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Assessment of the Impact of Pretreatment of Spent Coffee Grounds with Diluted Sulfuric Acid on the Efficiency of Methane and Lactic Acid Fermentation

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ABSTRACT

Lignocellulosic materials are composed of three major biocomponents such as cellulose, hemicellulose and lignin, which form a compact lignocellulosic complex. Characterized by high caloric content, lignocellulosic biomass, including coffee grounds, is a valuable energy source that can be efficiently used in various bioconversion and biotransformation processes. Due to the high consumption of coffee in the world, there is an increasing amount of coffee grounds, which is a rich waste and at the same time a valuable secondary raw material, and its use fits perfectly into a closed-loop economy. Coffee grounds biomass contains polysaccharides, mainly mannans, proteins, lipids, polyphenols, which will allow the development of different biorefinery strategies, the creation of new value-added products with reduced waste generation. The research describes the pre-treatment of coffee grounds with dilute sulfuric acid to evaluate the effect of acid concentration, hydrolysis time on biogas yield, including methane and lactic acid biosynthesis during anaerobic fermentations. A yield of 381.12 mL of CH_4/g-VS methane was obtained, accounting for 72.48% of the total biogas composition. It was found that the most efficient sample in terms of substrate pre-treatment for lactic acid biosynthesis was coffee grounds after 90 min hydrolysis with 1.5% H_2SO_4 at 121 °C.

KEYWORDS: spent coffee grounds, methane fermentation, lactic acid, Fourier transform infrared spectroscopy

Introduction

The world's second most traded commodity after oil is coffee, which is an agro-food commodity. The coffee processing process generates numerous wastes that have significant potential for use as valuable secondary raw materials. By-products include husks, peel, pulp, which together account for about 45% of the coffee fruit. Other by-products, including mucilage and parchment, are

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also generated during the industrial processing of coffee. As a result, the total amount of waste generated during coffee fruit processing exceeds 50% of its weight (Tsai *et al*., 2012). During the coffee brewing process, additional waste is generated, namely spent coffee grounds (SCG). According to the literature, one ton of green coffee beans produces 650 kg of spent coffee grounds (Murthy *et al*., 2012). Production waste and spent coffee grounds have significant potential in the context of the bioeconomy and sustainable development, including the pursuit of a closed-loop, cradle-to-cradle economy. The bioeconomy is a progressive economic model that deals with the production (using biochemical and biophysical processes) of environmentally friendly renewable biological resources based on the animate world (animals, plants, fungi, *Protista*, *Monera*), biomass and the transformation of these bioresources, as well as the value-added waste generated as a result. Closed-loop economy model on maximizing the use of raw materials and products so as to keep them in circulation for as long as possible. This is in contrast to the linear economy model. In terms of the bioeconomy, which is an integral part of the CE, the use of organic matter like spent coffee grounds (especially lignocellulosic biomass) for the production of biofuels, biopolymers, biosorbents, antioxidants, biochar is not only a way to obtain energy and value-added products, but also a strategy for sustainable development. These processes will make it possible to reduce the carbon footprint (Bevilacqua *et al*., 2023).

Spent coffee grounds are a lignocellulosic material that contains cellulose and hemicellulose in its structure. Spent coffee grounds (SCG) are rich in various polysaccharides, with mannans dominating the composition. Specifically, SCGs consist of 46.8% mannan, 30.4% galactose, 19.0% glucose and 3.8% arabinose, highlighting their complex polysaccharide structure (Mussatto *et al*., 2011). Agro-food waste such as spent coffee grounds contain 13.6% (w/w) of proteins. The protein content of spent coffee grounds exceeds that of coffee beans and fruit due to the accumulation of components not extracted during the manufacture of instant coffee. This can lead to an overestimation of protein content in SCG due to the presence of other nitrogenous compounds such as 1,3,7-trimethyl-xanthine, trigonelline, as well as free amines and, after protein degradation, amino acids (Delgado *et al*., 2008).

