Potato Peels and Mixed Grasses as Raw Materials for Biofuel Production

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Abstract—**Biogas and fuel ethanol are renewable energy sources, can be produced from complex organic materials that are decomposed by microorganisms in the anaerobic digestion method. Potato peels (PPs) and mixed *Lolium perenne* and *Dactylis glomerata* grasses were assessed as a potential substrate for biomethanation in a batch method under mesophilic condition (35°C) and ethanol fermentation. The first approach of this work was focused on pre-treatment of PPs using acidic and enzymatic hydrolysis to produce biogas and ethanol fermentation using *Saccharomyces cerevisiae* and *Safbrew S-33*. These experiments proved that enzymatic hydrolysis produced 1.2 g/L of ethanol involved 115 h of fermentation and 665 ml/h of biogas after 451 h of biomethanation, this was more than the outcomes of acidic treatment. The second approach was concentrated on ability of biogas and ethanol production from mixed grasses treated with different acid concentrations that produced 0.16 g/L ethanol over 8 days of fermentation and 500 ml/h of biogas after 13 days of methanation technique. In general, the results pointed out that PPs and combined grasses can be used as potential substrates with raw materials for biogas and ethanol production.**

Index Terms—**Acid hydrolysis, Biogas, Biomethanation, Enzymatic hydrolysis.**

I. Introduction

Biogases present alternative renewable energy and also decrease the dependence on fossil fuel sources (Aziz, et al., 2019). The conversion of complicated organic compounds to biogas is possible due to the cooperated role of many groups of microorganisms inducing hydrolysis, acidogenesis, acetogenesis, and methanogenesis steps (Mulat, et al., 2018). Bioethanol production from lignocellulosic biomass comprises different steps of pretreatment, hydrolysis, fermentation, and ethanol recovery (Tran, et al., 2019). Pre-treatment is a significant step to modify some structural components of biomass that inducing glucan and xylan accessibility for additional hydrolysis processes (Thangavelu, et al., 2016). Lignocellulosic biomass is the most plentiful feedstock with seasonal availability of over 220 billion dry metric ton. Such feedstocks can be cultivated in different environments and utilized to produce renewable energy with the great potential to sequester greenhouse gases, mainly CO_2 from the atmosphere (Phuttaro, et al., 2019). One source of lignocellulosic is grasses despite its rigid structure can be counted as a potential feedstock for biogas generation, due to its low water content and served as counter to other crops, and the fact that it can be grown in non-arable areas (Rodriguez, et al., 2017; Wagner, et al., 2018). Therefore, various methods based on the biodegradation and solubilization of lignin and hemicellulose have been developed to support efficient hydrolysis and promote optimal biogas production from grasses (Li, et al., 2012). On the other hand, waste of potato industry could be an economical carbon source for ethanol fermentation industry, this is due to their high contents of starch, cellulose, hemicellulose, lignin, and fermentable sugars to guarantee use as an ethanol feedstock (Izmirlioglu and Demirci, 2012). Potato peels (PPs) can be degraded by subsequential enzymatic hydrolysis and fermentation utilizing *Saccharomyces cerevisiae* to produce ethanol (Liang and McDonald, 2014).

This work aims to investigate renewable biogas and ethanol sources through the biological route of biomethanation process and ethanol fermentation using various biomass wastes (PPs and mixed grass), to analyze and determine the chemical composition of PPs and fresh mixed *Lolium perenne* and *Dactylis glomerata* grasses, and to investigate different strategies of chosen biomass substrate pre-treatment.

II. Materials and Methods

A. Activated Sludge (Methanogenic Consortium)

The activated sludge used as a standard inoculum in our experiments was supplied from methanogenic reactor for the treatment of water from bioethanol production at the ALMAGEST AD, 2063 Verinsko village Ichtiman municipality, Bulgaria.

B. Substrate Preparation

PPs

Potatoes were obtained from local market and manually peeled. Then, peels were crushed in a blender, to this 120 ml of D.W was added for better homogenization. The liquefied substrate was stored at 4°C (Sanchez and Cardona, 2008).

Grasses

Fresh, perennial ryegrass (*L. perenne*) and cocksfoot (*D. glomerata*) were harvested in the beginning of June 2018 at Studentski grad park, near UCTM University campus in Sofia, fresh grass was first cut to small pieces, then 10 g was weighed to evaluate the dry weight by oven drying at 105°C for 45 min to a constant weight (Sanchez and Cardona, 2008).

