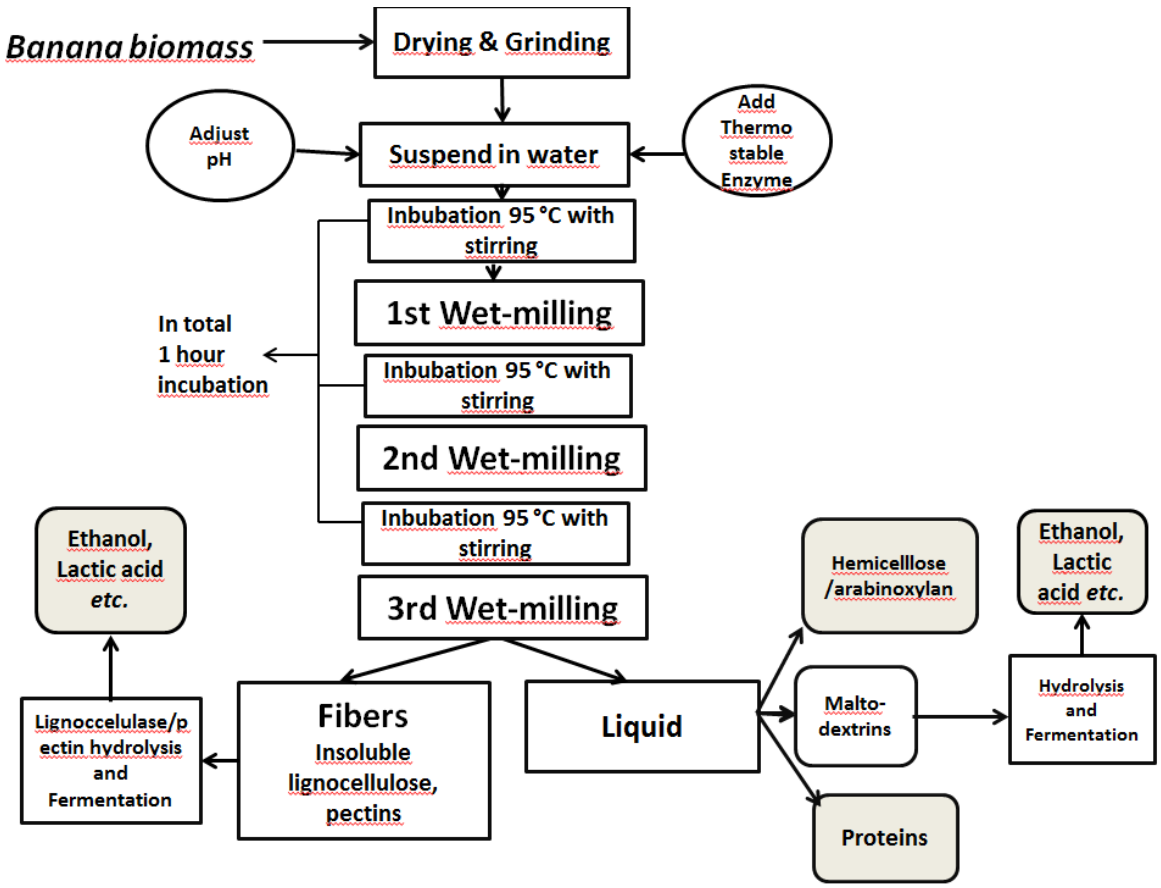

Figure 1 Diagram of the wet-milling process for value-added product extraction and ethanol production from banana biomass

The materials after wet-milling showed different extents of liquefaction (i.e. banana peels material EGBP and SABP showed a much lower extent of liquefaction than the banana flesh materials EGBF and SABF, Figure 2). Since the banana peels contain a high content of lignin (Table 1) or are lignocellulosic materials, cellulases were added to the material after wet-milling pretreatment. This addition made obvious improvements in the liquefaction (which can be observed by the naked eye) of all four kinds of banana biomass, especially on banana peels (i.e. SABP, EGBP) (Figure 2).