In the context of a circular economy, coffee grounds are becoming an important subject of scientific research and practical efforts to find effective solutions for their management. Accordingly, this article aims to increase knowledge about the benefits of using spent coffee grounds and other forms of managing these waste products.

Materials and Methods

Substrate and Inoculum

The biological material used in the study was coffee grounds from *Coffea arabica* L. The coffee grounds were dried at 55 °C for 24 hours, and the ground test material was stored at room temperature with silica gel (25 °C) for further analysis. The chemical composition of the ground coffee was as follows: (TS) dry matter 919.60 ± 4.91 g/kg and (VS) organic dry matter 886.65 ± 3.11 g/kg. The water-soaked liquid containing coffee grounds contained COD 1510 ± 15 mg/dm^3 O_2 and (RS) reducing sugars 10.95 ± 0.08 g/l $C_6H_{12}O_6$ and pH 5.90 ± 0.05.

The inoculum used was digested excess sludge with chemical composition of (TS) 20.02 ± 0.03 g/kg and (VS) 3.42 ± 0.19 g/kg.

Chemical pretreatment of biological material

Two different concentrations of H_2SO_4 were used: a 1.0% and a 1.5% solution in 250 ml flasks with an S:L (solid:liquid) ratio of 1:10. Each of the chemical treatments was carried out both at room temperature and at 121 °C (autoclave). Hydrolysis was performed for 60 and 90 minutes. After autoclaving, the mixture was cooled to room temperature and the solid fraction was separated from the filtrate. It was stored at –7 ± 2 °C until later analysis.

HPLC compound quantification

For carbohydrate analysis, determinations were performed at a mobile phase flow rate of 0.6 ml/min, temperature of 60–70 °C and eluent – dilute acid. Samples were separated on a Hi-Plex H column (7.7 × 300 mm, 8 µm, Agilent Technologies).

Fourier Transform Infrared Spectroscopy Analysis

A Nicolet 6700 FT-IR instrument and the OMNIC analysis program were used for the infrared spectrometric studies. For each measurement, spectra were obtained in the range of 4000 to 400 cm^{-1}. The instrument was cleaned with isopropanol between analyses.

For Methane Production

After careful analysis of various pre-treatment methods, optimal conditions were identified for use in the inoculum-based methane fermentation process. The process was carried out under mesophilic conditions, maintaining a constant temperature of 35 ± 1 °C. The inoculum and the analysed sample were combined in a 2:1 ratio, taking into account the dry organic matter. The weight of the inoculum was 500 g (Figure 1). Mixing of the media contents in the reactors was done manually. Methane was determined with a gas chromatograph.

Culture conditions for Lactobacillus plantarum and Lactobacillus brevis

They were cultured in liquid MRS medium and incubated at 37 °C for 24 hours. Initial cell concentrations for *Lactobacillus plantarum* and *Lactobacillus brevis* were 2×10^9 CFU/ml.

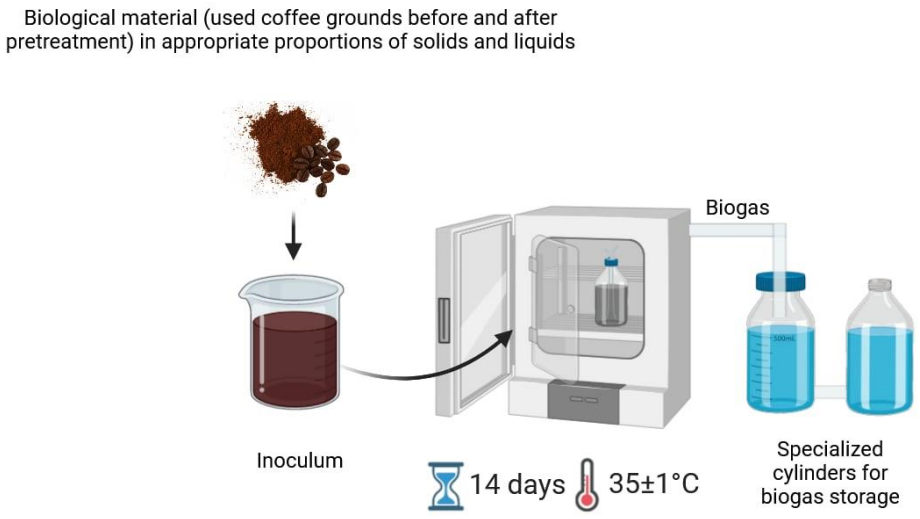

Figure 1. Laboratory set-up.