Microelements (S3 and S4)

S3: 19.62 mg of $(NH_4)_2 HPO_4$ dissolved in 100 ml of D.W.

S4: To prepare 100 ml of S4, each of the following salts was dissolved in 100 ml of D.W. $MnSO_4.H_2O$ (4.29 mg), H_3BO_3 (1.34 mg), NH_4Cl (104.63 mg), $CaCl_2. 2H_2O$ (63.67 mg), $ZnCl_2$ (0.57 mg), $CoCl_2.6H_2O$ (7.10 mg), KCl (344.14 mg), $CuCl_2.2H_2O$ (0.63 mg), $NaMo_4.2H_2O$ (0.69 mg), and $MgCl_2.6H_2O$ (376.00 mg).

C. Pre-treatment of Substrates

Acid hydrolysis of PPs

Acid hydrolysis of PPs was conducted using two different sources of acidity, which were 0.5 M HCl and 1% H_3PO_4. HCl hydrolysis was performed by digesting 40 g of PPs with 120 ml of 0.5 M HCl, the mixture was autoclaved (121°C and 1 atm for 15 min). In the case of H_3PO_4 hydrolysis, two various methods were carried out. The first pre-treatment was as follows: 30 g of PPs were digested with 120 ml of 1% H_3PO_4. The resulted liquid was heated at 100°C in a water bath for 5 h. The second pre-treatment was directed by digesting 30 g of PPs with 120 ml of 1% H_3PO_4 and the mixture was autoclaved.

During sterilization, the carbohydrates from PPs were degraded into fermentable sugars due to the action of acid hydrolysis (0.5 M HCl and 1% H_3PO_4) at high temperature of 121°C and 1 atm for 15 min. After sterilization, the pH was adjusted to 7.0 with 5 M NaOH.

Enzymatic hydrolysis of PPs

Forty grams of PPs were ground with 59 ml of D.W by a homogenizer, and the mixture was heated in a water bath for 1 h at 90°C. The produced substance was treated with either α-amylase (Novozymes) or amyloglucosidase (Novozymes) and Sanferm in two steps, namely, dextrinization and saccharification.

- Dextrinization process: One milliliter of α-amylase (Novozymes) in a process called liquefaction at 90°C and pH 5.8 for 1 h. Samples were taken every 20 min and subjected to glucose analysis using the 3,5-dinitrosalicylic acid method (Miller, 1959).
- Saccharification process: Achieved by cooling down the mixture to 35–40°C, then hydrated with water to the original volume. One milliliter of amyloglucosidase

(Novozymes), 54 ml of D.W., and 1 ml of Sanferm yield enzyme (Novozymes) were added to the mixture and treated at 45°C and pH 4.7 for 2 h.

Acid hydrolysis of mixed grasses

Acid hydrolysis of combined grasses was conducted using 3.5% or 4% HCl and 1% H_2SO_4. The HCl hydrolysis was achieved by mixing 50 g of fresh grass with 100 ml of D.W., then ground by a homogenizer, 50 ml of either 3.5% or 4% of HCl added, the mixture was autoclaved. H_2SO_4 hydrolysis was carried out by adding 0.5 ml of 1% H_2SO_4 to 50 ml of 3.5% HCl hydrolysate, then the mixture was autoclaved for 45 min at 121°C and 1 atm.

D. Ethanol Fermentation of Pretreated Substrates

Acid hydrolysate (0.5 M HCl) of PPs

Fermentation was carried out by mixing 40 mg of yeast (*Safbrew S-33*) with 2 ml of D.W. The mixture incubated at room temperature for 20–25 min in a 250 ml volume Erlenmeyer flask. Then, 50 ml of acid hydrolysate solution was added to the mixture. The flask was supplemented with tube contains 2 ml of sulfuric acid (H_2SO_4). Fermentation was continued for 5 days, every 24 h, the mass of the flask was measured to find the amount of ethanol g/L production.

Enzymatic hydrolysate of PPs

Hydrolysate solution of enzymatic hydrolysis of PPs was subjected to ethanol fermentation by *S. cerevisiae* in anaerobic condition at room temperature in a 250 ml Erlenmeyer flask. The inoculum was 60 mg of *S. cerevisiae*, 65 ml of enzyme hydrolysis solution, and 2 ml of H_2SO_4. Fermentation was continued for 4 days, every 24 h, the mass of the flask was measured to find the quantity of ethanol g/L production.

