

INVESTIGATION OF POTATO PEELS AS A RAW MATERIAL BASE FOR BIOFUELS PRODUCTION

SHAABAN OMAR^{1,2}, POLINA VELICHKOVA^{1*},
TODOR IVANOV¹, IVO LALOV¹

1 – Faculty of Chemical System Engineering, Department of Biotechnology, University of Chemical Technology and Metallurgy, Sofia, Bulgaria

2 – Faculty of Science and Health, Department of Medical Microbiology, Koya University, Iraq

**Corresponding author: p.velichkova_BT@uctm.edu*

Keywords: biochemical methane potential, biogas, bioethanol, bioenergy, biofuels, potato peels, fermentation

Abstract: This study deals with improvement of the energy potential of potato peels via different types of modification. Proper pretreatment methods can increase concentrations of fermentable sugars, thereby improving the efficiency of the whole bioconversion process. As pre-treatment methods, hydrochloric acid hydrolysis at high temperature and pressure and enzymatic hydrolysis at high temperature using an amylolytic complex were selected. Both modifications have led to an increase in biochemical methane potential of potato peels - 0.33 dm³CH₄/gVSS by 24 % in the acid hydrolysate (0.41 dm³CH₄/gVSS) and by 67 % in the enzymatic hydrolysate (0.55 dm³CH₄/gVSS), respectively. Additionally pretreatments of the potato peels resulted in expanded spectrum of produced biofuels, as it was proved by subjecting the modified substrates to successful alcohol fermentation. The total energy potential of the modified substrates was revealed using combined anaerobic bioconversion process. Such approach allowed us to determine that the energy density of the enzymatically modified potato peels exceeds that of the untreated biomass more than 2.2 times.

INTRODUCTION

The biorefinery applies many hybrid technologies from different fields, such as bioengineering, polymer chemistry, food science and agriculture. Similar to oil-based refineries, where many petrochemicals are produced from crude oil, biorefineries produce many different bio-products from biomass like bioethanol and biogas (Ohara, 2003).

Renewable energy is currently being developed as suitable alternative to conventional fossil energy in many countries of the world. Biofuels are among these alternatives that comprise various forms of fuels, obtainable from biomass. Biofuels are considered reliable substitutes in view of their intrinsic qualities such as renewability, sustainability, biodegradability, non-toxicity, and non-emissions of greenhouse gases (Olayinka, 2015).

Potato peel waste (PPW) is the major waste from the potato processing industry and a potential source of functional and bioactive compounds, including antioxidants, pigments, dietary fibers, vitamins and minerals (Teow *et al.*, 2007). The problem of the management of potato peel waste causes considerable concern to the potato industries in Europe, thus implying the need to identify an integrated, environmentally-friendly solution. Potato peel is a zero value waste from potato processing plants (Israilides *et al.*, 2008). While consumption of potatoes has decreased, processed products such as French fries, chips, and puree have experienced growing popularity. Losses, caused by potato peeling range from 15% to 40% of their mass depending on the procedure applied, i.e. steam, abrasion or lye peeling (Scieber *et al.*, 2001).

The PPW contains sufficient quantities of starch, cellulose, hemicellulose, lignin and fermentable sugars to warrant its use as an ethanol feedstock. Starch was traditionally hydrolyzed by acids, but the specificity of the enzymes, their inherent mild reaction conditions and the absence of secondary reactions have led to the widespread use of amylases as catalysts in this process. Enzymes possess numerous advantages compared to acidic hydrolysis because they work under mild conditions, are biodegradable, improve yields, reduce energy, water consumption and the amount of by-products (Arapoglou *et al.*, 2010). The strategy for the use of enzymes in the production of bio-ethanol from starch includes two stages: liquefaction and saccharification. In liquefaction, α -amylases are used to decrease viscosity in the slurry or produce dextrans. In saccharification the enzymes use dextrans to make glucose.

In this paper we investigate different types of potato peels hydrolytic pre-treatments in order to optimize their full energy potential as well as to expand the spectrum of the possible biorefinery products.

MATERIALS AND METHODS

Substrate preparation

The potatoes (obtained from a local market) were manually peeled in the lab. Twenty grams of potato peels were crushed into a blender with 60 ml water (for better homogenization). The solution was stored at 4°C until use.

Methanogenic consortia

Methanogens were obtained as activated sludge from a factory for bioethanol “Almagest”, Ihtiman, Bulgaria.

