Bioresource Technology 172 (2014) 58–67

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Characterisation and evaluation of a novel feedstock, *Manihot glaziovii*, *Muell. Arg*, for production of bioenergy carriers: Bioethanol and biogas

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HIGHLIGHTS

- Composition of wild cassava, *Manihot glaziovii* was determined for the first time.
- *M. glaziovii* was found to be suitable for bioethanol and biogas production.
- Supplementation of fermentation residue with peels achieved higher fuel energy.
- Co-production of bioethanol and biogas achieved maximum fuel energy.

ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form 18 August 2014
Accepted 19 August 2014
Available online 27 August 2014

Keywords:
Manihot glaziovii
Bioethanol
Methane
Bioenergy
Cassava

ABSTRACT

The objective of this study was to characterise and evaluate a wild inedible cassava species, *Manihot glaziovii* as feedstock for bioenergy production. Tubers obtained from 3 different areas in Tanzania were characterised and evaluated for bioethanol and biogas production. These bioenergy carriers were produced both separately and sequentially and their energy values evaluated based on these two approaches. Composition analysis demonstrated that *M. glaziovii* is a suitable feedstock for both bioethanol and biogas production. Starch content ranged from 77% to 81%, structural carbohydrates 3–16%, total crude protein ranged from 2% to 8%. Yeast fermentation achieved ethanol concentration of up to 85 g/L at a fermentation efficiency of 89%. The fuel energy of the bioethanol and methane from flour-peels mix ranged from 5 to 13 and 11 to 14 MJ/kg VS, respectively. Co-production of bioethanol and biogas in which the peels were added to the fermentation residue prior to anaerobic digestion produced maximum fuel energy yield of (15–23 MJ/kg VS).

1. Introduction

Among biofuels, bioethanol is the most frequently used in motor vehicles, with an annual worldwide production of 8.6×10^7 m^3 in 2011 (REN21, 2012). Bioethanol is currently being produced from starch and sugar crops (Frigon and Guiot, 2010) whereas biogas is produced from wastewater sludge, manure, food and agricultural waste and supplemented with energy crops (Mursec et al., 2009). According to the World Biogas association (WBA), 2014, global potential of biogas is estimated to cover ca. 6% of the global primary energy supply or one quarter of the present consumption of natural gas (fossil methane). Germany leads in production of biogas with more than 7400 plants which generated ca. 20 TWh (1 Nm^3 of methane = 9.97 KWh, 1 TWh = 10^{12} KWh) in 2013. On the other hand Sweden leads in the use of biogas in transportation with more than 44,000 vehicles (World Bioenergy Association (WBA), 2014).

The question of whether the production of bioethanol or biogas is the best use for a given sugar/starch energy crop has been debated in literature (Börjesson and Mattiasson, 2008). It has been argued that more bioenergy carriers can be generated from

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http://dx.doi.org/10.1016/j.biortech.2014.08.084
0960-8524/

sugar-/starch-rich energy crops by producing methane rather than bioethanol (Börjesson and Mattiasson, 2008). However, each of these products may be more appropriate than the other in certain areas of application and the use of either of these fuels also depends on the existing infrastructure. Therefore, in evaluating a new feedstock for bioenergy potential, it is imperative to explore both options. Since most of the sugar/starch energy crops have competing uses such as food or feed, the main focus is on the use of lignocellulosic biomass for the so called second generation bioethanol. However, the commercial lignocellulosic bioethanol is not yet optimised (Marvin et al., 2012) and lignocellulosic ethanol production is not yet economically viable. Therefore, the search for and characterization of inedible bioenergy crops with low requirement of agricultural input is important. Currently, starch based ethanol is produced from corn, wheat, barley, sweet sorghum, potato and cassava, although these feedstocks are considered as non food in some regions, in other regions they are main staple food. Thus, cassava in Thailand, Indonesia and China is considered non food but it is staple food in many areas in Africa where it is cultivated (Moshi et al., 2014). Cassava is a high-yielding crop that grows well in tropical and subtropical temperature. Its starch level is higher than in other stem tuber plants. Additionally, cassava has many desirable growing characteristics such as drought- and flood-tolerance and is suitable to grow on light sandy soils and medium texture soils. The starch content in cassava is higher than 30% and it is estimated that 7.2 tons of fresh cassava or 3.0 tons of dry cassava can produce 1 ton of fuel ethanol (Moshi et al., 2014). Therefore, the use of an inedible wild cassava species, *Manihot glaziovii* will offer many benefits compared to other non-food starchy feedstocks for bioethanol production. Such benefits include ability to grow in poor soils yet with minimum of agricultural inputs, more resistant to pests and disease infestation, high yield and high conversion efficiency (Moshi et al., 2014).

The extent of tuber formation observed in *M. glaziovii* is assumed to be due to hybridization with the *Manihot esculentum*. A similar observation has been reported elsewhere in which hybridisation with *M. esculentum* led to formation of a new species, *Manihot fortalezensis* (Nassar et al., 2011). Since *M. glaziovii* grows on marginal land with low requirement for agricultural inputs, it is a very attractive resource and potential feedstock for biofuels production.

In *Manihot* sp., tubers at, at present, the main targeted part for production of biofuels, thus tuber formation and composition are important characteristics to consider. For example the quantity and ratio of structural and non-structural carbohydrates determine the type of pre-treatment required prior to yeast fermentation or anaerobic digestion (AD).

Although, *Manihot* species grow widely in many countries especially in Latin America and in Africa there is a shortage of scientific literature on their potential as feedstock for biofuel production. Furthermore, *Manihot* sp. may contain varying amounts of protein and cyanide which may not have negative effect on yeast fermentation (Boonnop et al., 2009), but both may have adverse effect on methanogenesis in AD due to ammonia inhibition and cyanide toxicity (Pirc et al., 2012). Moreover, during starch and bioethanol processing from cassava huge amounts of waste consisting of peels and pulp are generated, ca. 0.47 ton for each ton of cassava processed (Aro et al., 2010), which is of great environmental concern especially in Asian countries where large-scale processing is undertaken (Akaracharanya et al., 2011). Therefore, in the development of *M. glaziovii* as feedstock for biofuels a production process which integrates use of peels and pulp will ensure both clean environment and improved process economics.

The aim of the present study was to characterise and evaluate a wild, inedible *Manihot* species, *M. glaziovii*, as potential feedstock for bioethanol and biogas. Also, a bioprocess was developed for co-production of bioethanol and biogas in which the peels are integrated in biogas production to ensure maximum fuel energy from the wild cassava as well as clean environment.