Acid hydrolysate (4% HCl) of mixed grasses

Thirty-five milligrams of *S. cerevisiae* powder were added to 250 ml Erlenmeyer flask that contains 40 ml of 4% HCl hydrolysate combined grasses. The pH adjusted to 7; then, the flask was incubated at room temperature supplemented with 2 ml of H_2SO_4 Erlenmeyer flask tube, the fermentation was continued for 12 days (Arapoglou, et al., 2010).

E. Biomethanation of Pretreated Substrates

Acid hydrolysate of PPs

After the pre-treatment of PPs with 0.5 M HCl, hydrolysate solution was subjected to a batch process methanation at 35°C by adding 20 ml of it pH 7 to 500 ml test bottle that previously filled with 300 ml activated sludge.

Enzymatic hydrolysate of PPs

After the treatment of PPs solution (liquefaction stage) by α-amylase and (saccharification stage) by amyloglucosidase and Sanferm. Yield enzyme was subjected to biomethanation process at 35°C by adding 20 ml of the final product to a test bottle containing 5.4 ml S4, 1 ml S3, and 300 ml activated sludge.

Acid hydrolysate of mixed grasses

After the pre-treatment of mixed grass with 4% HCl, 20 ml of neutralized mixed-grass hydrolysate solution (pH 7) was mixed with 300 ml of activated sludge in a test bottle to

perform methanation in a batch process fermentation under mesophilic temperature 35°C.

Biogas analysis

In this analysis, biogas production rate measurements were performed using a liquid replacement system (Mahmoodi, et al., 2018). The biogas composition was analyzed using the absorptive method (Mahmoodi, et al., 2018). A scaled cylinder is filled with 0.5 M of HCl and is reversibly submerged in a container containing the same liquid. A tube connected to a syringe was inserted into the headspace of the test bottle (which contains the activated sludge plus sample), whereas the other end of the tube was placed in the cylinder. At the connection site between the test bottle and the tube, there is a pin, which will be opened when the tube is located at the right spots at both ends. Then, the produced biogas will flow to the cylinder and replace an equal amount of liquid. The volume of the produced gas (Volume 1 in mL) was determined by measuring the height space in the cylinder. To measure the methane concentration, 5 N NaOH were added to the container to increase the pH (above 9). At this underlying condition, CO_2 and H_2S gas are adsorbed, and the volume of the gas is decreased (Volume 2 in mL). Therefore, the measured volume represents CH_4 content in the biogas mixture. It must be remarked that the variations between the first and the second volume (V1–V2) display the CO_2 content of the biogas because H_2S concentration is entirely negligible in the adsorbed gas.

Analytical methods

The dry weight of PPs and fresh mixed grasses was determined by oven drying at 105°C for 45 min to a constant weight. Biochemical methane potential (BMP) was measured (Chynoweth, et al., 1993). The released reducing sugar was measured by a standard method called dinitrosalicylic (Miller, 1959). Quantitative elemental analysis of PPs and mixed grasses for carbon, nitrogen, and hydrogen was measured by the automated Elemental Analyzer EA 3000 Euro Vector in the Central Research Laboratory – UCTM. The chemical oxygen demand (COD) was determined according to the American Public Health Association, 1995. The protein content was defined (Lowry, et al., 1951).

III. Results and Discussion

A. Characteristic of Substrates

PPs

PPs composition (Table I) contains 16.84% dry mass weight, 45.5% carbon, 3.16% nitrogen, 6.49% hydrogen, 0.84 mg/ml reducing sugar, and 10.2 gO$_2$/L COD. According to this data, PPs can be considered as a wealthy medium containing the most nutrients fundamental for microbial growth (Liang, et al., 2014). Furthermore, the richness of this waste with starch can be used as feedstock for ethanol production. However, the low fermentable reducing sugar content makes the fermentation of the raw material not practical. Therefore, initial hydrolysis (acidic or enzymatic) of carbohydrates is necessary (Pathak, et al., 2018).

