

Lactic Acid Production from Potato Peel Waste, Spent Coffee Grounds and Almond Shells with Undefined Mixed Cultures Isolated from Coffee Mucilage from Coatepec Mexico

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Abstract

Food waste is considered a non-valued material and the volume is increasing with population and these wastes can be used as raw materials to produce useful bioproducts. Potato peel waste (PPW), almond shells (AS) and spent coffee grounds (SCG) are such wastes and were used as feedstocks to produce lactic acid (LA) via fermentation. Two lactic acid producing bacterial consortia were screened/selected based on glucose and arabinose fed media and were each used for subsequent fermentations. The food wastes were subjected to either: (i) starch gelatinization, (ii) hydrothermal pretreatment, (iii) hydrothermal and cellulase pretreatment, and (iv) hydrothermal and cellulase pretreatment buffered with CaCO₃ prior to fermentation. The glucose selected consortia was better than the arabinose selected consortia for generating LA, and thus was used for further fermentations trials using pretreated biomass. The best LA yield (0.175 g LA g⁻¹ biomass) was from gelatinized AS than the hydrothermal plus cellulase pretreatment. LA productivity was improved for the hydrothermal plus cellulase pretreated biomass by addition of CaCO₃ as buffer to give LA production rates of 0.063 g LA g⁻¹ PPW, 0.045 g LA g⁻¹ AS and 0.049 g LA g⁻¹ SCG.

Keywords: Lactic acid; Fermentation; Microbial consortia; Spent coffee grounds; Almond shells; Potato waste

Introduction

Food waste is a growing problem with around 1.3 billion tons produced globally [1]. Generally, these organic food wastes are composted and applied to cropland or used as animal feed [2]. Furthermore, these wastes are also landfilled which have associated environmental and societal impacts [3]. Alternatively, food wastes could be used as a resource to obtain chemical co-products [1]. Some examples of food waste are vegetable and fruit peel [4], nut shells and coffee residuals.

Potatoes is the fourth major starch based crop behind corn, rice and wheat [5]. In the U.S. the majority of the potato crop 2.03×10^{10} kg [6] is processed into French fries and other food products [7] generating considerable amounts of potato peel waste (PPW). While, world coffee production in 2014 was 9.24×10^9 kg [8], with the majority of the spent coffee grounds (SCG) being discarded after brewing which contain a high proportion of carbohydrates (polysaccharides and monosaccharides) [9] plus lignin and polyphenolics [10,11]. Moreover, almond kernel production in 2014 was 9.5×10^8 kg in California, which generates about similar amounts of almond shells (AS) an agricultural by-product, that is mainly used as fertilizer or animal feed based on its nutrient composition [12]. The AS mainly contains protein (16-31%), fiber, carbohydrates (26%) and ash/minerals (P, K, Mg, Zn, Fe and Mn) [12-14]. Hence, there is a need to find fermentation technologies to convert these organic wastes into chemical building blocks, such as lactic acid (LA).

LA is produced biosynthetically (90%) by fermentation of sugars (e.g. glucose) using pure cultures of lactic acid producing bacteria [15,16]. LA is a natural hydroxyacid and is used extensively in the food industry (85% of the LA market) as an acidulant, flavoring or preservative agent. Furthermore, LA is used in the pharmaceutical industry as a pH regulator, and recently as the bioplastic, polylactic acid (PLA), for use in medical devices [17,18]. PLA is also gaining traction as a sustainable substitute for petroleum based plastics [19].

Fermentations are highly expensive with commercial media for certain type of microorganism, the use of inexpensive raw materials (e.g. PPW, AS and SCG) leads to a profitable process. These wastes contain compounds for bacteria growth to produce bioproducts [20].

The aim of this study was to use natural mixed microbial consortia isolated from coffee mucilage to ferment PPW, AS and SCG to yield LA. The effect of various biomass pretreatment regimes (starch gelatinization, hydrothermal pretreatment, hydrothermal and cellulase pretreatment with and without CaCO₃ buffer) were employed to maximize LA production. This simple and novel approach can potentially maximize the value of food wastes to produce LA.