2. Methods

2.1. Substrate collection and preparation

Tubers of *M. glaziovii Mueller Arg* (presumed to be a natural hybrid between *M. glaziovii* and *M. esculentum* (Rogers and Appan, 1973) were obtained from three districts in Tanzania namely, Kisarawe and Bagamoyo in the East coastal (Pwani) region and Muheza in the North-East coast (Tanga) region. Kisarawe is about 40 km from Dar-es-Salaam, located on Latitude: 6°54′00″S and Longitude: 39°04′00″E; Muheza is 385 km from Dar-es-Salaam and is located on latitude 5°10′00″S longitude 38°46′59″E; whereas Bagamoyo is ca.70 km from Dar-es-Salaam, and is located on Latitude: 6°25′59″S and Longitude: 38°53′59″E.

Composition data of flour and peels of these tubers are presented in Table 2. Samples of flour for tubers obtained from Kisarawe, Muheza and Bagamoyo and the reference material are denoted as MGK, MGMU, MGB and ME respectively, whereas samples of their corresponding peels are denoted as MGKP, MGMUP, MGBP and MEP respectively. The plants from which tubers were obtained were first identified using identification key, at the Botany Department, University of Dar-es Salaam. Afterwards the plants were earmarked at the beginning of the rainy season when starch formation commenced concomitantly with an increase in size of the tubers. The tubers were harvested 8 months later, when they had attained maximum size and starch content according to Moorthy and Ramanujam (1986) to ensure that tubers from all plants were of similar maturity. The tubers were cleaned, peeled and processed into chips of 5–15 mm thickness, within 24 h. Both chips and peels were dried in a solar dryer cabinet at 50–55 °C for 2–3 days to a moisture content of 10–12%, and subsequently milled and packed in polythene bags and subsequently, sieved through 850 μm steel mesh prior to use. Tubers of ME obtained from a local store in Dar-es Salaam, Tanzania were treated in the same way and were used as reference sample material.

In order to fully explore the bioenergy potential of *M. glaziovii*, bioethanol and biogas production were evaluated separately and combined in a sequential process. The entire experimental design and layout for energy evaluation is given in Fig. 1.

2.2. Composition analysis

The samples of flour/peels described in Section 2.1 were analysed for total solids (TS), ash content, volatile solids (VS) following the method described by Sluiter et al. (2008). Starch content was determined as per the method described by Holm et al. (1986), with some modifications. The pH of the water used in the analysis was adjusted to 7.0 by drop-wise addition of $CaCl_2$ (0.1 M) to pH 6.0, then neutralised to 7.0 ± 0.2 by adding NaOH (0.01 M) to enhance α-amylase catalysed hydrolysis. The released glucose was analysed using HPLC according to the method described by Crespo et al. (2012). Total starch was calculated according the following equation: $\text{Total starch} = \left\langle \frac{Gh \times Df \times TS \times R_v}{100 \times 1000} \right\rangle - (E_f + G_f) \times 0.9$ where, Gh is the amount of glucose (g) as analysed by HPLC, Df total dilution factor (where, total dilution factor is defined as the ratio of the final volume (obtained during sample preparation for HPLC analysis, buffers and volume of enzymes added during hydrolysis) of the aliquot divided by the initial volume of the aliquot). TS (total solids), R_v reaction volume, 0.9 (162/180) is hydro-factor for hexose (conversion factor for dehydration on polymerisation of glucose to starch i.e. a factor due to difference in

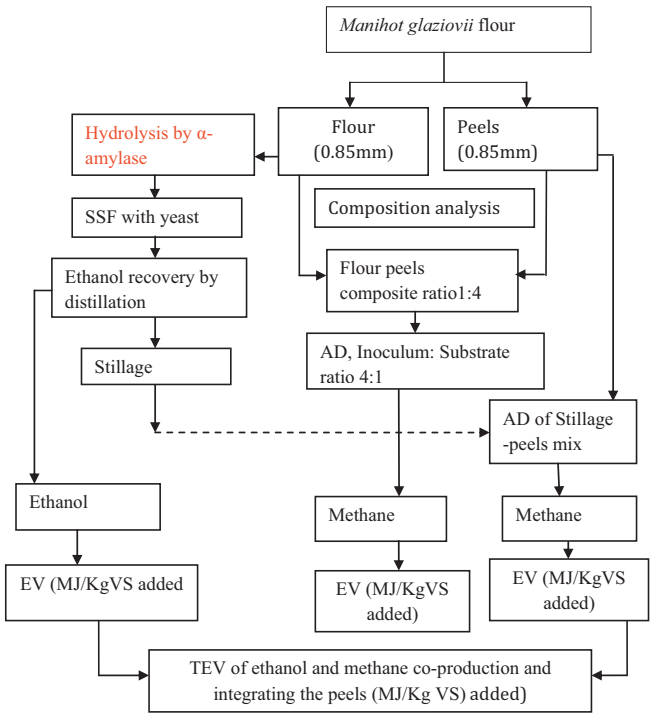

Fig. 1. Experimental design for evaluation of bioenergy potential of *Manihot glaziovii*: NB: AD refers anaerobic digestion, EV: energy value, TEV: Total energy value, stillage = refers to yeast fermentation residue.

mass between anhydroglucose ring and glucose), E_f is the amount of glucose in enzyme formulations obtained from controls i.e. enzymes without substrate and G_f is free glucose in cassava flour/peels obtained by treating the substrate without addition of enzymes in the hydrolysis step.

Structural carbohydrates and lignin were analysed following the method described by Sluiter et al. (2011), with some modifications to adapt it to substrate with high starch content. Briefly, the amount of glucose contributed by acid hydrolysis of starch residue was corrected by pre- and post- extraction determination of starch, following the protocol described in Section 2.2 and afterwards the amount of glucose contributed by starch residue were mathematically adjusted.

Total crude protein was determined by the micro Kjeldahl method in which total nitrogen (N) was converted to total crude protein by multiplying by a factor of 3.24 (Yeoh and Truong, 1996). The total cyanide expressed (as % of dry weight) was determined using linamarase loaded picrate paper (School of Botany and Zoology Canberra, ACT 0200, Australia) to absorb the cyanide in 100 mg of samples suspended in 0.5 mL deionised water, and afterwards the colour was extracted in 5.0 mL deionised water and the absorbance was read at 510 nm. Total cyanide (mg/kg of dry weight) was calculated.

