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Production of renewable fuel and value-added bioproducts using pineapple leaves in Costa Rica

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ABSTRACT

Pineapple, Ananas comosus, is one of the most important cash crops in Costa Rica with more than 44,500 ha of plantation. The pineapple industry contributes approximately 1.7% of the gross domestic product (GDP) of Costa Rica. Pineapple cultivation generates a large amount of plant residues (250 metric tons per hectare of wet plant residues mainly leaves). Current practices of the field residue handing include direct burning, in situ decomposition and removal of residue before planting, which are neither economically sound nor environmentally friendly. New approaches are urgently needed to utilize the residues and improve sustainability of pineapple production in Costa Rica. This study developed a simple, efficient process to convert the pineapple plant leaves into bioethanol, spent yeast proteins, and fibrous material (pulp). The residue was first treated by a mechanical extruder to generate juice and fibrous material. The juice was fermented by a yeast, *Kluyveromyces marxianus*, to produce ethanol and spent yeast proteins. Under the selected process conditions, the plant leaves (125 tons fresh weight per year) from 1 ha can generate 2.1 tons of bio-ethanol, 1.55 tons of spent yeast biomass, and 11.65 tons of dry fibrous material. The mass and energy balance analysis concluded that using the studied process, the pineapple plant leaves from 44,500 ha of pineapple plantation in Costa Rica can produce 93,043, 68,975, and 518,425 tons of bioethanol, spent yeast, and fibrous material per year, respectively. The amount of bioethanol is able to replace approximately 8.51% of transportation fossil fuel consumption in Costa Rica.

1. Introduction

Costa Rica is one of the biggest fresh pineapple producing countries in the world. Most of pineapples are exported to North America and the European Union with a total revenue of $941.5 million per year. Despite its importance in the local economy, there is an increasing concern about the negative environmental impacts of the large amount of waste generated while harvesting pineapple. There are approximately 44,500 ha of pineapple plantation [1,2], generating more than 5.6 million tons of wet pineapple plant residue per year [3]. Due to its growth cycle, pineapple plant is not profitable after two years. The on-farm plant residue must be completely removed to allow plantation of new pineapple. In addition, since the plant residue is a favorable substrate for the larvae of the blood-sucking stable fly (*Stomoxis calcitrans*), the exponential growth of the stable fly population places a serious health threat to nearby animal farms, particularly cattle farms, as it can decrease milk production [4,5]. Therefore, fast removal of the residues is critical to increase land efficiency of pineapple production and protect animals from the proliferation of the stable fly. Current practices of processing the residues are mainly in situ burning of pineapple residues, in situ decomposition of pineapple residues and removal before planting [6,7]. The in situ burning method is quick and easy to carry out but not environmentally sound since burning can lead to ground water and air pollution, and cause fire [6]. In situ decomposition takes a long time to start, which builds up partially decomposed residue, and increases the possibility of pests contamination and fire outbreak [6]. Removal of

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pineapple residues before planting has long term economic advantages if value-added products can be economically produced from the pineapple residues [7].

The pineapple plant residue contains relatively high levels of cellulose, hemicellulose, and soluble mono-sugars (Table 1), which represents a potential feedstock for microbial conversion to produce fuels (i.e., alcohol) and value-added products (i.e., proteins, organic acids, and enzymes) [8–10]. Pineapple residues have been used to produce biogas and fertilizer [11], protease of bromelain [12], growing media for mushroom [13,14], and substrates of fiber etc. [15,16]. Different pretreatment methods have been used to improve ethanol production [17, 18]. The use of pineapple leaf in combination with molasses for ethanol production was investigated by integrating steam explosion pretreatment and separated hydrolysis and fermentation (SHF) [19]. *Saccharomyces cerevisiae* has been widely used in the ethanol fermentation process. However, *S. cerevisiae* cannot utilize pentose from cellulosic materials. *Kluyveromyces marxianus*, another fast growing yeast, has attracted more attention due to its remarkable thermotolerance and capacity to utilize various agriculture residues and substrates, such as xylose and lactose [20–27]. Rocha et al. applied acid pretreatment on cashew bagasse to generate hydrolysate with high glucose and xylose content for *K. marxianus* fermentation [28]. In addition, *K. marxianus* can produce different enzymes (e.g. pectinase and inulinase) which contribute to the biological conversion of agricultural residues into value-added products [29–31]. Moreover, *K. marxianus* has potential as a probiotic yeast and can be used as a food additive for human food and animal feed production [32–35].