Mixed grasses

A fresh ryegrass (*L. perenne*) and cocksfoot (*D. glomerata*) have been identified as a promising feedstock candidate for bio-based alternative energy to create biomethane and ethanol. This is due to its high yield of dry mass weight 20.03%. The analysis of blended grasses was as follows: Carbon 43.63%, nitrogen 2.8%, hydrogen 5.83%, and protein 7 mg/ml (Table I). Based on these results, grasses can be identified as a promising feedstock candidate for the anaerobic digestion process to produce biogas and ethanol yield by batch procedure fermentation.

B. Pre-treatment of Substrates

Acid hydrolysis of PPs

Reducing sugar quantity was increased after acid hydrolysis with 0.5 M HCl, the first and second 1% H_3PO_4 treatment by 31.54, 11.47, and 2.45 folds, respectively (Table II). These findings suggest; first, the rate of hydrolysis boosts by increasing acid concentration which can be a consequence of hydrogen ions activity as a catalyst and second, the digesting of PPs by 1% H_3PO_4 at 100°C for 5 h in water bath produces a higher amount of reducing sugars compared with the autoclave method (121°C for 15 min). This is maybe due to the short retention time of the heating solution.

Accordingly, the optimum condition to hydrolyze PPs is utilizing with 0.5 M HCl at 120°C that can liberate high concentration of reducing sugar (26.5 mg/ml). When comparing our results to those of older studies, it must be pointed out that our method led to better values (Arapoglou, et al., 2010). However, even better results are reported by Sheikh, et al. (2016) when adding 0.5% HCl to a dry powder of PPs, then autoclave it at 121°C, for 15 min.

TABLE I
CHEMICAL COMPOSITION OF NATIVE POTATO PEEL AND MIXED GRASSES

Parameters	Potato peels	Mixed grasses
Dry mass weight (%)	16.84	20.03
Nitrogen (%)	3.16	2.8
Carbon (%)	45.5	43.63
Hydrogen (%)	6.49	5.83
Protein content	7.2 mg/ml	7 mg/ml
Reducing sugars	0.84 mg/ml	NA*
COD	10.2 gO$_2$/L	NA

NA*: Not applicable

TABLE II
CHEMICAL COMPOSITION OF HYDROLYSATE POTATO PEELS

Parameters	Hydrolysis by			
	0.5 M HCl	1st 1% H_3PO_4	2nd 1% H_3PO_4	Enzymatic
Reducing sugars before fermentation	26.5 mg/ml	9.64 mg/ml	2.06 mg/ml	14.98 mg/ml
COD	35.74 gO$_2$/l	35.74 gO$_2$/l	35.74 gO$_2$/l	32.68 gO$_2$/l
Protein content	2.8 mg/ml	2.94 mg/ml	1.2 mg/ml	3.49 mg/ml
Reducing sugars after fermentation	1.69 mg/ml	NA*	NA*	1.47 mg/ml
BMP	NA*	NA*	NA*	0.5 1CH$_4$/gCOD

NA*: Not applicable, COD: Chemical oxygen demand, BMP: Biochemical methane potential

Enzymatic hydrolysis of PPs

The degrading ability of the following enzymes to digest PPs carbohydrates and to fermentable reducing sugars was tested; Termamyl α-amylase, amyloglucosidase, and Sanferm. The use of Termamyl α-amylase was released 12.45 mg/ml of reducing sugar that has been used as a substrate for amyloglucosidase plus Sanferm yield enzymes, which, in turn, released 14.98 mg/ml of reducing sugar (Table II). These outcomes demonstrate that the saccharification stage alone is an insufficient step for bioethanol production. However, when carbohydrates complex converted into simple monomers, a better production was obtained. Hence, a preparatory dextrinization stage was required. For this reason, the utility of enzyme combination was necessary for compelling hydrolysis of PPs. These results confirm previous findings by Khawla, et al., 2014; Izmirlioglu and Demirci, 2012; and Sujeeta, et al., 2018.

Overall, our results confirmed that acid hydrolysis by 0.5 M HCl separates 26.5 mg/ml of fermentable reducing sugar and it has higher 35.74 gO_2/L COD compared to enzymatic hydrolysis, which were 14.98 mg/ml of reducing sugar and 32.68 gO_2/L of COD. These variations of production may due to the way of acid treatment that was carried out under a higher temperature of 120°C and utilizing mild acid concentration. However, enzymatic treatment leads to complete hydrolysis of the starch component and is highly substrate specific (de Souza, et al., 2019).