(The values were afterwards converted to percentage of dry weight of sample).

2.3. Bioethanol production from M. glaziovii

2.3.1. Microorganism, media and culture maintenance

Baker's yeast used for ethanol production was purchased from a local supermarket in Lund, Sweden. The yeast was cultivated on medium formula described by Postma et al. (1989). The cells were maintained on agar plate and incubated at 30 °C for 24–36 h. Subsequently, the plates were stored at 4 °C until required for use. The yeast was incubated in 250 mL conical flask with 100 mL reaction volume in shaking incubator at 30 °C, 100 rpm.

2.3.2. Bioreactor set up and operation for ethanol production

Bioethanol production from the *M. glaziovii* flour was evaluated in Automatic Gas Potential Test System (AGPTS) as described by Moshi et al. (2014). All flour samples (ca. 62 g) were suspended in (200 mL) deionised water neutralized to pH 7.0 ± 0.2 as described in Section 2.2. The slurry was then hydrolysed with α-amylase as detailed elsewhere (Moshi et al., 2014). Afterwards, the mixture was supplemented with a 10 times concentrated solution of growth medium 10% (v/v), and inoculated with 10% (v/v) of actively growing Baker's yeast to a final reaction volume of 250 mL and then pH was adjusted to 5.8 ± 0.2 with H_2SO_4 (1 M). The bioreactors were then purged with oxygen-free nitrogen through a sterile cellulose membrane (0.45 μm pore size) and then incubated for simultaneous saccharification and fermentation (SSF) in the AGPTS at 32 ± 2 °C, stirring speed of 140 rpm. Ethanol was monitored online stoichiometrically through CO_2 registered in the AGPTS software and confirmed by HPLC through samples taken regularly in the course of fermentation according to Moshi et al. (2014).

2.3.3. Ethanol distillation

After ethanol production, as detailed in Section 2.3.2, the fermentation residue was stored at −20 °C until when used for anaerobic digestion (AD). Subsequently, ethanol was stripped off in a water-jacketed glass bioreactor (17 cm height × 9 cm inner diameter) using a Laboratory suction pump (Heto Master Jet, Denmark) under mild vacuum at 80 °C for 30 min. Ethanol recovery was only 92%. Afterwards, during AD the methane yield contributed by the ethanol residue was corrected for according to procedure describer by Kreuger et al. (2011). When converting the ethanol to energy units a High Heating Value (HHV) of 29.7 MJ/kg was used.

2.4. Biogas production from M. glaziovii

2.4.1. Inoculum collection and preparation

An active inoculum was collected from a mesophilic anaerobic digester at a municipal wastewater treatment plant at Källby (Lund, Sweden). The inoculum was pre-incubated at 37 °C for 4 days to deplete the residual readily biodegradable matter. After pre-incubation, a representative sample of the inoculum was analysed in quadruplicate to determine TS, VS and pH.

2.4.2. Setup for anaerobic digestion

A multi-channel analyzer, the AMPTS as described by Badshah et al. (2012) was used to evaluate biogas potential of *M. glaziovii* (flour–peels mix) and peels alone. The flour and peels were mixed in a ratio of 4:1, a proportion as they occur in the tubers. Two sets of controls were run and treated in the same way as the test bioreactors. One control containing only inoculum to determine the background methane production from the inoculum, and another control was starch and cellulose (Avicel) in a ratio of 4:1 to mimic the ratio of starch and structural carbohydrates in the flour:peels mixture and ascertain the suitability of the inoculum to this type of substrate.

The inoculum to substrate ratio (in terms of VS) in all the bioreactors was 4:1. AD was carried out in 500 mL glass bioreactors with working volume of 300 mL. Bioreactors were purged with nitrogen for about 2 min and incubated for AD at 37 ± 0.5 °C in a water bath. Stirrers were set to operate intermittently at 46 rpm, 30 s on and 120 s off during the whole experiment.

2.4.3. Methane production from M. glaziovii fermentation residue

The stillage from the ethanol fermentation was mixed with peels in a ratio of 4:1 (w/w) and AD was carried out using the set-up described in Section 2.4.2 except that cellulose (Avicel) was used as a control. The methane potential was not experimentally determined for yeast, yeast extract and enzymes, but the

methane contributions were calculated based on the COD of these carbon sources according to Kreuger et al. (2011). A high heating value (HHV) of 55.5 MJ/kg and a density 0.7157 kg/m^3 were used to convert the volume of methane to energy units (Kreuger et al. (2011).

2.5. Analytical methods

All samples after hydrolysis and fermentation were analysed for glucose and fermentation products by HPLC as per Section 2.2. The volume of CO_2 (NmL) was registered online by the AGPTS software as described previously by Moshi et al. (2014). Dry cell mass was estimated as a function of optical density using a pre-established calibration curve.

3. Results and discussion

3.1. Substrate description

3.1.1. Introduction of M. glaziovii to Tanzania

M. glaziovii Mueller Arg was introduced to East Africa from Brazil by Germans in early 1900 for trial as plantation crop for rubber production. Quite extensive plantings were done with more than 100,000 acres in the then Tanganyika. However, this crop was abandoned before 1925 because of its poor resin and a better crop (*Hevea brasiliensis*) for rubber was found (Rogers and Appan, 1973). Since then the plant became wild and spread widely especially in coastal regions including Pwani, Tanga, Lind and Mtwara (Personal observation).

Table 1
Physical characteristics of *Manihot glaziovii*, and reference *Manihot esculentum* tubers.

Parameters	MGK	MGMU	MGB	ME
Weight (kg)	0.1–1.5	2.3–4	5–8	2–3.2
Length (cm)	16–36	30–40	56–122	16–34
Diameter (cm)	0.15–2.50	10–14	9–16	9–12

All values are range for 50 tubers.

3.1.2. Plant phenotypic description and tubers physical characteristics

The plants, *M. glaziovii*, found in the three areas (as described in Section 2.1) were phenotypically different. The plants observed could clearly be categorised as subshrub occurring in clusters and early branching with no tubers, and slender tall single apical branching plants with significant tubers. The tubers obtained from the three areas (Kisarawe, Muheza and Bagamoyo) were different in both physical dimensions and in composition (Tables 1 and 2).