Pineapple plantations in Costa Rica are in a ranging from 100 ha to 2000 ha. Technology development must satisfy such operational scales as well as consider local economic and environmental conditions. In response to this technical demand, this study developed a simple and efficient process to convert the pineapple plant residue into bioethanol, spent yeast protein, and fibrous material (Fig. 1). The field pineapple residues (mainly leaves) were first treated by a mechanical extruder to generate juice and the fibrous material. The juice was fermented by *Kluyveromyces marxianus* to produce ethanol and spent yeast as a protein source. The process does not use corrosive chemicals or expensive enzymes to pretreat the residue. A simple mechanical juice extraction and a robust yeast fermentation are two main unit operations in the process, which has technical advantages of good scalability and stable performance. With multiple products of bioethanol, spent yeast protein, and fibrous material, economic performance would be enhanced as well. Therefore, the studied process could be an alternative technology to facilitate sustainable plant residue management for local pineapple plantations in Costa Rica.

2. Material and methods

2.1. Feedstock

Pineapple leaf samples were collected from a pineapple plantation located in San Carlos, Costa Rica. The leaves were collected right after the fruits were harvested. The leaves were cleaned by wiping off the soil in the Laboratory of Agricultural Microbiology at University of Costa Rica and vacuum packed and frozen at −70 °C. The frozen leaves were then shipped to Michigan State University (MSU) and stored at −20 °C.

Table 1
Major components in the pineapple leaves, juice and fibrous material.

Parameter	Whole leaf	Juice	wet fibrous material
% TS	13.8	6.2	51.6
Cellulose (% TS)	22.6	–	36.8
Hemicellulose (% TS)	26.1	–	28.1
Lignin (% TS)	7.3	–	5.1
Crude Protein (%TS)	6.9	14	5.7
Crude Fat (%TS)	3.0	3.5	4.0
Ash (%TS)	6.1	10	1.7
Potassium (%TS)	2.6	3.8	0.6
Total carbohydrates (%TS)	–	72.5	–

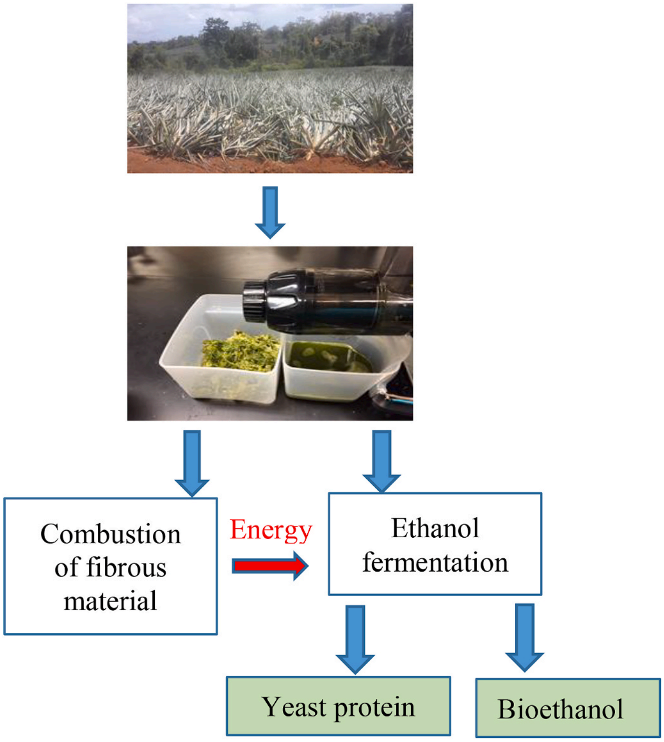

Fig. 1. Process flowchart of pineapple leaf utilization.

2.2. Preparation of pineapple leaf juice

The frozen pineapple leaves were thawed at room temperature (25 °C) and cut into small pieces (3–4 cm each). A juice maker (Omega NC900HDC Juicer Extractor, Florida, USA) was then used to process the pineapple leaves and produce juice and fibrous material. This juice maker includes a juice screen, a homogenizer cone, and five extrusion nozzles, which allows for automatic pulp-ejection and continuous juicing. The juice and fibrous material were collected from two separated ports. The juice was stored at −20 °C, while the fibrous material was dried at 60 °C overnight and then stored at room temperature. The characteristics of the pineapple leaves, fibrous material, and juice were listed in Table 1.