These results showed that both strategies were successfully recovered reducing sugar from PPs. Financially acid hydrolysis was more beneficial that requires severe conditions such as high temperature (120°C) and low pH (4). However, such conditions support the use of expensive corrosive-resistant equipment. Furthermore, they are restricted by the requirements of a neutralization step before fermentation and the generation of inhibitory by-products such as furfural and 5-hydroxymethylfurfural. Hence, enzymatic hydrolysis is more beneficial due to its low toxicity and low corrosion; no inhibitory is formed and requires less energy and mild environmental conditions (Maurya, et al., 2015).

Acid hydrolysis of mixed grasses

Acid pretreatment is likely to be the preferred choice for fresh grass because of the progressed degradation of xylan, which is the crucial component of hemicellulose. The quantity of reducing sugar increased 2-fold using 4% HCl, if we compare it with 3.5% HCl pre-treatment (Table III) which drive us to conclude that the production of glucose can be increased through increasing the concentration of treated acid. A slight increase in reducing sugar production was observed by adding 1% H_2SO_4 to hydrolysate solution of 3.5% HCl. Our results are broadly in line with other studies (Tanangteerapong, et al., 2017; Valva and Tichagwa, 2013).

C. Bioethanol Production from PPs and Mixed Grasses through Anaerobic Fermentation

Acid hydrolysate of PPs

Acidic hydrolysis produced 26.5 mg/ml reducing sugar. The produced sugar had been subjected to fermentation by

TABLE III
CHARACTERISTIC OF ACID HYDROLYSATE OF COMBINED GRASSES *LOLIUM PERENNE* AND *DACTYLIS GLOMERATA* SPECIES

Parameters	Hydrolysis by		
	3.5% HCl	4% HCl	1% H_4SO_4
Reducing sugars	14.98 mg/ml	28.8 mg/ml	16.92 mg/ml
Protein content	7 mg/ml	6.5 mg/ml	6.1 mg/ml
COD	45.8 gO_2/l	45.8 gO_2/l	45.8 gO_2/l
BMP	0.54 1CH_4/gCOD	0.54 1CH_4/gCOD	0.54 1CH_4/gCOD

COD: Chemical oxygen demand, BMP: Biochemical methane potential

Safbrew S-33, and 1.69 mg/ml of reducing sugar remained and fermentable reducing sugars were 24.81 mg/ml. These results indicate that the yeast efficiently utilized most of the reducing sugar as a nutrient source for fermentation. Fig. 1 shows the investment of ethanol generation (g/L) during PPs hydrolysate fermentation. The maximum ethanol produced was 0.4311 g/L after 98 h fermentation, along these lines leveling off to 98 h with a slight diminish from that point. When comparing our results to the previous studies, it shows lower values than those of Arapoglou, et al., 2010 and Hashem and Darwish, 2010.

The low yields of ethanol are likely due to the presence of microorganism inhibitors such as furfural and 5-hydroxymethylfurfurals within the hydrolysate. This is causing slow xylose and hexose consumption during fermentation. Furthermore, *Safbrew* lacks the mechanism to take up pentose sugars as a substrate in acid hydrolysate, which probably contains a high amount of five-carbon sugar (Robak and Balcerek, 2018).

Enzymatic hydrolysate of PPs

The quantity of reducing sugar after enzymatic hydrolysis of PPs was 14.98 mg/ml. The combination of enzymatic hydrolysates solution was directed to fermentation to assess the ethanol generation. After fermentation, the total amount of reducing sugars was 1.47 mg/ml. From the results, it is clear that *S. cerevisiae* produced high quantities of ethanol on PPs (Fig. 1) that were previously treated with α-amylase, amyloglucosidase, and Sanferm. The highest quantity of ethanol production was achieved after 42 h of fermentation (1.2 g/L).

Results indicate that 19.6 g/L of ethanol was produced after 33 h of fermentation using a combination of Termamyl α-amylase and amyloglucosidase (Khawla, et al., 2014). Higher quantity of ethanol was obtained, which was 30.99 g/L after 48 h of fermentation through enzymatic hydrolysis using a combination of 1 ml α-amylase at 95°C for 3 h and 0.2 ml amyloglucosidase at 30°C for 48 h (Izmirlioglu and Demirci, 2012).