Tuber formation is a feature which has not been reported for *M. glaziovii* before; the extent of tuber formation observed in this study makes this plant interesting for study as bioenergy crop. If *M. glaziovii, Muell. Arg* is explored as a bioenergy crop it has many advantages compared to the domesticated cassava, *M. esculentum*. It is very hardy, a fast grower, free from insect and fungoid attacks, requires little or no attention when once established and thrives in poor, dry and rocky soils unsuited to almost any other crop (Rogers and Appan, 1973). Moreover, the types recently found in Tanzania possess huge tubers with high content of readily degradable carbohydrates up to 89% (Table 2) of dry matter. Furthermore, it is inedible because it is presumed to contain fibrous roots and is very bitter compared to *M. esculentum.*

3.2. Composition of M. glaziovii

The compositions of three types of *M. glaziovii* samples mentioned in Section 2.1 are given in Table 2. The composition analysis disclosed that MGK and MGMU flour have high starch content; comparable to that of the reference (ME), whereas MGB flour was more fibrous with lower percentage of starch. The peels of MGK and MGMU contained starch in comparable amounts to that of the peels of the reference (MEP) ca. 45%, whereas MGBP displayed very low starch content.

Table 2
Composition of three types of *M. glaziovii, Mueller, von Argau*, found in Tanzania.

Sample code		ME	MEP	MGMU	MGMUP	MGK	MGKP	MGB	MGBP
Moisture content (%)		12.0	11.0	11.0	11.0	12.0	12.0	12.0	10.0
		(0.1)	(0.1)	(0.8)	(0.0)	(0.1)	(0.5)	(0.0)	(0.1)
Total solids (%TS)		88.0	89.0	89.0	89.0	88.0	88.0	88.0	90.0
		(0.1)	(1.1)	(0.8)	(0.0)	(0.1)	(0.5)	(0.0)	(0.1)
Structural sugars (%)	Glucose	3.1	9.3	8.5	6.2	1.5	2.3	13.7	12.4
		(0.1)	(0.3)	(3.3)	(0.8)	(0.9)	(0.2)	(0.5)	(1.5)
	Xylose	0.0	0.4	0.0	0.6	0.0	0.4	1.5	1.0
		(0.0)	(0.0)	(0.0)	(0.3)	(0.0)	(0.0)	(0.0)	(0.0)
	Galactose	0.0	1.7	1.6	1.5	1.1	1.6	1.6	1.7
		(0.0)	(0.)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.4)
	Arabinose	0.4	1.1	0.5	1.0	0.5	1.0	0.7	1.7
		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
TSC (%)		3.2	11.1	9.5	8.2	2.8	4.8	15.7	15.1
		(0.0)	(0.3)	(3.3)	(0.0)	(0.9)	(0.2)	(0.5)	(1.5)
Total lignin (%)		1.5	16.0	3.5	15.1	4.4	17.3	34.6	44.6
		(0.4)	(0.0)	(0.4)	(2.4)	(0.7)	(1.1)	(0.5)	(1.9)
Total starch (%)		81.4	45.4	76.6	46.7	80.1	33.2	28.2	6.1
		(0.4)	(0.7)	(1.1)	(0.2)	(1.1)	(1.0)	(0.4)	(1.1)
Free glucose (%)		0.3	0.5	0.4	0.3	0.2	0.5	0.10	0.1
		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Extractives (%)		11.6	16.9	9.9	20.2	14.3	31.2	24.5	24.2
		(0.4)	(0.7)	(1.1)	(0.2)	(1.1)	(1.0)	(0.4)	(1.1)
Total crude protein (%)		1.3	8.1	5.2	7.4	2.4	7.0	5.3	8.0
		(0.0)	(0.5)	(0.2)	(0.3)	(0.2)	(0.3)	(0.5)	(0.2)
Total cyanide*10^{-3} (%)		2.1	39.1	2.6	21.6	8.1	16.6	8.1	7.0
		(0.1)	(0.0)	(0.2	(0.1)	(0.2)	(0.0)	(0.2)	(0.1)

NB: MGK, MGMU, MGB, MGKP, MGGMUP and MGBP refer to *M. glaziovii* flour and peels obtained from Kisarawe, Muheza and Bagamoyo Tanzania respectively. ME and MEP flour and peels of the reference *M. esculentum*, TSC: Total structural carbohydrate (cellulose and hemicelluloses). Values in brackets refer to standard deviation for triplicate analysis.

The total amount of starch observed for ME is in agreement with the range reported in literature, 71–85% (Muzanila et al., 2000). Cassava starch is preferred for bioethanol production more than starch from other sources, because it has a lower gelatinization temperature (e.g. in comparison to corn) (Sánchez and Cardona, 2008). This fact is advantageous as mild heat treatment would cause granules to swell, which enhance enzymatic digestibility (Shariffa et al., 2009). Furthermore, cassava starch can be readily hydrolysed by lower dosage of enzymes as compared to starch from other sources (Ocloo and Ayernor, 2010).

In addition to starch, MGK and MGMU contained amounts of cellulose and hemicelluloses similar to that of ME whereas MGB demonstrated higher levels of cellulose, hemicelluloses and lignin (Fig. 2A). The proportion of cellulose, hemicellulose and lignin determines the degree of recalcitrance of the material to hydrolysis (Xu et al., 2012).

The observed ratios of cellulose:lignin:hemicelluloses for MGK, MGMU and MGB were 1:3:1, 1:2:1 and 4:12:1 respectively, whereas that of ME was 8:4:1 (Fig. 2A). High proportion of cellulose and lignin content has a strong negative synergistic effect on biomass digestibility (Xu et al., 2012). From this argument it is plausible to say that the degree of recalcitrance to hydrolysis increases in the direction ME → MGK → MGMU → MGB (Fig. 2A). With regard to peels, (MGKP, MGMUP, MGBP and MEP) the proportion of cellulose, lignin and hemicelluloses are; 1:7:1, 1:7:1, 3:13:1 and 3:6:1, respectively (Fig. 2B) and following the same argument as for the flour, the peels which contain higher proportions of cellulose and lignin than the flour (Table 2), are more resistant to hydrolysis.

When considering the suitability and appropriate process for each type of *M. glaziovii*, MGMU and MGK with 83 and 80% of total degradable carbohydrate of which 95 and 97% is starch respectively, are more readily enzymatically hydrolysable for bioethanol production via yeast fermentation. Conversely, MGB whose TDC is 44% and out of which 65% is starch will require elaborate pre-treatment to remove the lignin shield and is recommended for biogas production via AD.

The peels displayed lower percentage of total degradable carbohydrate (21–56%) and high lignin content. They are recommended for biogas production via AD. They could also be utilized in co-production of bioethanol and biogas with mild pre-treatments. The residue from AD can be used as bio-fertiliser.