2.3. Aerobic seed cultivation and anaerobic ethanol fermentation

A thermophilic yeast, *Kluyveromyces marxianus* ATCC 12424 obtained from American Type Culture Collection (ATCC, Manassas, VA), was used for pineapple juice fermentation. *K. marxianus* stored in the 15% glycerol solution at −80 °C was first activated on the yeast extract/peptone/dextrose (YPD) medium (ATCC Medium No.1245) for 24 h in a shaker (Thermo Scientific™ MaxQ™ 4000 Benchtop Orbital Shakers, NY, USA) at a temperature of 30 °C and a shaking speed of 200 rpm. One mL of activated yeast biomass solution was then inoculated into 100 mL pineapple juice medium and aerobically cultured in 250 mL flasks at the same conditions for 24 h. During the seed preparation of the aerobic culture, samples were taken at the 0, 3, 6, 9, 12, and 24-h for glucose, xylose and ethanol analyses. The resulted yeast solution was used as the seed for anaerobic ethanol fermentation on pineapple juice at the 10% (v/v) inoculum. Three different culture temperatures (35 °C, 40 °C and 45 °C) were tested to study the impacts of temperature on yeast fermentation. The fermentation was carried out in 250 mL Wheaton bottles for 24 h without shaking. Screw caps with rubber septa were used to seal the bottles. Needles were used to penetrate the septa to release carbon dioxide. During the bioethanol production of the anaerobic culture, samples were also taken at the 0, 3, 6, 9, 12, and 24-h for glucose, xylose and ethanol analyses. The selected temperature was verified to run the culture in 500 mL Wheaton bottles for 24 h.

2.4. Analytical methods

Ethanol, glucose and xylose concentrations were determined using an Agilent 1100 HPLC system equipped with a Bio-rad Aminex HPX-87H analytical column (300 × 7.8 mm, catalog number 125–0140) and a refractive index detector. The mobile phase used was 0.005 M sulfuric acid with a flow rate of 0.6 mL/min and column temperature of 65 °C and pressure of 30 kPa [36]. Total reducing sugars in the fermentation broth were also determined by 3,5-Dinitrosalicylic acid (DNS) method [37]. Total solid content (TS) was analyzed according to APHA [38]. Fiber composition (cellulose, xylan, and lignin) of the pineapple leaves was measured according to the National Renewable Energy Laboratory's (NREL) analytical procedure for determination of structural carbohydrates and lignin in biomass [39]. Gross energy (gross calorific value) of dried fibrous material expressed as calories per gram (kJ/g) was detected using an IKA C2000 basic Calorimeter System (IKA Works, Inc. Wilmington, NC).

3. Results

3.1. Juice and fibrous material preparation from pineapple leaves

The juice and fibrous material were efficiently separated by the juicer. Yields of the juice and fibrous material were 77% and 18% (w/w), respectively, with about 5% loss of the wet pineapple leaves during juice extraction. The characteristics of juice and fibrous material were listed in Table 1. The juice contained 72.5% (TS) of carbohydrates. Glucose and xylose accounted for 60% of the total carbohydrates in the juice. The concentrations of glucose and xylose in the juice were 10.6 g L^{-1} and 16.4 g L^{-1}, respectively. The juice also contained 14% crude protein, 3.5% crude fat, 3.8% potassium and 0.9% of other minerals of calcium magnesium, iron, zinc, and manganese etc. It is apparent that the juice can be a good nutrient source to support microbial growth. The yeast, *Kluyveromyces marxianus* ATCC 12424 that is able to utilize both glucose and xylose, was used by this study to directly ferment the juice to produce ethanol and protein-rich yeast biomass.

The fibrous material is another product from pineapple leaves. It has a relatively high content of cellulose (37%) and hemicellulose (28%) and a low content of lignin (5%). Meanwhile, its energy content is relatively high too. The high heating value (HHV) of the dried fibrous material was 19.4 MJ kg^{-1}, which is close to the HHV of lignin (22.2 MJ kg^{-1} to 28.5 MJ kg^{-1}) and represents a good feedstock for thermal energy generation.

3.2. K. marxianus seed culture under aerobic condition

The productions of yeast biomass and bioethanol and consumptions of glucose and xylose during the seed culture under aerobic condition were shown in Fig. 2. Both glucose and xylose were quickly consumed within 9 h, while the exponential growth of yeast started at the 3rd hour and approximately ended at the 11th hour. The ethanol production was relatively low due to the aerobic condition. The ethanol production reached the highest value of 2% v/v at the 11th hour and then decreased with the increase of cultivation time. The decrease of ethanol may be caused by the vaporization of ethanol during aerobic agitation. The pineapple juice has 9 g L^{-1} crude protein, 45 g L^{-1} carbohydrates and 6 g L^{-1} minerals. No other nutrients were added into the culture medium during aerobic cultivation. The data indicated that the yeast grew very well on the juice without additional nutrients. Thus, the juice of pineapple leaves can be used as the sole nutrient medium to support yeast growth.