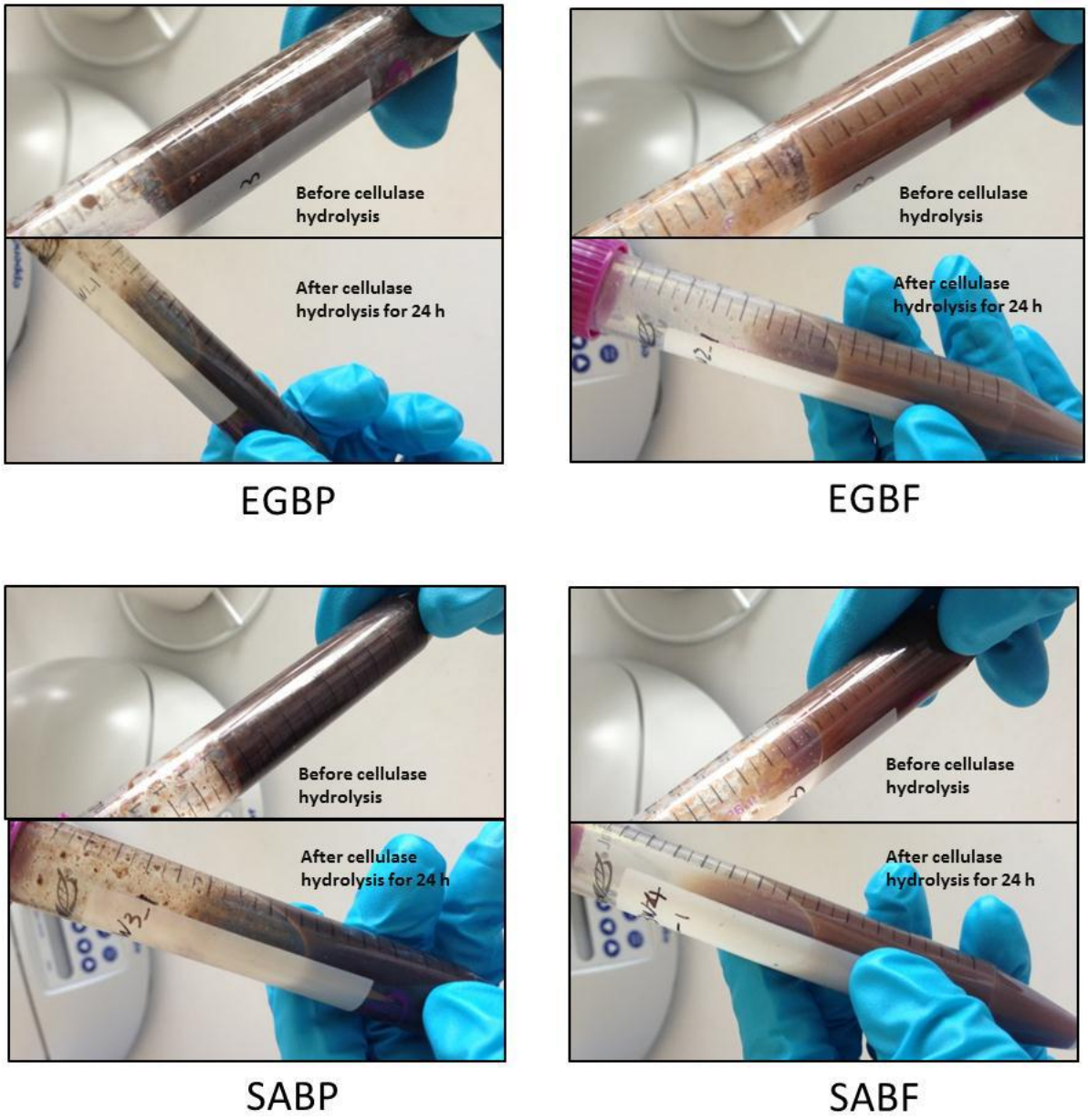

Figure 2 Observation of liquefaction of four banana biomass (i.e. EGBP, EGBF, SABP, and SABF) during the cellulase hydrolysis