Lactic fermentation

The experiment was performed in 3 variants: (A) inoculated with *Lactobacillus plantarum*, (B) inoculated with *Lactobacillus brevis*, (C) a consortium of *L. plantarum* and *L. brevis*. The fermentation process was carried out in the SHF system, 5% (v/v) inoculum for each strain was added simultaneously to the suspension of hydrolysed biomass in a ratio of 1:1. For variants (A) and (B), 5% (v/v) inoculum of *L. plantarum* or *L. brevis* was added to the fermentation medium. Samples were incubated at 37 °C for 72 hours. Batch fermentation was performed on a laboratory scale in a 2-liter bioreactor. The anaerobic fermentation process was maintained at a pH of 6.0 ± 0.4 with an optimal process temperature of 37 °C.

Results and Discussion

Products for the pretreatment of spent coffee grounds from Coffea arabica L.

After chemical pretreatment at different parameters of hydrolysis time and temperature, the wet biomass was separated from the liquid. It was shown that the content of dry organic matter decreases with increasing concentration of sulfuric acid (VI) and hydrolysis time. From the results of the chemical oxygen demand by the dichromate method, it can be concluded that the existence of a negative correlation between the analysed variables is indicated by correlation coefficients approaching –1. This confirms that there is a strong negative relationship between the variables. This means that an increase in the concentration of COD in the filtrate is associated with a decrease in the concentration of COD in the biomass. Such a relationship indicates a process of translocation of organic compounds from the biomass to the liquid phase. More favourable results were obtained for hydrolysis at 121 °C. There were more organic compounds in the filtrate that were oxidized. In contrast, there were fewer organic compounds in the biomass after both chemical pretreatment and biological treatment. It is concluded that sulfuric acid (VI) partially hydrolyses hemicellulose to simple sugars and leads to improved cellulose availability by degrading cell structures. The hemicellulose fraction, due to its structure, is a heterobiopolymer that is extremely susceptible to the action of acids, leading to its effective degradation to monosaccharides (Dziekońska-Kubczak *et al.*, 2016). The highest COD value was obtained for the filtrate, where 33.412 mg/dm^3 was obtained for coffee grounds after 90 min hydrolysis with 1.5% H_2SO_4 at 121 °C. This is an increase of 104.21% with respect to the control sample (90 min, 121 °C). When the acid concentration is increased from 1.0% to 1.5%, there is an increase in reaction efficiency of 16.93% after 90 minutes of hydrolysis at 121 °C.

Chemical pretreatment studies have shown that high temperature is not a major factor in the degradation of the lignocellulosic complex, whereas the use of dilute sulfuric acid (VI), according to the analysis, leads to the depolymerisation of hemicellulose (xylose, arabinose and galacturonic acid formed by the oxidation of galactose) and the decrystallisation and depolymerisation of cellulose, leading first to cellobiose and then to glucose. In addition, with increasing temperature, acid concentration and hydrolysis time, there are inhibitors derived from the degradation of pentoses (furfural to formic acid) and hexoses (HMF to formic acid) and from the hydrolysis of acetyl groups of hemicellulose acetic acid (Domański *et al.*, 2016; Janković *et al.*, 2023).