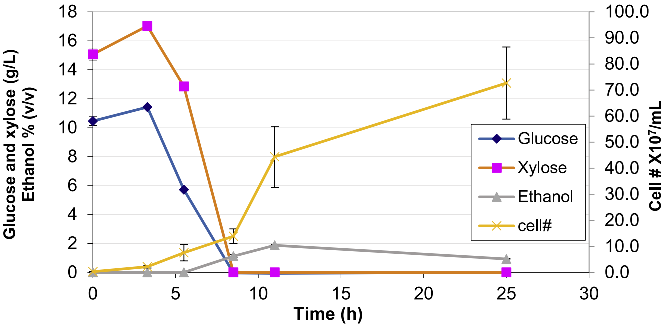

Fig. 2. *K. marxianus* seed culture under aerobic cultivation*.
*: cell numbers were counted using a hemocytometer observed under a light microscope and calculated taking account of dilution factor and counting chamber volume.

3.3. Effect of temperature on anaerobic fermentation of ethanol production

The effect of temperature on ethanol fermentation was investigated. As shown in Fig. 3, the sugars were completely consumed in 12 h by the yeast under three temperatures (35 °C, 40 °C and 45 °C), while the ethanol concentration reached at 3.2% (v/v) at the 12th hour and then leveled off. Statistical analysis indicated that there was no significant difference on ethanol accumulation from the operation temperature range of 35 °C–45 °C (p-value >0.05).

3.4. Verification of the ethanol fermentation

A verification fermentation was carried out in 500 mL Wheaton bottles to verify the fermentation performance and generate spent yeast biomass (Fig. 4). The reducing sugars in the juice were about 27 g L^{-1}. Based on the amount of the reducing sugar, the theoretical ethanol production is supposed to be 1.74% (v/v) in the broth. However, the real ethanol concentration reached to 3.6% (v/v) in the broth (Fig. 4), which indicated that there were certainly other carbon sources (i.e., small cellulose and hemicellulose particles) in the juice contributing to the ethanol production. At the end of the 24-h fermentation, the broth was centrifuged to separate liquid and solid. The liquid portion, 75.6% of the total medium weight, can be further distilled to produce bioethanol. The solid portion includes both yeast biomass and unutilized fiber, which were dehydrated to produce the spent yeast biomass.

3.5. Mass and energy balance

According to the above experimental results, a mass balance was conducted on the proposed conversion process (Fig. 5 and Table 4) based on 1 ha of pineapple plantation. As a perennial plant, 250 metric tons of fresh pineapple plant residues (wet basis, mainly leaves) are removed every other year per hectare. After the extraction, two streams of juice (192.5 tons) and fibrous material (45 tons) were obtained. Juice (containing 6.2% total solids, 14% protein, 10.6 gL^{-1} glucose, 16.4 gL^{-1} xylose, and other soluble and insoluble nutrients) was used as the sole medium for both seed culture and ethanol fermentation. The aerobic seed culture was conducted at 30 °C for12 h. The conditions for the anaerobic ethanol fermentation were 35 °C and 12 h. The mass balance demonstrated that the studied process is able to generate 5.3 m^3 of ethanol, 3.1 tons of dry yeast biomass, and 23.3 tons of dry fibrous fiber from 250 metric tons of the pineapple plant residues.

The energy balance was conducted based on the mass balance results of processing 250 metric tons of pineapple residues (Table 5). The energy values used for the analysis were from the lab results and available commercial bioethanol production data [40]. Two unit operations of leaf extraction and ethanol fermentation require energy inputs of 99 GJ and 133 GJ, respectively. Due to the relatively low ethanol concentration in the fermentation broth (3.6% v/v), the energy input for ethanol production (133 GJ) is bigger than the energy output of ethanol (112 GJ). However, since the process generates a large amount of fibrous material (23.3 tons), the combustion of the fibrous material can generate 271 GJ of energy. This energy can be used to distill ethanol from the fermentation broth and power other unit operations of the process. The total energy output was 446 GJ, which includes fibrous material (271 GJ), ethanol (112 GJ), and yeast biomass (63 GJ). The net energy overall was 214 GJ. The process has a positive energy balance, which can be used to cover other pineapple farm operations such as energy consumption of pineapple cultivation (0.6 GJ per hectare for 2 years of pineapple plantation) [41].