Materials and Methods

Raw materials

The PPW used during the experiment was provided by the potato plant of JR Simplot Company (Caldwell, ID) and stored at -20°C in Ziploc bags [21,22]. The SCG was collected from Starbucks (Moscow, ID) and the AS was provided by Alldrin Brothers (Turlock, CA). Both

(SCG and AS) were Wiley milled (Thomas Scientific, N, USA) to pass a 3 mm screen and stored in plastic bags.

Biomass characterization

Samples of SCG and AS (5 g of known moisture content, in duplicate) were Soxhlet extracted with CH_2Cl_2 (150 mL) for 16 h according to American Standard Test Method (ASTM) D 1108-9623 and lipids were determined gravimetrically. Total lignin content (Klason + acid soluble lignin) were performed on extractives free samples according to ASTM D 1106 and Schoening and Johansson [23], respectively. Extractives free biomass (200 mg) was incubated in 72% H_2SO_4 (2 mL) for 1 h at 30°C, then diluted into 4% H_2SO_4 , and subjected to a secondary hydrolysis in an autoclave (117 KPa and 121°C) for 30 min. Klason lignin was determined gravimetrically. The hydrolysis filtrate was made up to 250 mL and an aliquot portion taken to determine acid soluble lignin content at 205 nm using an absorption coefficient of $110 \text{ L g}^{-1} \text{ cm}^{-1}$ (Biomate 5 spectrometer, Thermo Electron Corp). Carbohydrate content was determined using a modified sulfuric acid method for cellulosic samples [24]. Specifically, extractives free biomass (10 mg) was weighed into a glass tube to which 77% H_2SO_4 (100 μL) was added and mixed for 5 min. Then 5% phenol in water (1 mL) plus conc. H_2SO_4 (5 mL) were added to the mixture, vortex mixed, incubated at 30°C for 30 min and absorbance measured at 490 nm. C and N content was determined on a Costech 4010 elemental analyzer and protein content estimated by multiplying N by 6.25 [25]. Fatty acid methyl ester (FAME) derivatives of the CH_2Cl_2 extracts (~1.8 mg) were prepared by methanolysis ($\text{CH}_3\text{OH}/\text{H}_2\text{SO}_4/\text{CHCl}_3$ (1.7:0.3:2.0 v/v/v) for 90 min at 90°C [26]. CHCl_3 contained naphthalene acetic acid (0.13 mg mL^{-1}) as an internal standard. The FAME derivatives were analyzed by GC-MS (FOCUS-ISQ, Thermo Scientific) with a temperature profile of 40°C (1 min) to 320°C at 5°C min^{-1} and a GC capillary column: (ZB5 ms, 30 m, 0.25 mm \varnothing , Phenomenex).

Materials pretreatment

Three different biomass pretreatments were assessed for the study:

Biomass samples in a flask were placed in boiling water (100°C) for 30 min, to gelatinize any starch, and then cooled in an ice bath to ambient temperature [27].

Biomass samples were hydrothermally treated (HT) to disrupt the cellular structure of the biomass. Biomass (50 g) and water (250 mL) were heated to 200°C for 20 min in a 500 mL pressure reactor (Model 4740, Parr Instrument Co) connected to temperature controlled heater. The reaction vessel took 15 min to reach temperature. The reactor was then cooled in an ice-bath and the sample was diluted with hot water (1500 mL, 90°C) [28].

Pretreated biomass solution from (ii) was enzymatically hydrolyzed with a cellulase cocktail. The diluted pretreated biomass (1 L) was incubated for 2 days at 50°C (water-jacket) with magnetic stirring (200 rpm) in a 2 L flask upon addition of 5% w/w Novozyme Cellic® C-tec2 (Novozymes North America Inc, NC, USA) enzyme. Samples were taken every 24 h to determinate the amount of sugar released from the biomass [28].