Impressive results were observed for ethanol productions from the four types of pretreated banana biomass, ethanol yields in the range of 80-90% of theoretical values (based on total glucose available in the substrate) were achieved within the first 24 hours of fermentation (Figure 3 and Table 2). The high final ethanol concentrations from banana flesh samples (EGBF and SABF) as well as the ethanol yield obtained from the peels indicate good potential for industrial-scale production for whole fruit conversion. The results agreed with the previous report of Joshi et al. (2001) in a fermentation study with flocculating yeast (*S. uvarum*) observed that waste banana peels can provide sufficient sugar for the fermentation and hence can be economic feedstock for ethanol production The relatively lower final ethanol concentrations from banana peel substrates are due to the relatively lower glucose content in the banana peel samples (Table 1) and the lesser amount of dry samples mixed with water (because of the poor liquefaction of banana peel samples during the wet-milling process). An increase in ethanol concentrations could be achieved by improving technologies to

increase DM water mix during wet-milling and fermentation of whole fruit banana (flesh and peels), eventually with added cellulases.

All fermentations were completed after only 24 hours without the addition of any other nutrient supplements according to the observed CO_2 loss (bubble production in yeast locks) and the simultaneous weight loss (Figure 3). According to the previous studies and protocols for lignocellulosic and starch-based biomass fermentation, nitrogen is the most important nutrient, and the addition of a nitrogen source such as urea, corn steep liquor, or yeast extract is often supplemented into the biomass hydrolysate and needed to carry out successful yeast fermentation (Gutiérrez et al. 2012). However, in the case of banana biomass tested in this study, no addition of extra nitrogen was needed in the experiments. Moreover, no toxic compounds such as furfural and HMF (which are well-known degradation products from sugar polymers after harsh pretreatment conditions) were presented after the gentle wet-milling process at 95°C. Such compounds can inhibit microbial growth and cause a long lag phase and the reduction of ethanol yield and activity (Hou and Shuo 2012). The quick fermentations observed without any lag phases and with high ethanol yields imply a very low amount of inhibitors in all four wet-milled pretreated banana substrates and also supported the hypothesis that sufficient nutrients especially nitrogen sources were available for fermentations. The determination of the total nitrogen source will be carried out in a future study. Moreover, by analyzing the final ethanol concentrations by GC, all the estimated ethanol concentrations by weight loss were considered reliable within the 10% error range, as shown in Figure 3. This result suggests the suitability of the weight loss measurement as a quick, easy, and cheap way to monitor the lab-scale yeast fermentation process.