Analytical methods

The moisture was determined by oven drying at 105°C to a constant weight. Protein was estimated by Lowry method (1951). Reducing sugars were determined as glucose by using dinitrosalicylic acid (DNS) reagent by the method described by Miller, 1959. Mono-, disaccharide compositions and furfural content were determined by HPLC. Volatile suspended solids (VSS) were determined according to APHA, 1992. Gas production rate measurements were performed using a manual constant pressure liquid displacement system. The biogas composition was estimated using the absorptive method by Lalov et al., 2015. The elemental analysis of nitrogen, carbon and hydrogen was performed with an automated EuroEA 3000 analyzer. The amount of produced bioethanol was determined using an enzymatic ethanol assay kit (Megazyme) by UV-method at a wavelength of 340 nm.

Acidic hydrolysis

In the present study HCl was used to achieve acidic hydrolysis. Hydrochloric acid is usually used for complete hydrolysis of carbohydrates of plant origin to simple reducing sugars, with no adverse effects on the material. Forty grams of PPW, containing 83% moisture and 6.8 g dry matter (17%) was added together with 120 ml HCl 0.5 M to a 250 ml Erlenmeyer flask with fermentation trap. The mixture was sterilized at 121°C for 15 min. During sterilization, the carbohydrates from potato peel were transformed into fermentable sugars due to acid hydrolysis. After sterilization the pH was corrected to 7.00 with 5 M NaOH.

Enzymatic hydrolysis

Potato peels (40 g) were crushed in a blender with 59 ml distilled water. The solution was heated to about 90°C and 1 ml solution of α -amylase Lp Hera by Novozymes (liquefaction process) was added. The heating was continued for 1 hour and samples were taken every 20 minutes and were subject to glucose analysis using the 3,5-dinitrosalicylic acid method, described by Miller (1959). The solution was then cooled to 35-40°C, hydrated with water to the original volume and 1 ml of amyloglucosidase solution, Saczyme Plus 2x by Novozymes and a mixture of Sanferm Yield enzymes by Novozymes (saccharification process) were added. The solution was stored in a refrigerator at 4°C.

Ethanol fermentation

Starch hydrolysates, obtained by both enzymatic and acidic hydrolysis and native potato peels were subjected to ethanol fermentation by *Saccharomyces cerevisiae* under anaerobic agitated conditions in a 250 ml Erlenmeyer flask with fermentation trap. Forty milligrams of lyophilized yeast were hydrated with 2 ml of distilled water at a temperature about 30°C for 20-30 minutes. They were then added to 50 ml of an aqueous solution of native potato peels or derived

therefrom hydrolysates. The conditions of process were: temperature around 25 °C, fermentation time 4-5 days (or lack of new bioethanol produced).

Determination of biochemical methane potential (BMP) by biogas production

Biochemical methane potential was determined by the procedure described by Velichkova et al., 2017. Ten grams of potato peels were placed as a substrate, crushed and diluted with distilled water to a volume of 20 ml and 20 ml of each potato hydrolysate. All substrates were previously neutralized to pH 7.0 with 5N NaOH.

RESULTS AND DISCUSSION

Characteristics of potato peels

In order to characterize this industrial waste as a substrate for a bio-refinery platform and to study its full energy potential, a number of analyzes were carried out. The results are summarized in Table 1.

Table 1 Main characteristics of potato peels

Parameter	Value
Reducing sugars, g/kg	4.36
Protein, g/kg	6.12
Dry weight, g/kg (% dry weight)	152.8 (15.28)
Moisture content, % dry weight	84.72
Ash, %	1.92
Organic content, gVSS/kg (% dry weight)	133.6 (87.43)
Nitrogen, % dry weight	3.16
Carbon, % dry weight	45.50
Hydrogen, % dry weight	6.49
D-cellobiosis, g/kg	0.03
D (+) xylose, g/kg	0.016
D (+) mannose, g/kg	1.74
D (+) glucose, g/kg	-
Furfural, g/kg	-

The results are in good correspondence with those reported by other studies (Arapoglou et al., 2010; Schieber, Saldana, 2009). The solid waste sample has a relatively high organic content (133.6 gVSS/kg), with ash content being negligible (below 2%). The results also show that the content of mono- and disaccharides is insignificant, which in turn means that they are still organized as polymers. This fact naturally implies the need for different types of pre-treatment aimed at hydrolyzing polymer compounds and increasing the “fermentability” of the material.

Modification of feedstock

A number of hydrolysis processes under different conditions (type of catalyst, temperature, pressure) was investigated. Most promising results were obtained

by hydrolysis with HCl at high temperature and pressure (PPH_{HCl}) and hydrolysis with an enzymatic amylolytic complex at high temperature (PPH_{enzyme}). The highest amount of reducing sugars was obtained under these conditions: 26.5 and 36.8 g/l respectively. Both types of modified substrate were further subjected to analysis in order to determine the individual sugar content and the results of these analyzes are presented in Table 2.