4. Discussion

In this study, we developed a simple, efficient process to convert the pineapple plant leaves into bioethanol, spent yeast proteins, and fibrous material. *Kluyveromyces marxianus* was selected as the yeast species for ethanol production. *K. marxianus* is a thermophilic yeast which grows well in a temperature range of 30 °C–45 °C [42]. Our data also indicated the high ethanol production during the culture temperature of 35 °C to 45C°(Fig. 3). Taking account of the heat generated during fermentation and the ambient temperature of Costa Rica during pineapple harvesting seasons (31 °C [43]), it is especially beneficial for implementing such ethanol fermentation in Costa Rica as cooling control is not necessary. Moreover, *K. marxianus* produces different enzymes such as inulinase [27,44], beta-galactosidase [22,31] and fructanase [26,45], which could contribute to the conversion of juice carbon sources into ethanol. As shown in Fig. 2, glucose and xylose were slightly increased at the beginning of the culture, which may be caused by the enzymatic hydrolysis of oligosaccharides or polysaccharides in the juice. The real ethanol concentration in broth (3.6% v/v) compared with the theoretical calculations (1.74% v/v) from the detectable reducing sugars indicated that the yeast may utilize other carbon sources (such as pectin, oligosaccharides etc.) besides glucose and xylose in the juice (Fig. 4). The mass and energy balance analysis concluded that using the studied process, the pineapple plant leaves from 44,500 ha of pineapple plantation in Costa Rica can produce 93,043, 68,975, and 518,425 tons of bioethanol, spent yeast, and fibrous material per year, respectively. The amount of bioethanol is able to replace approximately 8.51% of transportation fossil fuel consumption in Costa Rica (8.7 million barrels of gasoline 91 and 95 were consumed in 2019 [46]), which could make great contributions towards carbon neutrality as the biggest obstacle to carbon neutrality in Costa Rica is the transportation sector.

Meanwhile, the other products, spent yeast and fibrous material, further strengthened the feasibility of the process. The chemical analysis indicated that the spent yeast had high gross energy (20.3 MJ kg^{-1}) compared with major staple foods. The protein content (23%TS) was higher than major staple foods such as corn, rice, wheat, potato, sweet potato, yams, sorghum and plantain although lower than soybean (Table 2). The contents of minerals showed that the spent biomass is also a good source of potassium (2470 mg/100 g dry matter), magnesium (260 mg/100 g dry matter), and phosphorus (440 mg/100 g dry matter) (Table 2). Moreover, the analysis of essential amino acids in the yeast biomass indicated that it is a good protein source compared with common food source proteins (Table 3). The lysine content (125 mg/g yeast protein) is higher than it is in beef and eggs. Thus, the spent yeast biomass can be used as a nutritious ingredient for human and animal foods.

On the other hand, in this study, the fibrous material was considered as a bioenergy feedstock for combustion to generate electricity and heat to power on-farm ethanol production. The resulted char/ash after