The peels contained high amount of total crude protein and cyanide for both *M. glaziovii* and *M. esculentum* (ME) Table 2. The observed values for ME agree with those reported by Muzanila et al. (2000). Also the amount of total crude protein compared closely to those reported for 4 other wild tuberous species (Nassar, 2000). Conversely, the total cyanide was comparatively lower in *M. glaziovii* than in the other wild species (Nassar, 2000). However, total crude protein reported in literature for cassava should be taken with precaution because the traditional conversion factor of 6.25 used on assumptions that all nitrogen detected by Kjeldahl analysis is from protein which contains 16% nitrogen may not be valid for cassava because of the presence of significant amounts of non-protein nitrogen. Yeoh and Truong (1996) observed that nitrogen-to-protein conversion factor based on nitrogen recovered from total amino acid analysis ranged from 4.75 to 5.85. The same authors reported that conversion factors of 15 varieties of cassava based on Kjeldahl nitrogen ranged from 2.49 to 3.67 and recommended an average factor of 3.24 ± 0.31 for cassava. Therefore, in this study a conversion factor of 3.24 was used.

Cyanide at the concentrations found has no effect on yeast fermentation because it is eliminated during fermentation (Ray and Sivakumar, 2009). The fact that yeast fermentation is an effective way of reducing cyanide in cassava is a well established fact. For example, cyanide levels are reduced from 10–49 ppm to 5.4–29 ppm during yeast fermentation (Ray and Sivakumar, 2009). However, AD can be inhibited by cyanide due to high sensitivity of methanogenic bacteria to cyanide (Pirc et al., 2012). In this study when an inoculum:substrate ratio of 2:1 was used, AD was inhibited after 32 h of incubation and this could partly be explained by the presence of high cyanide content and partly due to pH drop (7.5 to 6.4 after 32 h of incubation). The drop in pH in the bioreactor shows that there was rapid conversion of released sugar into VFAs causing inhibition of methanogenesis (Raposo et al., 2009). When the inoculum:substrate ratio was increased to 4:1, only slight inhibition occurred in the first day of AD for MEP in which the highest cyanide content (ca. 0.04% of dry matter) was observed, but was quickly overcome Fig. 3A. Therefore, one disadvantage of using cassava in AD is that low inoculum substrate ratio is difficult to achieve because of cyanide inhibition. The composition analysis displayed the quantity and the ratio of different degradable carbohydrates and lignin, total crude protein and total cyanide in each type of *M. glaziovii*. This information is useful in choosing an appropriate pre-treatment technique for each type for either bioethanol or biogas production.

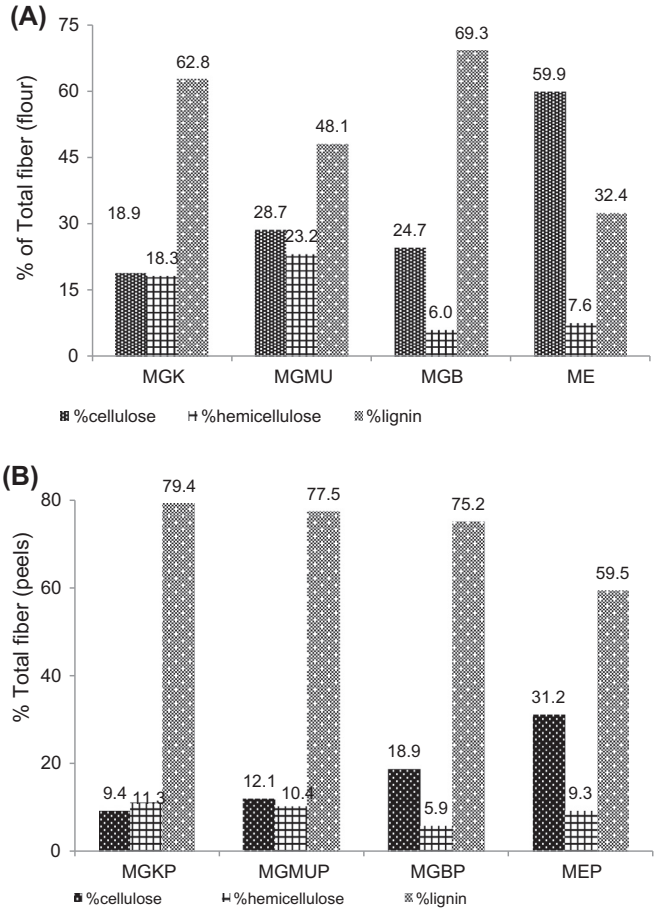

Fig. 2. (A) Proportion of cellulose, hemicellulose and lignin in flour expressed as % of total fibre. (B) Proportion of cellulose, hemicellulose and lignin in peels expressed as % of total fibre. MGK, MGMUP, MGB,ME, MGKP, MGMUP, MGBP and MEP refers to *Manihot glaziovii* obtained from Kisarawe, Muheza, Bagamoyo, and the reference *Manihot esculentum* and their corresponding peels respectively.

3.3. Bioethanol potential of M. glaziovii

Ethanol concentration from two types of *M. glaziovii* (MGK and MGMU) and the reference ME were 78.5 g/L, 84.5 g/L and 77.3 g/L,