The filtrate was analysed for reducing sugars. The highest value was obtained for coffee grounds after hydrolysis with 1.5% H_2SO_4 at 121 °C for 90 min. 34.02 ± 0.11 g/l $C_6H_{12}O_6$ was obtained, which is an increase of 1128.16% with respect to the control sample, while increasing the acid concentration from 1.0% to 1.5%, there is an increase of 11.55% in the amount of reducing sugars after 90 minutes of hydrolysis at 121 °C. High temperature plays a key role in increasing the yield of reducing sugars, especially when dilute sulfuric acid (VI) is used. This process contributes to the precipitation of some lignin and the efficient hydrolysis of hemicellulose to simple sugars (Galbe and Zacchi, 2012).

Because the results obtained showed a significant amount of organic compounds in the filtrate, which correlated with the results of organic dry matter, which decreased with increasing temperature, hydrolysis time and sulfuric acid (VI) concentration. The filtrate was subjected to HPLC analysis, which showed that the glucose content obtained after chemical hydrolysis of coffee grounds was 7.29 to 25.72 times higher in comparison with samples of grounds treated with water for 60 minutes (control sample) (Table 1). It was shown that the maximum concentration of cellobiose reaches 0.54 g/L when treated with 1.5% H_2SO_4 solution for 60 minutes at 121 °C, while the lowest reaches 0.02 g/L for coffee grounds after 90 minutes of hydrolysis with 1.0% H_2SO_4 at room temperature. This shows that the amount of cellobiose decreases and the amount of glucose increases with hydrolysis time, sulfuric acid concentration, and process temperature (Saini *et al*., 2015). This indicates that H_2SO_4 contributed to the partial depolymerisation of cellulose homopolymer into glucose monomers.

Table 1. Effect of coffee grounds pretreatment with different concentrations of sulfuric acid

Sample Type: H_2SO_4 Concentration, Hydrolysis Time, Hydrolysis Temperature	Cellobiose [g/L]	Glucose [g/L]	Xylose [g/L]	Arabinose [g/L]	Glicerol [g/L]	Acetic acid [g/L]	Formic acid [g/L]	Furfural [g/L]
1.5% H_2SO_4 for 90 minutes at 121 °C	0.50	2.9	9.94	1.22	0.09	1.21	0.13	0.12
1.5% H_2SO_4 for 60 minutes at 121 °C	0.54	2.5	9.61	1.24	0.08	0.58	0.08	0.05
1.0% H_2SO_4 for 90 minutes at 121 °C	0.52	2.6	9.88	0.7	0.06	0.64	0.10	0.05
1.0% H_2SO_4 for 60 minutes at 121 °C	0.53	2.6	8.74	0.8	0.06	0.57	0.06	0.04
1.5% H_2SO_4 for 90 minutes at room temperature	0.11	0.39	0.15	0.17	0.04	0.03	0.01	–
1.5% H_2SO_4 for 60 minutes at room temperature	0.07	0.13	0.11	0.12	0.08	0.01	0.01	–

Table 1(continued). Effect of coffee grounds pretreatment with different concentrations of sulfuric acid

Sample Type: H_2SO_4 Concentration, Hydrolysis Time, Hydrolysis Temperature	Cellobiose [g/L]	Glucose [g/L]	Xylose [g/L]	Arabinose [g/L]	Glicerol [g/L]	Acetic acid [g/L]	Formic acid [g/L]	Furfural [g/L]
1.0% H_2SO_4 for 90 minutes at room temperature	0.03	0.05	0.16	0.01	0.02	0.02	0.01	–
1.0% H_2SO_4 for 60 minutes at room temperature	0.05	0.08	0.24	0.01	0.06	0.02	0.01	–
Control sample for 90 minutes at 121 °C	0.04	0.82	0.46	0.11	0.09	0.19	0.08	0.01
Control sample for 60 minutes at 121 °C	0.01	0.63	0.33	0.04	0.06	0.08	0.04	0.01

The highest amount of glucose was for a 1.5% solution of H_2SO_4 for 90 min at 121 °C and was 2.9 g/L, while 0.4 g/L less glucose was obtained for the sample after 60 min of hydrolysis at 121 °C (Table 1). The highest xylose concentration (9.94 g/L) was observed in samples hydrolysed with 1.5% sulfuric acid for 1.5 h at 121 °C. The highest value for lactic acid was obtained for coffee grounds after 90 min hydrolysis with 1.5% H_2SO_4 at 121 °C (3.07 g/L). This is 168.31% higher than the sample at room temperature (90 minutes, 1.5% H_2SO_4) and 194.05% higher than the control sample (90 minutes, 121 °C, no sulfuric acid).