Mixed cultures

The lactic acid producing bacterial consortia were obtained and isolated from coffee mucilage provided by the ecological benefit

Tecoxolco (Coatepec, Veracruz, Mexico), before it was cultured by adding aqueous acetic acid (3.96 mL L^{-1}) to the mucilage, then fermented at 45°C for 48 h, and subsequently cultured for 48 h through a selection media fed either glucose (G consortia) or arabinose (A consortia) and freeze-dried. The consortia were cultured for 48 h at 37°C in a *Lactobacillus* media. The composition of the media for isolation and culture was (g L^{-1}): 10 g Bacto peptone, 5 g yeast extract, 20 g glucose or arabinose, 6 g KH_2PO_4 , 2 g $(\text{NH}_4)_2\text{H}_2\text{C}_6\text{H}_5\text{O}_7$, 25 g CH_3COONa , 0.575 g MgSO_4 , 0.12 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.034 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g polysorbate 80, 1.32 g acetic acid. The bacterial cultures were examined after Grams staining by light microscopy (Olympus BX51) in bright-field mode with 1000X magnification.

Fermentation

Air-locked glass Erlenmeyer flasks (250 mL) were used for experiments with a solution volume of 100 mL. The temperature of 37°C was controlled by a water-jacket. All the bioreactors were run in duplicate and inoculated with 5% (5 mL) of either G or A activated consortia.

There were four different fermentation operating factors for the PPW, SCG and AS substrates: (i) gelatinized at 100°C; (ii) hydrothermal pretreatment; (iii) hydrothermal pretreatment with cellulase addition (48 h at 50°C); (iv) hydrothermal pretreatment with cellulase and CaCO_3 addition to the media (0.5 g).

Operating factors: fermentation (i) was performed in batch mode (100 mL) using two different bacterial consortia (G or A) for 10 days; fermentations (ii), (iii), and (iv) were performed as a batch reactor (100 mL) using bacterial consortia G.

Fermentation analytical methods

LA, acetic acid, propionic acid, ethanol, glucose and other carbohydrates were quantified by HPLC analysis, using a Rezex ROA organic acid column (7.8 mm \times 30 cm, Phenomenex) and a Waters 510 HPLC pump and TSP AS2000 autosampler equipped with differential refractive index detector (ERC-5710, ERMA, Japan), on elution with 0.005 N aqueous sulfuric acid (0.5 $\text{mL} \cdot \text{min}^{-1}$) at 65°C. HPLC data were acquired and analyzed using N2000 chromatography software (Surwit Science & Technology, China). DO and pH were measured with Orion-3-Star meter (ThermoScientific).

The LA yield was calculated by with the formula: $Y_{\text{LA}} = (C - C_0) V / M$

YLA is the lactic acid yield, C is the concentration of LA quantified on the HPLC, C_0 is the initial LA concentration, V is the volume used in the bioreactor and M is the mass of solid on the bioreactor. All the measurements were tested in duplicate and the average values are reported and analyzed using Excel.

Results and Discussion

Biomass composition

The CSG and AS biomass samples were analyzed for C, N (protein), lipids, lignin and carbohydrate contents and the PPW composition has been previously reported (Table 1) [21]. Carbohydrate is the major component in AS, SCG and PPW (35 to 51%) and values were comparable to that reported in the literature [9,14,26,29,30]. SCG was shown to have a high lipid content of 15% and was consistent with the findings (16%) by Vardon et al. [31], however, more than 5-fold higher

than that reported by Ballesteros et al. [11]. PPW and AS had low lipid contents similar to reported values [14,26,29]. The PPW and SCG biomass contained a considerable amount of protein (18-23%) while the AS had 3-4 fold less protein and this is consistent with the literature [11,14]. AS and SCG contained a considerable amount of recalcitrant lignin (25-28%) at values about 20% higher than reported [11,14]. The composition of these feedstocks makes them amenable to fermentation [27].