This result shows that enzymatic hydrolysis leads to high yield of ethanol generation compared to acidic hydrolysate. It reveals that utilizing *S. cerevisiae* can efficiently ferment both pentose and hexose sugars more than *Safbrew* yeast. The capacity of every microorganism to ferment of reducing sugars, which includes glucose, xylose, arabinose, galactose, and mannose, after hydrolysis of cellulose and hemicellulose are different (Yang, et al., 2014). Our finding on ethanol
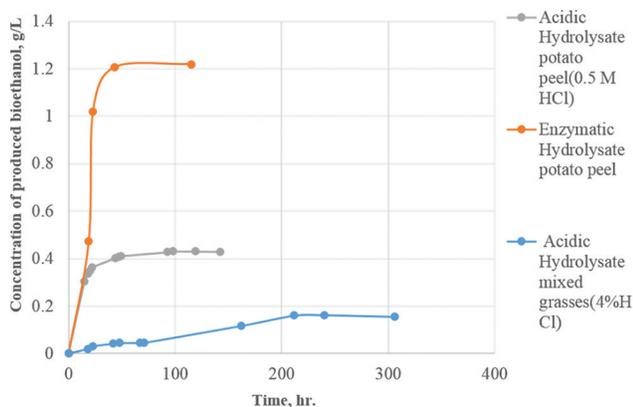
Fig. 1. Kinetics of bioethanol production from hydrolysate potato peels and combined grasses.
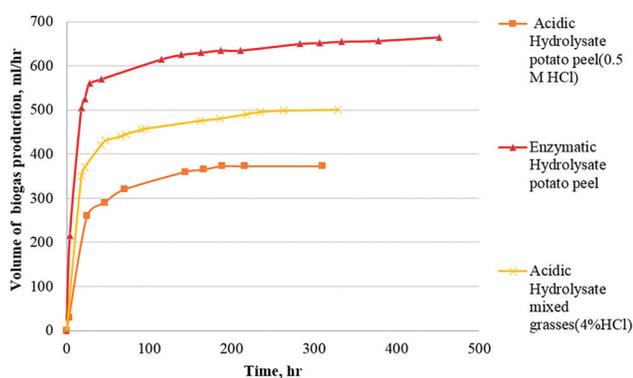
Fig. 2. Batch processes of biogas production from native and pretreated potato peels and mixed grasses.

generation at least provides some critical parameters for ethanol production from PPs which were the enzyme combination, the dose and the residence time of hydrolysis. A high yield of ethanol was obtained after liquefaction, saccharification, and fermentation by *S. cerevisiae*. The method of producing ethanol in this work is promising and shows that PPs of the potato industry treated with a locally produced enzyme, features an appealing feedstock for the bioethanol production. Moreover, bioconversion gives a reasonably priced and secure approach to produce cheap, clean energy and decreases the environmental pollutants caused by PPs.

Acid hydrolysate of mixed grasses

The quantity of reducing sugar was decreased 14.76-fold (from 28.8 mg/ml to 1.95 mg/ml) after fermentation in the presence of *S. cerevisiae*. This suggests that the yeast to grow utilized almost all of the reduced sugar (26.85 mg/ml). In contrast, the production of ethanol was elevated over 8 days of fermentation from 0.05 g/L to 0.16 g/L (Fig. 1). A similar pattern of results with higher values was obtained by Eliana, et al., 2014.

The low level of ethanol production can be returned to many factors, the first is the end product of acid pre-treatment such as acetic acid, furfural, and 5-hydroxymethylfurfural which are yeast growth inhibitors. This problem can be

solved through neutralization and then detoxification of hydrolysates. The second factor can be a result of the inability of *S. cerevisiae* to utilize 5-carbon sugars that include in the mixed grass (Tesfaw and Assefa, 2014).

D. Biogas Production from PPs and Mixed Grasses through Batch Process Methanation

Acid hydrolysate (0.5 M HCl) of PPs

After pre-treatment of PPs with 0.5 M HCl, hydrolysate solution was subjected to a batch process methanation under mesophilic condition 35°C. A rapid degradation rate of organic matter, protein, and carbohydrate with considerable biogas (260 ml/h) was obtained from the 1st day of batch process methanation (Fig. 2). These results suggest a methanogenic phase highly active from the 1st day of anaerobic digestion. After few days, the rate of biogas production steadily increased. The highest average volume of biogas was 373 ml/h after 188 h of methanation process, after that remained constant. This maximum yield of biogas production shows that the highest intake of organic matter was observed on the last day of the methanation process. In comparison with the enzymatic hydrolysate of PPs, low yield of biogas production was obtained from 0.5 M HCl treatment of PPs. The reason can be returned to lack of supplementary microelement, formation of bacterial inhibitor, and production of toxic compounds which, in turn, suppress bacterial growth.