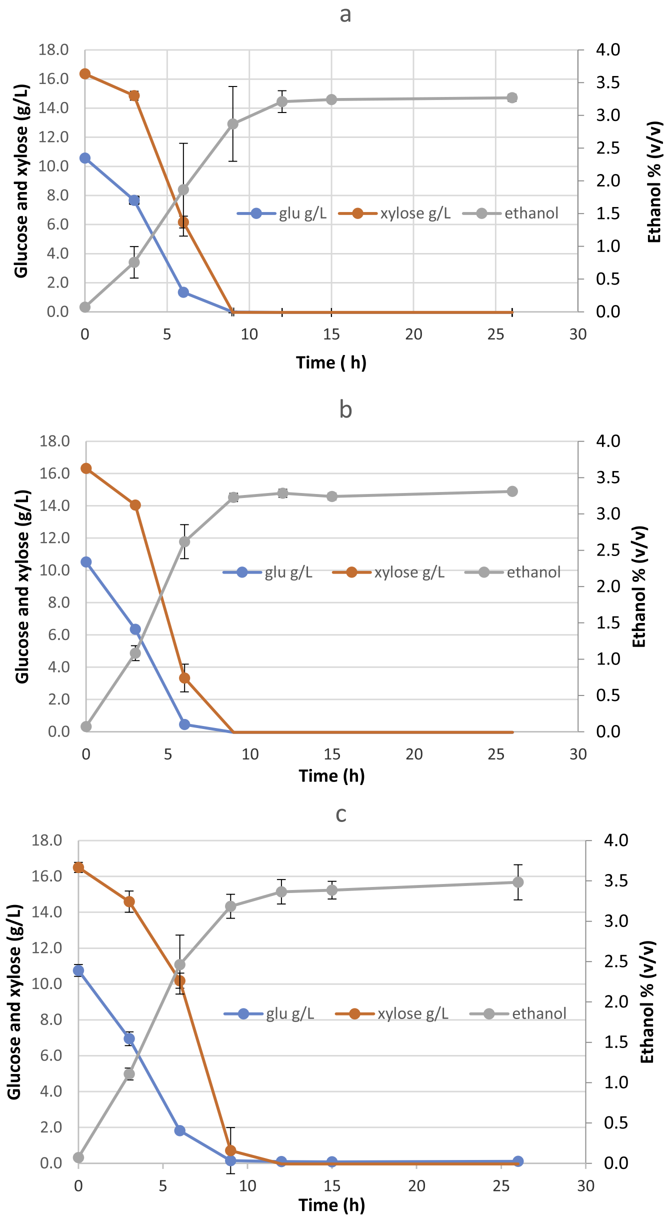

Fig. 3. *K. marxianus* fermentation of pineapple juice under anaerobic condition: (a) 35 °C; (b) 40 °C; (c) 45 °C.

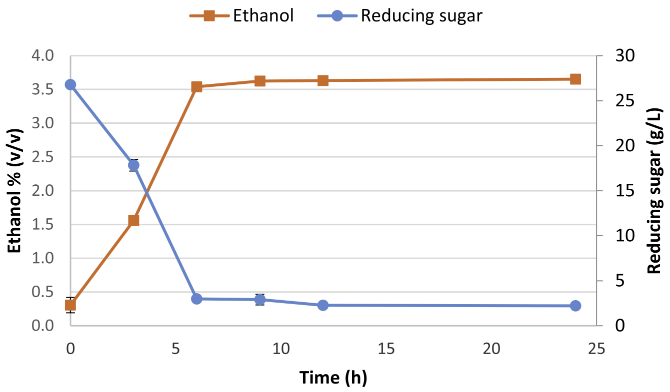

Fig. 4. Verification of ethanol production and sugar utilization of *K. marxianus* fermentation on pineapple juice in 500 mL Wheaton bottles at 35 °C.

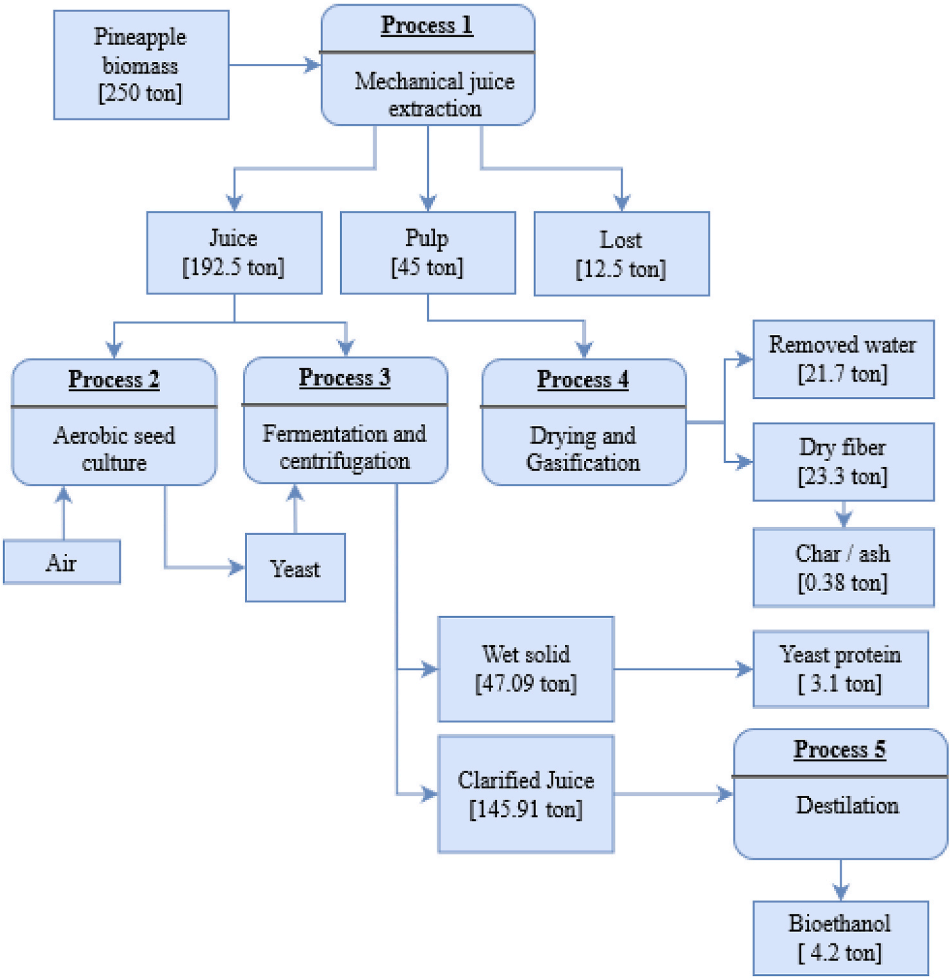

Fig. 5. Mass balance of pineapple waste utilization system based on 1 ha pineapple land [a].
a: Pineapple is a perennial plant. The 250 tons plant wastes per hectare is based on a 24-month plantation.