Analyte	SCG	AS	PPW ^a
C (%)	54.4	48.4	
N (%)	2.85	0.89	3.65
Protein (%)	17.8	5.6	22.8
Lipids (%)	15.3	1.5	2.1
Total lignin (%)	29.2	24.8	16
Total carbohydrate (%)	35	48.3	51.3

Table 1: Chemical composition of spent coffee grounds (SCG), almond shells (AS) and potato peel waste (PPW).

Since the SCG contained a considerable amount of lipids the composition of the extracts were determined as their FAME derivatives (Table 2). The main fatty acids found in the extracts were palmitic, linoleic, oleic, and stearic acids with a minor amount of eicosanoic acid and these were in agreement with previous reports [26,31,32].

FAME	M (m/z)	RT (min)	SCG	AS	PPW
			(% of extract)		
Palmitic acid (C16:0)	270	31.85	47.7	6.8	4.8
Linoleic acid (C18:2)	294	35.02	30	0.1	5.2
Oleic acid (C18:1)	296	35.14	10.6	1.4	3
Heptadecenoic acid (C18:1)	296	35.21	0.7	0.4	
Stearic acid (C18:0)	298	35.61	9.1	1.6	0.9
Eicosanoic acid (C20:0)	326	39.07	1.9		
Lignoceric acid (C24:0)	382	45.2			0.3
Hexacosanoic acid (C26:0)	410	47.92			0.6
Montanoic acid (C28:0)	438	50.49			6.3
Nonacosylic acid (C29:0)	452	51.71			0.6
Melissic acid (C30:0)	466	52.9			1.9
Total			100	10.3	23.7

Table 2: Fatty acid composition of the CH₂Cl₂ extracts by FAME analysis from spent coffee grounds (SCG), almond shells (AS) and potato peel waste (PPW).

Furthermore, in the PPW extract higher fatty acids (C24-C30) were also detected. Fatty acids accounted for all the SCG extract while only 10% and 24% in the AS and PPW extracts, respectively. In the AS extract nonanal and lauraldehyde were detected (not quantified) and have been previously observed [32].

Bacterial consortia

The isolated consortia was cultured at 45°C to obtain *Lactobacillus* while suppressing other lactic acid producing bacteria such as *Lactococcus*, *Leuconostoc* or *Pediococcus* at this temperature [33].

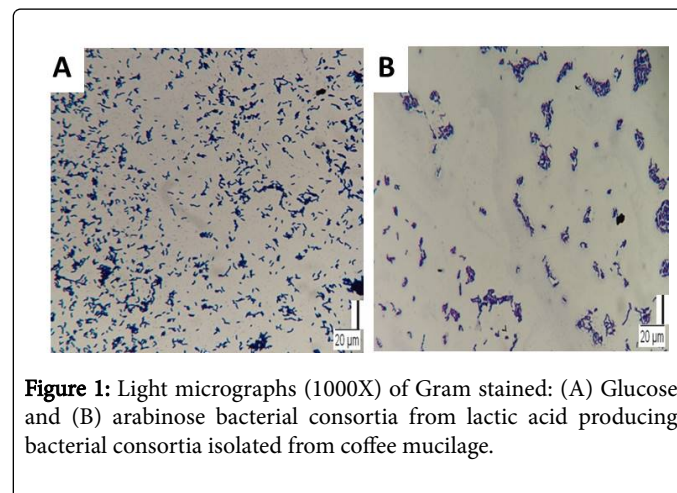


Figure 1: Light micrographs (1000X) of Gram stained: (A) Glucose and (B) arabinose bacterial consortia from lactic acid producing bacterial consortia isolated from coffee mucilage.

The cultures were examined by microscopy after Gram staining showing the presence of Gram positive consortia, mainly bacilli (Figure 1). The glucose (G) consortia once cultured appeared to have more growth and different morphology than the arabinose (A) consortia. However, the selection was favored through the addition of acetic acid, which favors lactic acid producing bacteria [34].

LA fermentation from gelatinized biomass using isolated microbial consortia

The biomass samples (PPW, SCG, AS) were heated to 100°C to gelatinize any starch present to aid its conversion to sugars by the bacterial consortia during fermentation [27].