Enzymatic hydrolysate of PPs

The resulted PPs solution from liquefaction and saccharification stages was subjected to biomethanation process at 35°C. The fast degradation rate and intake of organic matter were seen over 24 h of methanation process (Fig. 2) with a considerable amount of biogas investment 215 ml/h, which increased more than 2-fold after 48 h (570 ml/h) of the process.

These results demonstrate two facts; first, the methanogenic phase activates from the 2nd day of anaerobic digestion. Second, the highest quantity of biogas can be acquired after 451 h of methanation process.

Enzymatic hydrolysate of PPs gives higher quantity (2-fold) of biogas production compared to acid hydrolysate, this can be returned to the ability of used enzymes to degrade PPs and make it amenable to bacterial methanogenic fermentation. It is worth mentioning that the presence of microelements in the hydrolysis process has positive effect on enhancing the rate of biogas production, and no inhibitory by-product is formed in enzymatic hydrolysis.

Depending on the obtained data, enzymatic hydrolysis has several benefits compared to acidic hydrolysis. They include works beneath moderate conditions, are biodegradable, enhance yields, decrease energy, water utilization, and the amount of by-products like furfural.

Acid hydrolysate (4% HCl) of mixed grasses

The results of methanization of mixed grasses hydrolysate (4% HCl) of perennial ryegrass (*L. perenne*) and cocksfoot (*D. glomerata*) indicate that the rapid degradation rate of organic matter content was noticed from the 1st day (Fig. 2)

TABLE IV
MEASUREMENT OF BIOGAS COMPOSITION

Substrates	% biogas	% CO_2	% CH_4
0.5 M HCl hydrolysis (PPs)	37.3	4	33.3
Enzymatic hydrolysate (PPs)	66.5	2	64.5
4% HCl hydrolysis (mixed grass)	50	2	48

PPs: Potato peels

with a significant quantity of biogas production (350 ml/h). Biogas yields were raised from the 2nd day to the 9th day of batch process methanation. The highest volume over the 13th day of batch process methanation was 500 ml/h. This is consistent with what has been found in the previous studies by Mahnert, et al., 2005 and Ngumah, et al., 2013.

This high yield of biogas may be due to 4% HCl pre-treatment of lignocellulosic combined grasses, and it could hydrolyze most of the hemicellulose into its element sugars. The following values were determined after acid hydrolysis of combined grasses; reducing sugar (28.8 mg/ml), protein content (6.5 mg/ml), COD (45.8 gO_2/L), and BMP (0.54 1CH_4/gCOD). These results confirm that pre-treatment strategies of grasses for biogas manufacturing enhance and boost up the degradation process attaining high biogas yields.

Biogas composition

The two main components of biogas are CH_4 and CO_2, also can include small quantities of nitrogen, hydrogen, hydrogen sulfide, and water vapor (Mahmoodi, et al., 2018).

The ratio of biogas content was produced by the anaerobic digestion process from PPs and combined grasses substrate (Table IV). Methane production was determined after the biogas passed through a cylinder containing 5 N NaOH solution to increase the pH. This alkaline solution adsorbs CO_2 and other gas from the biogas mixture, and the gas volume is decreased. Therefore, the measured volume represents CH_4 content in the biogas mixture. It was noted that the variation between the biogas and methane volume displays the CO_2 content of the biogas because H_2S concentration is entirely negligible in the adsorbed gas. Determination of biogas composition needs to perform to qualify biogas as natural gas which environmentally friendly and safe for health.

IV. CONCLUSION

The main conclusion that can be drawn from this work is that PPs and mixed grasses are valuable substrates for biomethanation, which can be applied in small and big scales.

Importantly, our results proved on the one hand that the enzymatic treatment of PPs produces ethanol and biogas after fermentation much more than the acidic treatment, which was 1.2 g/L ethanol after 42 h of fermentation and 665 ml/h biogas over 451 h of batch process methanation. On the other hand, this work argued that 4% HCl is the best way to hydrolyze blended grass, which released 28.8 mg/ml of reduced sugar, produced 0.16 g/L ethanol over 8 days of fermentation and 500 ml/h of biogas after the 13th day of batch process methanation.

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