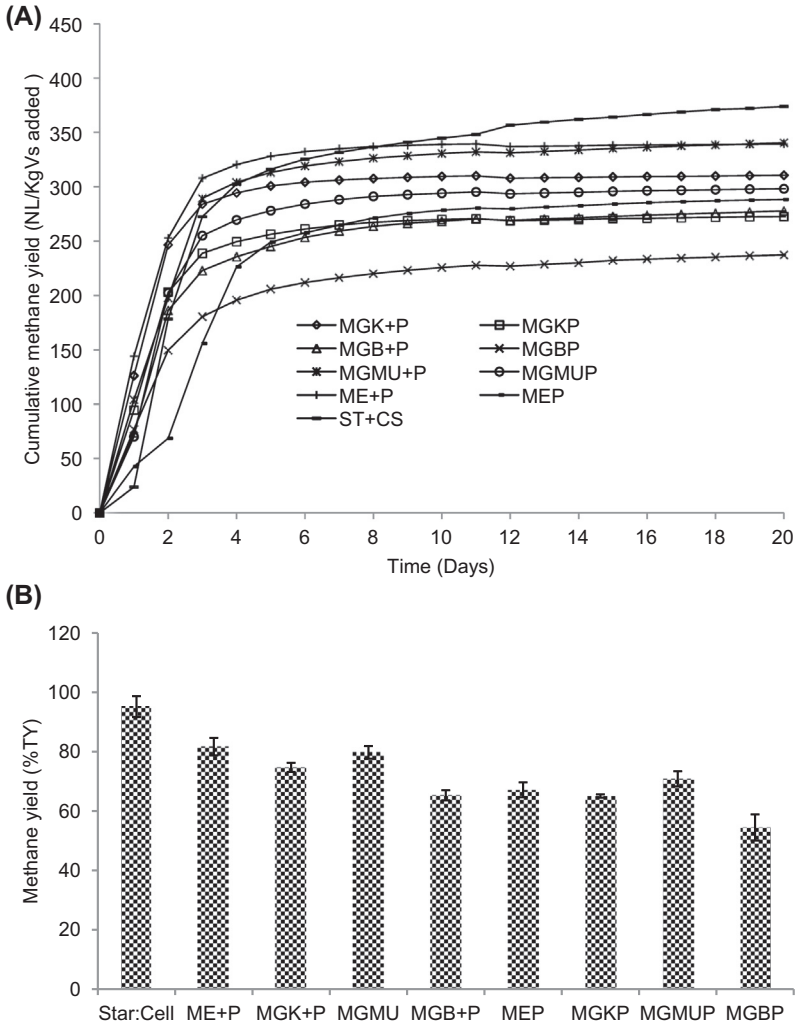

Fig. 3. Cummulative methane yield and yield percentage of theoretical yield after anaerobic digestion of different types of wild cassava (*M. glaziovii*) (flour–peel mix and peels). (A) Accumulated amount of methane produced vs. time (days) after anaerobic digestion. (B) Methane yield (%TY). MGK + P, MGMU + P, MKG + P, ME + P, and MGKP, MGMUP, MGBP MEP refers *Manihot glaziovii* flour + peels and peels from Kisarawe, Muheza and Bagamoyo and the reference *Manihot esculentum* flour and peels respectively. Whereas ST and CS refers to starch and cellulose respectively.

respectively. These corresponded to yields of 81%, 87%, and 80% (based on expected theoretical glucose (g/L) in the bioreactors at fermentation efficiency of 83%, 89% and 82%, respectively (based on actual and expected ethanol concentration). Ethanol concentration and fermentation efficiency for ME were comparable to values reported elsewhere at similar flour concentration (250 g/L) (Lin et al., 2011) which is 75.6 g/L with fermentation efficiency of 76%. Despite high fermentation efficiency (94%) ethanol yield from MGB was expectedly very low (26 g/L which corresponded to a yield of 47%) due to high fibre and lignin content (Table 2). No attempt was made to hydrolyse the cellulosic component of this feedstock to fermentable sugars for the sake of process simplicity and economics. The cellulosic fraction left was used as substrate for biogas in the subsequent AD (as described in Section 2.4.3). In this study, ethanol titres of 10–11% (v/v) were achieved using mild liquefaction conditions and SSF in Automatic Gas Potential Testing System, focusing on the starch component and saving the structural carbohydrate and other components for biogas production. The mild conditions employed in hydrolysis and SSF (i.e. 90 °C followed by SSF at 32 °C, saves energy that would otherwise be used in starch extraction, cooking and saccharification.

3.4. Biogas production from M. glaziovii

3.4.1. Biogas production from flour–peel mix and peels alone

The amount of methane accumulated per day during AD of flour–peels mix and peels alone of wild cassava and the reference domesticated cassava are given in Fig. 3A whereas the methane yields expressed as percentage of theoretical yield are given in Fig. 3B. The control, (starch:cellulose in a ratio of 4:1) reached 95% of the theoretical yield (Fig. 3A) after 36 h (when production ceased) showing that the inoculum was relevant to this kind of substrate. Nevertheless, the methane yield increase for the control from day 20 to 36 was less than 5%, therefore no energy value was calculated beyond day 20. Different flour–peels mixtures and peels alone reached constant yield between 10 and 20 days (Fig. 3A).

The methane yield from the flour–peels mix (MGK + P and MGMU + P) and corresponding peels alone were comparable to the methane yield from the reference flour–peels mix (ME + P) and peels alone (MEP), respectively, whereas the MGB + P mix and corresponding peels alone (MGBP) produced relatively low yield (Fig. 3B). This could be explained by the high lignin content observed for MGB and MGBP, (Table 2). The methane yields observed in this study both from peels of wild and domesticated