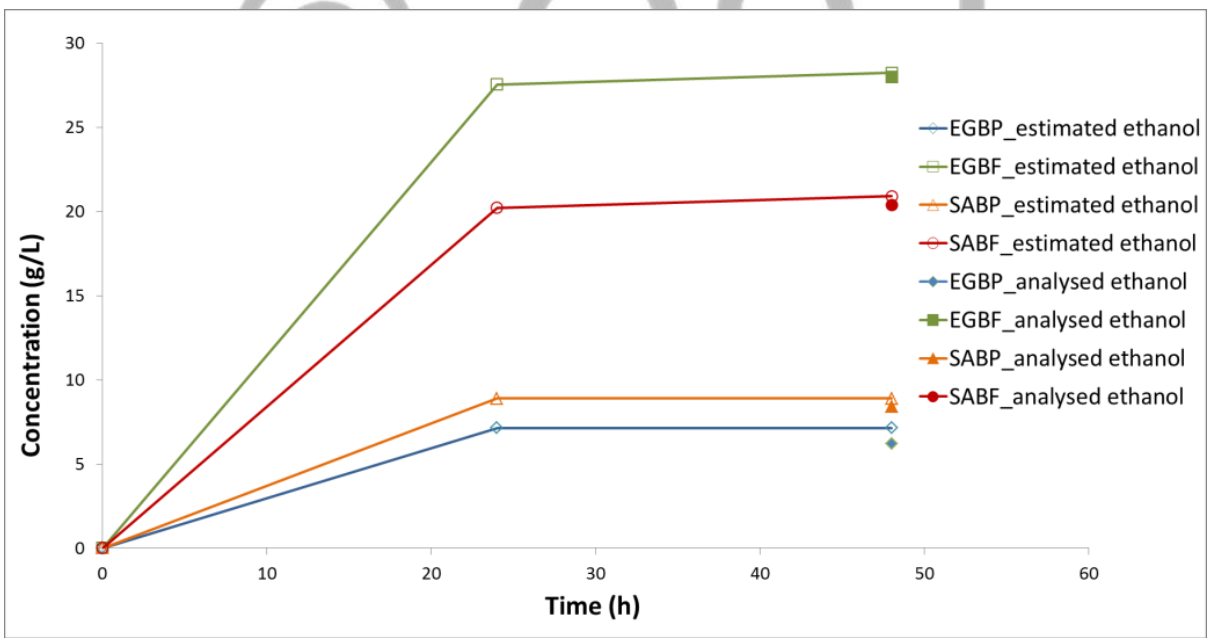

Figure 3 Time course of ethanol production during the fermentation on four banana waste substrates.
The estimated ethanol concentrations were calculated according to the measured weight loss, following the formula equation of $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$, and the assumption of all the weight loss coming from the CO_2 production. The final ethanol concentrations accurately analyzed by GC were also marked out as a comparison.

Table 2 Final ethanol concentration and yield from four different banana waste samples after wet-milling and enzymatic hydrolysis (GC results) after 48 hours of fermentation

Samples	Final ethanol concentration (g/L)*	Ethanol yield (% theoretical value)
EGBP	6.2	80.9
EGBF	28	90.3
SABP	8.4	82.0
SABF	20.4	84.1

*Ethanol concentrations were determined by GC-MS

Conclusions

All four tested banana biomass contain relatively high concentrations of glucose for bioethanol production (i.e. 44.3% in the flesh of the over-matured banana 21.1% in the peel of the over-matured banana, and 82.2% in the flesh of the green banana, 34.5% in the peel of green banana) which indicate their potential as a good substrate for ethanol fermentation in all four substrates. The results support wet milling as a promising method to pretreat banana biomass for ethanol production. Satisfactory ethanol yields in the range of 80-90% theoretical yield were obtained from the four kinds of banana biomass. Final ethanol concentrations of 6.2 g/L, 28 g/L, 8.4 g/L, and 20.4 g/L were obtained, after 48 hours of fermentation of the peel of green banana from Egypt, flesh of green banana from Egypt, the peel of over-matured banana from South Africa and flesh of over-matured banana from South Africa, respectively. The result indicated that it would be possible to treat “whole crop” banana (flesh and peel together) in one step. on the other hand, All four tested banana biomass contain relatively high concentrations of glucose and sufficient amounts of ash (nutrients) to facilitate microbial performance, and therefore these four banana biomass could be promising substrates for bioethanol production by yeast fermentation. However, technology requires to be further improved to pretreat higher DM wet-milling pretreatment on the high-lignin-content banana peels, to get higher final ethanol concentration to add more economic benefit to the whole process.

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References

1. Azeddin El Barnossi , Fatimazhrae Moussaid, Abdelilah Iraqi Housseini (2021). Tangerine, banana, and pomegranate peels valorization for a sustainable environment: A review. Biotechnology Reports , Vol(29), e00574.