Table 2 Sugar content of two types of potato peels hydrolysates

Parameter	PPH_{HCl}	PPH_{enzyme}
D (+) glucose, g/l	12.081	23.644
D-cellobiosis, g/l	3.943	0.156
D (+) xylose, g/l	1.308	1.364
D (+) mannose, g/l	0.392	0.01
Total sugars, g/l	17.724	25.174

No presence of furfural, galactose and arabinose was detected in the studied hydrolysates. The results demonstrated the higher content of fermentable sugars in acidic and enzymatic hydrolysates, especially glucose (respectively 12.081 and 23.644 g/l). The increase in the fermentable sugars content of both hydrolysates is an objective criterion both for improving the overall energy potential of the substrate and for expanding the spectrum of the products obtained from the modified feedstock. The main differences in the characteristics of the two substrates were expressed on the one hand in the doubly higher glucose content of PPH_{enzyme} compared to PPH_{HCl} and on the other hand in better degradation of the cellulose and hemicellulose compounds in PPH_{HCl} which is evident from the content of cellobiose (respectively 3.943 and 0.156 g/l) and mannose (0.392 and 0.01 g/l).

Further, the increase of the energy potential of modified substrates was explored, as well as the possibility of expanding the spectrum of obtained biofuels.

Biogas production and BMP determination

The study of the effect of different types of pretreatment of substrate on the increase of its total energy potential consisted of two separate stages. Comparative study of the BMP of modified and native potato peels were carried out as a first stage. A comparison between BMP of modified and native substrates is shown on Fig. 1.

Obviously, both types of pre-treatment lead to an increase in biochemical methane potential of the studied raw material. This increase reached 67% in the enzyme-modified substrate and only 24% in the acid-modified substrate. At the same time, an increase in the rate of biomethanation has been observed. Such observation more fully expresses the importance of pre-hydrolysis for the increase

of the overall efficiency of biomethanation process. In this case the highest rate of the process was recorded in the acidic hydrolysate, where the biomethanation was completed for 195 hours, while in the enzymatic hydrolysate for the same period the conversion rate reached 88 % and in the untreated potato peels - 56 %.

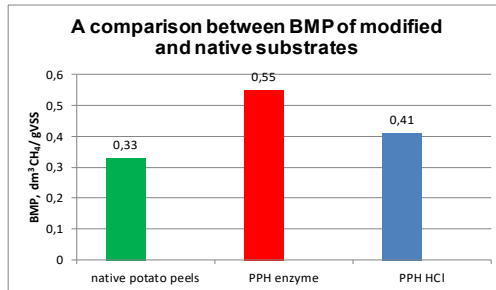


Fig. 1 Biochemical methane potential of a native and modified potato peels

These facts are consistent with the earlier observations that acidic hydrolysis is more complete, affecting both the starch and lignocellulosic contents in substrate while the enzymatic treatment in turn leads to a more complete hydrolysis of the starch component. The significant increase in biotransformation rate is obviously due to the shortened step of hydrolysis of biomass as a result of the applied pre-treatment. This stage is usually speed-limiting, and often not complete because of the complex character of the biomass.

Co-generation of liquid and gaseous biofuels production

Expansion of the spectrum of biorefinery products that can be obtained from the studied raw material as a result of its modification has been investigated by subjecting the unmodified and modified substrates to an alcoholic fermentation process. This process was used as a criterion for enhancing the fermentability of the raw material as a result of its modification. Figure 2 shows a comparative graph between the production of bioethanol from potato peels and pre-treated potato peels. Analysis of the residual fermentable sugars content in the medium showed that in all three cases they were almost exhausted, indicating the effective course of the process and consequently the lack of inhibiting substances in the raw material both before and after its modification.

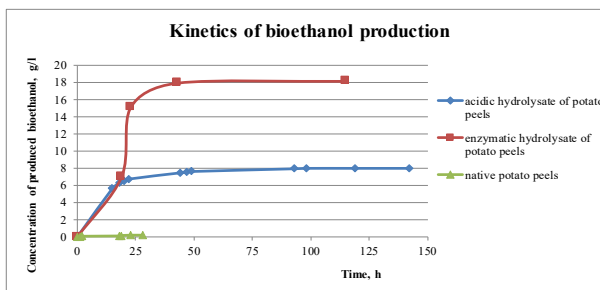


Fig. 2 Kinetics of bioethanol production from native and modified potato peels

Maximum experimental bioethanol yields was 0.003, 0.18 and 0.42 g/gVSS (or 0.179, 8.02 and 18.185 g/l) respectively for native potato peels, acidic hydrolysate and enzymatic. From the results it can be concluded that the enzyme pre-treatment enables the optimal generation and utilization of fermentable sugars.