Table 2
Comparison of nutrient content of the spent yeast with major staple foods per 100 g dry solids[a].

nutrient	Maize (corn)	Rice, white	Wheat	Potatoes	Cassava	Soybeans, green	Sweet potatoes	Yams	Sorghum	Plantain	Spent Yeast[b]
Gross Energy (MJ kg^{-1})	17.0	17.4	15.7	15.3	16.8	19.2	15.7	16.5	15.6	14.6	20.3
Protein (%TS)	10.4	8.1	14.5	9.5	3.5	40.6	7.0	5.0	12.4	3.7	22.8
Fat (%TS)	5.3	0.8	1.8	0.4	0.7	21.3	0.2	0.6	3.6	1.1	11.1
Carbohydrates (TS%)	82.2	90.9	81.6	81.0	95.0	34.4	87.0	93.3	82.4	91.4	16
Fiber (%TS)	8.1	1.5	14.0	10.5	4.5	13.1	13.0	13.7	6.9	6.6	41.6
Sugar (%TS)	0.7	0.1	0.5	3.7	4.3	0.0	18.2	1.7	0.0	42.9	3.3
Minerals											
Calcium (mg)	7.8	31.8	33.3	57.1	40.0	615.6	130.4	56.7	30.8	8.6	390
Iron (mg)	3.0	0.9	3.7	3.7	0.7	11.1	2.7	1.8	4.8	1.7	57.8
Magnesium (mg)	141.1	28.4	144.8	109.5	52.5	203.1	108.7	70.0	0.0	105.7	260
Phosphorus (mg)	233.3	130.7	331.0	271.4	67.5	606.3	204.3	183.3	315.4	97.1	440
Potassium (mg)	318.9	130.7	417.2	2004.8	677.5	1937.5	1465.2	2720.0	384.6	1425.7	2470
Sodium (mg)	38.9	5.7	2.3	28.6	35.0	46.9	239.1	30.0	6.6	11.4	25
Zinc (mg)	2.5	1.2	3.0	1.4	0.9	3.1	1.3	0.8	0.0	0.4	6.4
Copper (mg)	0.3	0.3	0.5	0.5	0.3	0.4	0.7	0.6	–	0.2	2.5
Manganese (mg)	0.5	1.2	4.6	0.7	1.0	1.7	1.1	1.3	–	0.0	18.1
Selenium (μg)	17.2	17.2	81.3	1.4	1.8	4.7	2.6	2.3	0.0	4.3	–

[a] : The 10 major stable foods nutrient content from “Nutrient data laboratory”. United States Department of Agriculture. Retrieved August 10, 2016.
[b] : Analysis was conducted by Forage Testing Laboratory, Dairy One Inc., NY, USA.

combustion rich in potassium (34% in the ash) can be used as a fertilizer for pineapple plantation. Besides its applications of energy feedstock and fertilizer, this fibrous material exhibits excellent mechanical properties and has a large potential to be used for production of multiple products such as a reinforcement in composites and epoxy resins [16] and a raw material for manufacture of yarns and fabrics for textile products [47]. Futhermore, as it is derived from the plant wastes, it could reduce the pressure caused by manufacturing of natural fiber

Table 3
Comparison of amino acid profiles in common protein (mg AA g^{-1} protein)[a].

Amino Acid	Human Milk	Cow Milk	Egg	Beef	Soy	Whey	Biomass of yeast *K. marxianus*
Histidine	26	27	22	34	19	16	26
Isoleucine	46	47	54	48	49	54	65
Leucine	93	95	86	83	82	89	88
Valine	55	64	66	50	48	82	85
Lysine	66	78	70	89	64	88	125
Methionine	42	33	57	40	26	32	38
Tyrosine[b]	72	102	93	80	92	65	46
Threonine	4	44	47	46	38	65	38
Tryptophan	17	14	17	12	14	22	n/a
TOTALS	421	504	512	482	432	513	511

[a] : https://www.hammernutrition.com/knowledge/endurance-library/protein-debate-which-protein-is-best/.
[b] Tyrosine = phenylalanine + tyrosine.