Samples treated with 1.5% sulfuric acid after 90 min hydrolysis at 121 °C yielded the highest inhibitor concentrations of acetic acid (1.21 g/L), formic acid (0.13 g/L), HMF (0.09 g/L), and furfural (0.12 g/L) of all samples tested. In contrast, for samples hydrolysed for 60 min with the same parameters, fewer inhibitors were obtained with 52.11% acetic acid, 41.01% formic acid, 87.05% HMF and 60.41% furfural (Table 1). The obtained inhibitors inhibit the growth of microorganisms responsible for anaerobic fermentation, including methane and lactic fermentation. The resulting increase in acetic acid was due to the hydrolysis of acetyl groups, which are part of the hemicellulose fraction. The concentration of furfural was shown to increase with the concentration of H_2SO_4, which correlates with the content of xylose and arabinose (simple sugars after hemicellulose depolymerisation). The resulting reaction inhibitors and value added products such as HMF and furfural after liquid phase separation indicate that there has been a loss of sugars (pentoses and hexoses). This can affect the yield and quality of anaerobic fermentation (Zabed *et al*., 2016; Janković *et al*., 2023).

Spent coffee grounds before and after chemical treatment were subjected to FT-IR analysis to confirm efficacy. Figure 2 shows the spectra of the different test samples. To further analyse the raw material of coffee grounds, the coffee beans were analysed after grinding. The physical treatment of coffee beans by grinding increases the availability of the substrate surface, which is critical for

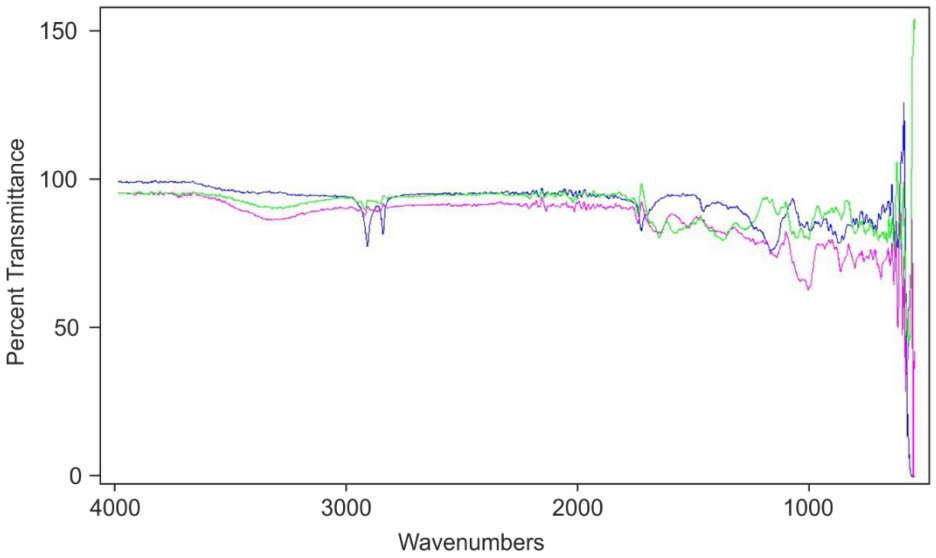

Figure 2. FT-IR spectrum of selected samples after chemical pretreatment. (—) control sample *Coffea arabica*, ground beans, 100% purity.SPA; (—) *Coffee arabica* ground 100% pure, pretreated with 1.5% sulphuric acid (VI), at room temp.SPA; (—) *Coffee arabica* ground 100% pure, pretreated with 1.5% sulphuric acid (VI), at 121 °C.SPA.