2. Fabrizio Saladini , Said A. Vuai , Benard K. Langat, Mathias Gustavsson, Richard Bayitse, Andrew B. Gidamis, Mohammed Belmakki, Amal S. Owis, Conanani Rashamuse, Daniel N. Sila and Simone Bastianoni (2016). Sustainability assessment of selected biowastes as feedstocks forbiofuel and biomaterial production by emergy evaluation in five African countries. Biomass and Bioenergy, 85: 100-108.

3. Abo-State, M. A., A. M. E. Ragab, N. S. El-Gendy, L. A. Farahat and H. R. Madian (2013). "Effect of different pretreatments on Egyptian sugarcane bagasse saccharification and bioethanol production." Egyptian Journal of Petroleum **22**(1): 161-167

4. Arumugam, R. and Manikandan, M. (2011) Fermentation of Pretreated Hydrolyzates of Banana and Mango Fruit Wastes for Ethanol Production Asian J. Exp. Biol. Sci. 2 (2) 246-256.

5. Campo, Id., Alegria, I., Zazpe, M., Echeverria, M., & Echeverria, I. (2006). Diluted acid hydrolysis pretreatment of agri-food wastes for bioethanol production. *Indust. Crops and Prod* 42: 214 221

6. DAFF (2010). A profile of the South African Banana market value chain.www.daff.gov.za/docs/AMCP/BananaMVCP2010-11.pdf (accessed 13/09/2011)

7. FAOSTAT (2013) Final 2011 Statistics Data of banans production (World, Africa, Egypt & South Africa), http://faostat.fao.org

8. Fernandes U, Costa M. Potential of biomass residues for energy production and utilization in a region of Portugal. Biomass Bioenergy 2010;34:661–6.

9. Francis Kemausuora,, Andreas Kampb, Sune Tjalfe Thomsenb,Edem Cudjoe Bensahc,d, Hanne Østergård (2014) Assessment of biomass residue availability and bioenergy yields in Ghana [online] available from http://www.journals.elsevier.com/resources-conservation-and-recycling

10. Gutiérrez A, Chiva R, Sancho M, Beltran G, Arroyo-López FN, Guillamon JM (2012), Nitrogen requirements of commercial wine yeast strains during fermentation of a synthetic grape must, Food Microbiol 31(1):25-32. doi: 10.1016/j.fm.2012.02.012. Epub 2012 Mar 3

11. Hammond, J. B., R. Egg, D. Diggins and C. G. Coble (1996). "Alcohol from bananas." Bioresource Technology **56**(1): 125-130.

12. Happi Emaga, T., R. H. Andrianaivo, B. Wathelet, J. T. Tchango and M. Paquot (2007). "Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels." Food Chemistry **103**(2): 590-600.

13. Happi Emaga, T., C. Robert, S. N. Ronkart, B. Wathelet and M. Paquot (2008). "Dietary fibre components and pectin chemical features of peels during ripening in banana and plantain varieties." Bioresour Technol **99**(10): 4346-4354.

14. Hou X, Shuo Y (2012) Improved inhibitor tolerance in xylose fermenting yeast Spathaspora passalidarum by mutagenesis and protoplast fusion, Appl Microbiol Biotechnol，Appl Microbiol Biotechnol 2012 93(6): 2591-601

15. IEA Bioenergy. From 1st- to 2nd-generation biofuel technologies: an overview ofcurrent industry and R&D activities. Paris, France: IEA Bioenergy; 2008. Available online < https://www.iea.org/publications/freepublications/publication/2nd_Biofuel_Gen.pdf

16. IEA (International Energy Agency). World energy outlook; (2012). Available online from: http://www.iea.org/ (accessed 14.02.14).