Table 4
Juice and pulp preparation and ethanol production from pineapple leaves harvested from 1-ha plantation.

Pineapple field	One hectare
Total fresh leave amount (ton)	250
Juice (ton)	192.5
Pulp (ton)	45
Lost (ton)	12.5
Juice yield (%)[a]	77.3 ± 5.3
Wet fibrous material yield (%)[a]	18 ± 3.5
Lost (%)[a]	4.7 ± 3.2
Ethanol fermentation on juice	
Liquid yield from juice after centrifugation (% w/w)	75.6 ± 1.7
Wet solid yield from juice after centrifugation (% w/w)	24.4
Ethanol (m^3)	5.3
Ethanol (ton)	4.2
Ethanol concentration in fermentation broth (% v/v)[a]	3.6 ± 0.04
Ethanol yield from leaf biomass (%)[b]	1.67
Solid spent yeast biomass (ton)	3.1
Solid spent yeast biomass yield from leaf juice (%)[a]	1.7 ± 0.04
Solid spent yeast biomass yield from leaf biomass (%)[c]	1.24
Energy and char production from fibrous material	
Dry fibrous material (ton)	23.3
Char/Ash (ton)	0.38
Gross energy (MJ/kg dry pulp)	19.4
Heat (GJ)[d]	271
Electricity[d] (MWh)	22.62

[a] Data are average of three replicates with standard deviation obtained from the scale-up test.
[b] Ethanol yield from leaf biomass = Ethanol amount in the fermentation broth/the amount of leaf used to produce the juice for ethanol fermentation x 100%.
[c] Solid spent yeast biomass yield from leaf biomass = dry solid spent yeast obtained from the fermentation broth/the amount of leaf used to produce the juice for ethanol fermentation x 100%.
[d] Assume Electricity yield is 30% and heat yield is 60% from combustion of pulp.

products from forest and agriculture plants.

5. Conclusions

As one of the largest pineapple producers in the world, Costa Rica produces large amounts of both fresh pineapples and pineapple plant residues. This study concluded a simple, efficient utilization process to convert pineapple plant residues into value-added protein, renewable energy, and fibrous fiber. Pineapple leaves were first extruded to produce juice and fibrous material. The juice was fermented by a yeast, *Kluyveromvces marxianus*, to produce ethanol and spent yeast proteins. The mass balance indicated that the annual production of ethanol, spent yeast and fibrous pulp are 93,043 tons, 68,975 tons and 518,425 tons, respectively. The energy balance analysis further resulted in a positive net energy output of 216 GJ per hectare including 112 GJ biofuel, which would significantly contribute to Costa Rican's carbon neutrality goal. Meanwhile, the environmental problems, such as the plague caused by proliferation of stable flies on pineapple leaves, would be also eliminated. It will change pineapple plant residues management strategies from treatment-based approaches to utilization-based approaches. Therefore, the studied process creates a win-win-win solution for the pineapple industry, biofuel refinery, and the environment.

Table 5
Energy balance of ethanol production on 250 tons pineapple plant residues from 1-ha plantation[a].

System components	The studied process (GJ)[a]
Pineapple leaf extrusion	
Energy input	−99[b]
Energy output from the fibrous material	271[c]
Ethanol production from the juice	
Energy input	−133[d]
Energy output from ethanol	112[e]
Energy output from spent yeast biomass	63[f]
Total energy input[g]	−231
Total energy output	446
Net energy	**214**

[a] Energy input is negative. Energy output is positive. Data for the energy balance analysis were from lab-scale experiments. The energy unit is gigajoule (GJ).
[b] Total amount of feed to the proposed process is 250 metric ton fresh pineapple leaves. The dry matter in the feedstock is 13.8%. The total energy input was calculated based on the electricity consumption of the juice extrusion of 394 kJ kg^{-1} leaf.
[c] The gross energy of dry pulp is 19.4 MJ $kg^{-1}$from the lab test. The dry pulp of 23.3 ton is produced from leaf extrusion.
[d] A year-round average temperature of feed is assumed to be 31 °C. The operational temperature for the fermentation is 35 °C. Since ethanol fermentation will generate some heat, not thermal energy is needed during fermentation. The required energy for ethanol production was 31.76 MJ kg^{-1} ethanol that is derived from a reference [29].
[e] The heating value of ethanol is 26.7 MJ kg^{-1}.
[f] The heating value of spent yeast is 20.3 MJ kg^{-1} from the lab test.
[g] The energy input is the processing energy does not include the energy content in the pineapple leaves.

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