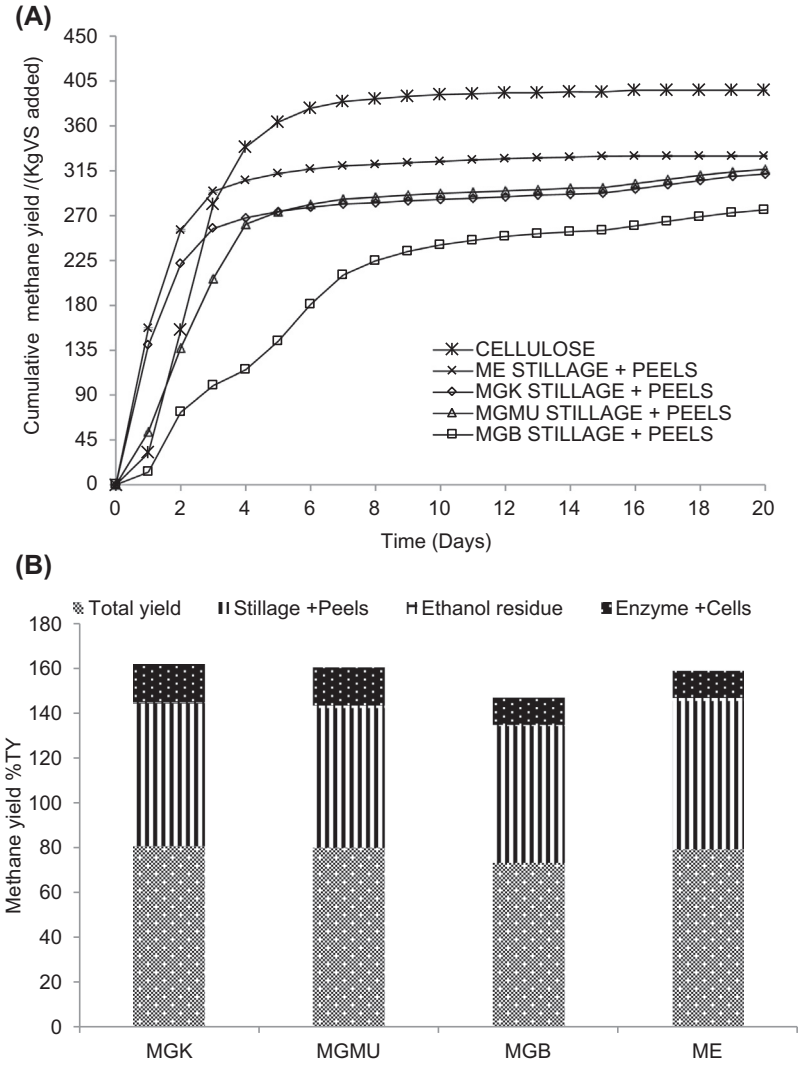

Fig. 4. Cumulative methane yield and yield percentage of theoretical yield after anaerobic digestion of yeast fermentation residue (stillage) of wild cassava mixed with corresponding peels in a ratio of 4:1. (A) Accumulated amount of methane produced vs. time (days) after anaerobic digestion. (B) Methane yield (%TY). MGK, MGMUP, MGB and ME refers to flour of *Manihot glaziovii* from Kisarawe, Muheza, and Bagamoyo and the reference *Manihot esculentum* respectively.

cassava were generally higher than that reported by Zhang et al., 2011 for *M. esculentum* of around 260 NL/kg VS added. This result revealed that mixing of flour and peel(s) results in more energy and allows the utilization of the whole tuber and significantly reduces waste that would otherwise pollute the environment. The amount of methane produced from peels alone which was up to 71% of theoretical (Fig. 3B) justifies their suitability as substrate for methane production in a scenario whereby the flesh is desired for other products such as bioethanol or glucose syrup. Methane from the peels alone or mixed with pulp may be used to fuel bioethanol or starch factory to reduce production cost.

3.4.2. Biogas potential of fermentation residue supplemented with the peels

The amount of methane accumulated per day during AD of these mixtures is given in Fig. 4A, whereas total yield and yield attributed to fermentation residue–peels mix, enzyme + cells and ethanol residues as percentage of theoretical yield are given in Fig. 4B. The control, Avicel cellulose reached 95% (396 L/kg VS added) of the theoretical yield of 415 NL/kg VS added showing that the inoculum used was appropriate for this kind of substrate. The control and the reference (ME fermentation residue + peels) reached constant yield after around 16 days whereas the wild type reached constant yield after 30 days. Probably, this could be attributed to presence of a high proportion of components such as cellulose, hemicelluloses, lignin and yeast cells that are poorly degradable by the microbial consortium in AD.

Carbon balance analysis on yeast fermentation revealed that fermentation of MGK and MGMU resulted in high cell mass (biomass) compared to those reported for ME and MGB.

3.5. Transport fuel energy from bioethanol and biogas from M. glaziovii

3.5.1. Yield of bioethanol fuel energy

Conversion of MGK, MGMU and ME achieved comparable amounts of fuel energy (MJ/kg VS added), whereas products from MGB represented very low energy (MJ/kg VS added) (Fig. 4B). The ethanol fuel energy value based on HHV (ca. 11–13 MJ/kg VS) was obtained for MGK and MGMU (Fig. 5A) which is attributable to their high content of degradable carbohydrates (Table 2). According to this analysis and based on global and Tanzanian cassava yield per ha which is 12.2 and 9.7 tonnes (dry weight) (FAOSTAT, 2006), about 145 GJ/ha and 115 GJ/ha, can be produced,

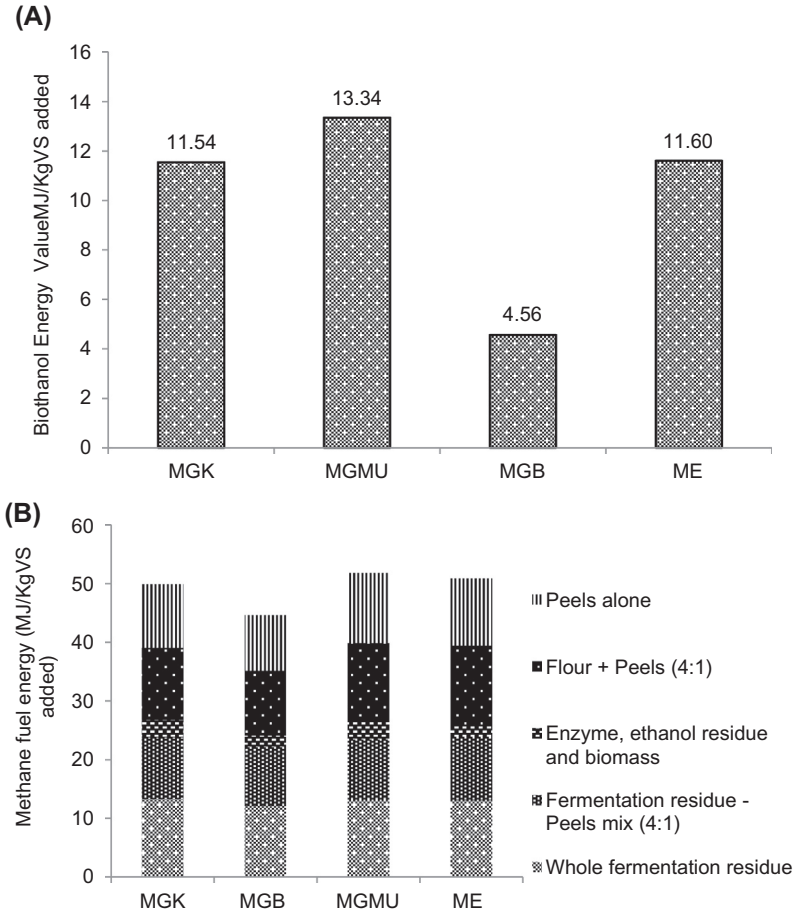

Fig. 5. Energy values of produced bioethanol and biomethane during batch simultaneous saccharification and fermentation, and anaerobic digestion of wild cassava and the reference *M. esculentum* with *S. cerevisiae*. (A) Energy values for bioethanol, (B) Energy values for biomethane.

respectively. To determine the exact energy yield per ha from *M. glaziovii* further studies are recommended to study tuber production per ha, harvesting cycles and harvesting times for this new feedstock.