17. Isarankura-Na-Ayudhya, C.,Tantimongcolwat,T., Kongpanpee,T., Prabkate, P., & Prachayasittiku l,V.(2007).Appropriate technology for the bioconversion of water hyacinth (Eichhornia crassipes) to liquid ethanol: Future prospects for community strengthening and sustainable development. EXCL Journal 6: 167 176

18. Joshi, S.S., Dhopeshwarkar, R., Jadhav, U., Jadhav, R.,D'souza, L.,&Dixit, J.(2001). Continuous ethanol production by fermentation of waste banana peels using flocculating yeast. *Ind. J Chem Tech* ., 8: 153 156

19. Juarez-Garcia, E., E. Agama-Acevedo, S. G. SÁYago-Ayerdi, S. L. RodrÍGuez-Ambriz and L. A. Bello-PÉRez (2006). "Composition, Digestibility and Application in Breadmaking of Banana Flour." Plant Foods for Human Nutrition **61**(3): 131-137.

20. Mkhize NM, Sithole B (2013) Potential Amounts Of Biomass Available For Biorefinery Processing In South Africa, p.24

21. Moreau, R. A., V. Singh, M. J. Powell and K. B. Hicks (2009). Corn kernel oil and corn fiber oil. Reprinted with permission from Gourmet and Health-Promoting Specialty Oils. R. A. Moreau and A. Kamal-Eldin. Urbana, Illinois, AOCS Press. **409-429**.

22. Nahman A, De Lange W, Oelofse S, Godfrey L (2012) The costs of household food waste in South Africa, Waste Management 32: 2147-2153

23. Oberoi, H. S., P. V. Vadlani, L. Saida, S. Bansal and J. D. Hughes (2011). "Ethanol production from banana peels using statistically optimized simultaneous saccharification and fermentation process." Waste Manag **31**(7): 1576-1584

24. Petersson, A., Thomsen, M.H., Hauggaard-Nielsen, H. & Thomsen, A.B. (2007). Potential bioethanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean. *Biomass and Bioenergy* 31: 812- 819.

25. Ramírez EC, Johnston DB, McAloon AJ, Singh V (2009), Enzymatic corn wet milling: engineering process and cost model, Biotechnology for Biofuels 2009, 2:2 doi:10.1186/1754-6834-2-2

26. Ranzani, M. R., G. L. Sturion and M. H. Bicudo (1996). "[Chemical and biological evaluation of ripe banana peel]." Arch Latinoam Nutr **46**(4): 320-324.

27. Renewable Fuels Association (RFA) (2013). "World Fuel Ethanol Production 2012." http://ethanolrfa.org/pages/World-Fuel-Ethanol-Production#sthash.upsSJiPK.dpuf.

28. RFA(Renewable FuelsAssociation). Industrial statistics. <http://www. ethanolrfa. Org / industry / statistics> 2007.

29. Rutz, D. and R. Janssen (2008). Biofuel Technology Handbook. Germany, WIP Renewable Energies.

30. Sarkar, N., S. K. Ghosh, S. Bannerjee and K. Aikat (2012). "Bioethanol production from agricultural wastes: An overview." Renewable Energy **37**(1): 19-27.

31. Sharma, N., K. L. Kalra, H. S. Oberoi and S. Bansal (2007). "Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by simultaneous saccharification and fermentation." Indian J Microbiol **47**(4): 310-316.

32. Smeets, E.M.W, Faaij, A. P. C, Lewandowski, , I. M and Turkenburg, W. C (2007) "A bottom-up assessment and review of global bio-energy potentials to 2050," *Progress in Energy and Combustion Science*, vol. 33, no. 1, pp. 56–106,

33. Valerie Thomas*, Andrew Kwong (2001) Ethanol as a lead replacement: phasing out leaded gasoline in Africa Energy Policy 29 1133–1143 [online] available from http://www2.isye.gatech.edu/people/faculty/Valerie_Thomas/ethanol.pdf

34. Zhang, P., R. L. Whistler, J. N. BeMiller and B. R. Hamaker (2005). "Banana starch: production, physicochemical properties, and digestibility—a review." Carbohydrate Polymers **59**(4): 443-458.

35. http://www.kzndae.gov.za/LinkClick.aspx?fileticket=A2tGm_U6fQM%3D&tabid=273 (accessed 27/09/2011).