further extraction processes. As a result of grinding, the degree of polymerization and crystallization of lignocellulosic materials is reduced, which facilitates access to the structural components of the bean during brewing. When ground coffee is brewed, the extraction process uses conditions analogous to those used in steam explosion and steam pretreatment methods. Both processes are characterized by an intensive thermomechanical treatment, during which hemicellulose and lignin are degraded without the formation of reaction inhibitors, as confirmed by the results of control tests. A significant advantage of the "steam explosion" method is its minimal environmental impact and efficiency in degrading structural polymers, making it an attractive alternative in biomass processing. However, this method requires specialized equipment and strict technical requirements. In the context of home coffee brewing, the process is carried out on a much smaller scale, resulting in coffee grounds as a by-product. These coffee grounds represent a partially processed phytobiomass. In the spectra, characteristic bands for cellulose were observed in the region of 3220–3400 cm^{-1}, corresponding to the bending vibrations of OH groups (Ibrahim *et al*., 2015; Gieparda, 2019). The stretching vibration bands of CH groups were shown at 2916 cm^{-1}. Most of the bands in the range of 1720–890 cm^{-1} were shown at lower intensity, which may indicate that the acid pretreatment process of cellulose leads to a decrease in the degree of crystallinity, an increase in the volume of microfibers, and at the same time intensifies the transformation of the cellulose structure towards an amorphous form (Ibrahim *et al*., 2015). This change may be due to the breaking of hydrogen bonds in the crystalline regions of cellulose, leading to an increase in the mobility of the polymer chains and an increase in their amorphousness. The FTIR absorption spectrum of the

pretreated biomass showed the presence of characteristic stretching bands at 1731 cm^{-1}, typical of carbonyl groups in hemicellulose. This shows a clear correlation with the results obtained from the analysis of the chemical composition of the pretreated biomass.

Conclusions

Biogas, methane yields of selected samples tested

After analysing the biological material after chemical pretreatment with sulfuric acid (VI), the most efficient sample was analysed. The highest yield of biogas and methane after inoculum methane fermentation was obtained for coffee grounds after 90 min hydrolysis with 1.5% H_2SO_4 at 121 °C, amounting to 525.83 mL/g-VS biogas, including 381.12 mL CH_4/g-VS methane, which accounted for 72.48% of the total biogas composition. This is 130.50% higher biogas yield than in the control experiment (90 min, 121 °C) and 116.91% higher biomethane yield than in the control experiment where 175.70 mL CH_4/g-VS was obtained. For the digestion, a pH of 7.60 ± 0.14 was obtained for all test samples. Girotto and co-authors obtained 392 mL of CH_4/g-VS after pretreatment of coffee grounds with NaOH (8% w/w) (Girotto *et al*., 2018). Luz and co-authors co-digested coffee grounds with cow manure without pretreatment, and used cow manure with water as a reference sample. Luz and co-authors showed that for co-digestion of substrate (SCG) with cow manure, it showed a higher biogas yield of 48% and 50% more biomethane compared to the experiment with manure input with water. A yield of 305 ml CH4/g-VS was obtained for the co-digestion (Luz *et al*., 2017; Girotto *et al*., 2018).

The most effective yield of lactic acid during anaerobic lactic fermentation

The study showed that the most efficient sample in terms of substrate pretreatment for lactic acid biosynthesis was coffee grounds after 90 min hydrolysis with 1.5% H_2SO_4 at 121 °C. The highest lactic acid yield was shown for the consortium of *Lactobacillus plantarum* and *Lactobacillus brevis* and is 0.51 ± 0.09 g lactic acid per gram biomass. The consortium data yielded 20 g of lactic acid produced per litter per hour. Lactic fermentation with coffee grounds inoculated only with *Lactobacillus plantarum* showed the most 0.47 ± 0.04 g of lactic acid per gram of coffee grounds for the best pretreatment. This is a 7.84% lower lactic acid gain per gram of substrate.

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