3.5.2. Methane fuel energy yield of M. glaziovii

The methane energy yield of *M. glaziovii* was evaluated in two stages. First, the flour–peels mix in a ratio as they occur in nature and peels alone and secondly fermentation residue from yeast fermentation of cassava flour supplemented with peels in a ratio of 4:1. Methane energy yield for the two approaches are presented in Fig. 5B. The (MGMU + P) and (ME + P) yielded comparable fuel energy (MJ/kg VS added), whereas MGK + P and MGB + P yielded slightly lower fuel energy (MJ/kg VS added).

When the peels were used as a standalone substrate during AD, they attained 86–88% of the cumulative methane produced by the flour–peels mixture in the ratio of 4:1. This is attributable to the high content of degradable carbohydrate (Table 2). In addition, the peels contain up to 8% (w/w) crude protein of the dry matter which is also convertible to methane by the microbial consortium in AD.

The amount of energy carriers obtained by fermentation of residue–peels mix was ca. 10 MJ/kg VS added (Fig. 5B), which is less than that from ethanol from flour or methane from flour–peels mix in a ratio of 4:1 or peels alone. However, by including the methane contributed by ethanol residue, recycled enzymes and yeast cells the yield was 12–13 MJ/kg VS added (Fig. 5B).

As mentioned earlier, some bottlenecks in processing cassava to biofuels are the higher level of cyanide which inhibits e.g. bacteria in ethanol fermentation or in AD. Also, cassava tubers are highly perishable and need quick processing and their bulkiness may impose transport and storage problems. Therefore, to be profitable, logistics need to be carefully considered e.g. conversion of tubers to dry chips right at the field and transport the latter to factory may significantly reduce the production costs and hence increase the net energy value.

Cassava processing into different products and in different regions takes diverse approaches and consequently handling of the waste stream. For instance, specifically, in Africa cassava is processed into gari (fermented cassava product common in West Africa) or flour and the flour is often used as feedstock for starch and bioethanol processing. Therefore the peels can be mixed with stillage for biogas production through AD. Conversely bioethanol factories e.g. in Thailand where cassava tubers are chopped and dried to chips prior to ethanol fermentation (Nguyen et al., 2007), the stillage is rich in structural sugars from the peels and pulp can be used for methane production. Likewise, in starch processing factories the solid residues consisting of peels and pulp is an excellent substrate for both bioethanol and biogas. This approach offers a strategy for waste management plus substantial amount of methane to fuel the bioethanol factory and hence reduce cost of bioethanol production.

3.6. Comparison of bioenergy yields from different scenarios

A comparison of the amount of bioenergy carriers produced using the different approaches is given in Fig. 6. Methane fuel energy values from the flour–peels mix in a ratio of 4:1, peels alone

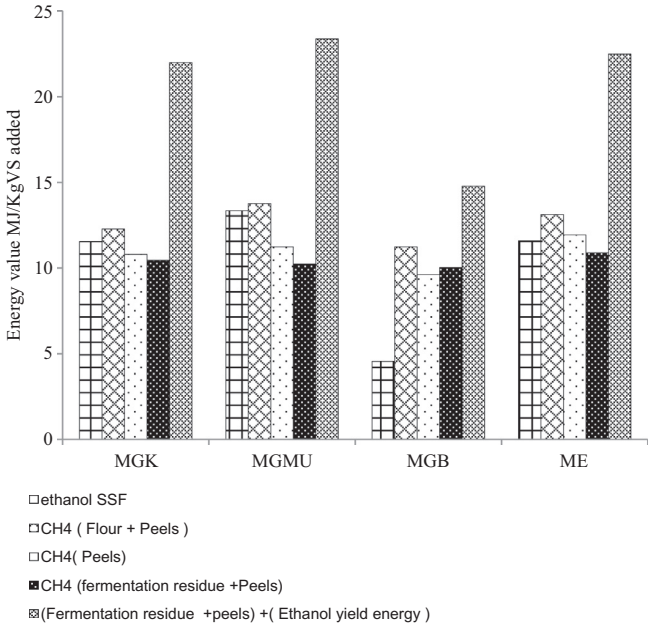

Fig. 6. Comparison of Energy yield (MJ/kg) from wild cassava (*Manihot glaziovii*) and the reference (*M. esculentum*) using different approaches.

and ethanol from the flour alone were in the range 11–14, 10–12, and 5–13 MJ/kg VS added, respectively. Ethanol production from the flour and afterwards methane production from fermentation residue combined with peels achieved maximum energy value in the range 15–23 MJ/kg VS added. This approach also saves cost of enzymes for hydrolysing the cellulosic component in the tubers for yeast fermentation if bioethanol alone is produced from tubers and peels or otherwise significant amount of biomass will be left unutilised. Therefore, this approach does not only offer a way of reducing green house gas from waste but also reduce the fossil fuel dependency by utilizing methane produced during an AD step. As Tanzania is at an infant stage of developing her biofuels industry, this result provides guidance to the type of processes that can be established to maximise both economical and environmental benefits. However, a study on maturity index and pilot plant up-scaling as well as Life Cycle Assessment on bioenergy carriers production from this new feedstock are proposed prior to any commercial production of bioenergy carriers from this new feedstock.

4. Conclusion

A new inedible feedstock, *M. glaziovii* was characterised and evaluated for bioethanol and biogas production. Starch content ranged from 46% to 81%, structural carbohydrate ranged from 3% to 16%, protein ranged from 2% to 8% and total cyanide ranged from 0.02% to 0.04% of dry weight. Ethanol concentrations of 78 and 85 g/L (from MGK and MGMU) were achieved at fermentation efficiencies of 82–89%. The sequential fermentation and AD which incorporated the peels produced energy carriers with 23 MJ/kg VS. This approach achieves high energy content and contributes to clean environment. Further process optimisation on the hydrolysis and fermentation may further improve energy yield.

Acknowledgements

This research has been supported by the Swedish International Development Cooperation Agency (Sida) and the Swedish Agency for Research and Cooperation with Developing Countries (SAREC) as part of the collaborative project Renewable Energy between Sweden and Tanzania. Carla F. Crespo was supported by SIDA-SAREC as part of collaborative project Microbial Diversity between Sweden and Bolivia. Malik Badshah was supported by a fellowship granted by higher education commission of Pakistan and EU Programme BIOGASSYS. Emma Kreuger of Biotechnology division Lund University is gratefully acknowledged for lending us equipment for composition analysis.

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