These results are in good correspondence to those obtained by other authors. For example, according to Arapoglou et al. (2010), enzymatic pre-treatment is better than acidic. The ethanol yields as well as sugar conversion degree both in the present study and in cited paper exceed 90 % of the theoretical values (for enzymatic and acidic pre-treatment). This is another proof of better conversion of sugars by enzymatic treatment of that type of waste products.

The total energy potential of a given raw material can objectively be expressed as a sum of the energy content (the energy density multiplied by the amount of the fuel) of all biofuels obtained from its processing. For this reason, at the last stage of this study, the possibility of increasing the energy potential of acidic and enzymatically modified potato peels was studied by applying sequential biotechnological processes (alcohol fermentation and biomethanation) transforming raw materials into energy carriers.

After fermentation of pre-treated peels, ethanol was removed and the residue was subjected to methanation. Negligible bioethanol production was observed during fermentation of native potato peels so they were not subjected to a combined process.

The kinetics of biomethanation processes of the two non-alcoholic residues are presented in Figure 3. Based on the results obtained from the two periodical processes as well as the data for the organic content of the enzymatic and hydrochloric acid residues, respectively 16.85 mgVSS/ml for PPH_{enzyme} after fermentation and 18.14 mgVSS/ml for PPH_{HCl} after fermentation, their biochemical methane potential was also calculated.

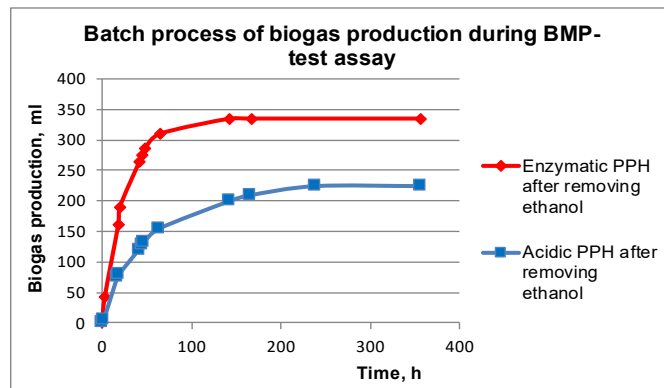


Fig. 2 Batch processes of biogas production from native and modified potato peels after removing ethanol from solutions

The results for the biochemical methane potential of the two substrates (respectively 0.31 dm³CH₄/gVSS for the acidic and 0.50 dm³CH₄/gVSS for the enzymatic residues) allowed the quantification of their full energy potential as a result of their processing in two successive anaerobic processes. Data on the evaluation of the energy potential of modified and untreated potato peels obtained in one-step (biomethanation) or two-step (alcohol fermentation and biomethanation) energy biotransformation of substrates are summarized in Table 3.

The hydrochloric acid modification of the potato peels allows an increase of the energy potential of the raw material by about 24%, as can be seen in Table 3, which however does not change in its combined treatment. The later only contributes to expanding the spectrum of generated fuels. On the other hand, the energy potential of enzymatic modified potato peels in their two-stage conversion exceeds more than 2.2 times that obtained for the unmodified raw material.

Table 3 Estimated total energy content of modified and non-treated potato peels

Substrate	Estimated energy density	
	bioconversion	bioconversion
Potato peels	11,99 kJ/gVSS	-
PPH _{enzyme}	19,98 kJ/gVSS	26,67 kJ/gVSS
PPH _{HCl}	14,93 kJ/gVSS	14,17 kJ/gVSS

CONCLUSIONS

One of the most serious challenges to the development of biorefinery platforms is the determination of the real energy potential of the raw material. The present study deals with the possibilities of increasing the energy potential of potato peels. It has been found that the two most successful substrate pretreatment methods are hydrochloric acid hydrolysis at high temperature and pressure and enzymatic hydrolysis at high temperature using an amylolytic complex. The biochemical methane potential of the substrate increased by 24 % due to the acid modification and by 67 % in the enzyme pre-treatment. For the determination of the full energy potential, a combination of two consecutive anaerobic processes (alcohol fermentation and biomethanation) was applied. It was found that the combined process only leads to the expansion of the spectrum of the produced biofuels in the acid modified substrate, whereas in enzymatically modified potato peels it allows 2.2 times the increase of the energy potential.

Acknowledgements: This work was supported by scientific project №11755 with UCTM- Sofia, Bulgaria

CONFLICT OF INTEREST: The authors declare no existing conflict of interest.

AUTHORS CONTRIBUTION STATEMENT: PV, TI and IL created the conception and designed the study. SO, PV, TI and IL did the experimental work and analyzed the data. PV wrote the manuscript. TI and IL made the critical revision of paper.

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