Homolactic fermentation appears to be the main process due to very low levels of ethanol detected in the broth [35]. LA concentration peaked around day 2-3 and then decreased since all the available carbohydrate was consumed and an increase in organic acids leads to bacterial death [27].

Batch fermentation studies were performed to establish which bacterial consortia (G or A) would result in higher LA yields. LA (Figure 2) and AA (Figure 3) were the main products biosynthesized during fermentation, while propionic and ethanol were present in low concentration, as determined by HPLC.

The G consortia showed significantly better LA yield for each of the biomass types than that produced from the A consortia (Table 3 and Figure 2).

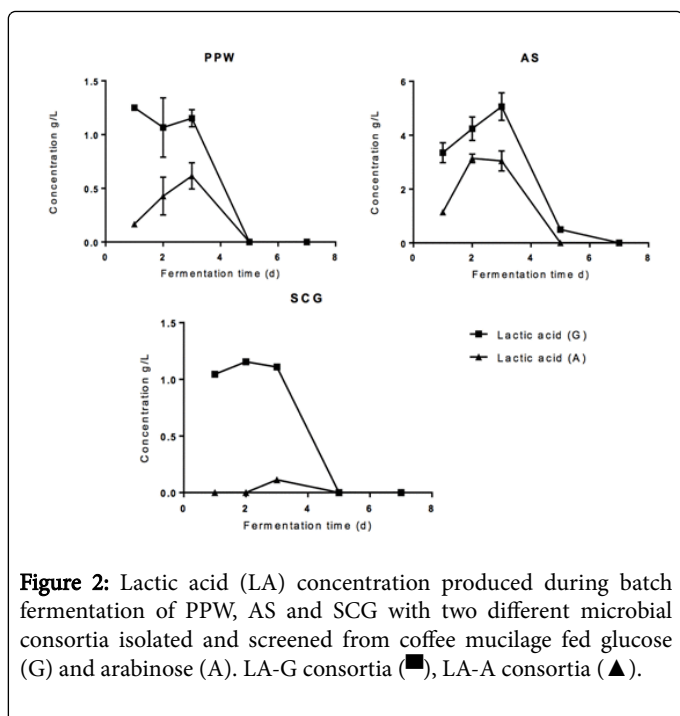


Figure 2: Lactic acid (LA) concentration produced during batch fermentation of PPW, AS and SCG with two different microbial consortia isolated and screened from coffee mucilage fed glucose (G) and arabinose (A). LA-G consortia (■), LA-A consortia (▲).

Very poor LA yields were obtained from SCG using the A consortia.

Substrate/Consortia	Lactic acid (g.L ⁻¹)	Acetic acid (g.L ⁻¹)	Day with maximum LA yield	LA yield (g.L ⁻¹)
PPW (G)	1.2 ± 0	0.9 ± 0	1	0.042
PPW (A)	0.6 ± 0.1	1.1 ± 0	3	0.021
AS (G)	5 ± 0.4	0.8 ± 0.1	3	0.175
AS (A)	3.1 ± 0.1	0.8 ± 0	2	0.108
SCG (G)	1.1 ± 0.1	0.9 ± 0	2	0.038
SCG (A)	0.1 ± 0	1 ± 0.1	3	0.003

Table 3: Yields of lactic acid (LA) and acetic acid (AA) in batch fermentations of from spent coffee grounds (SCG), almond shells (AS) and potato peel waste (PPW) with G and A consortia.

However, AA was shown to be an important by-product in PPW and SCG produced from the A consortia. The LA concentration produced, using the G consortia, were similar for PPW (~1.2 g.L⁻¹) and SCG (1.1 g.L⁻¹), but significantly larger for AS (5.0 g.L⁻¹). The yields were lower than those reported by Liang et al. for PPW [27]. The highest LA production yield was for AS at 0.175 g LA g⁻¹ biomass which was comparable to those values reported by Liang et al. but with a different solid loading [27].

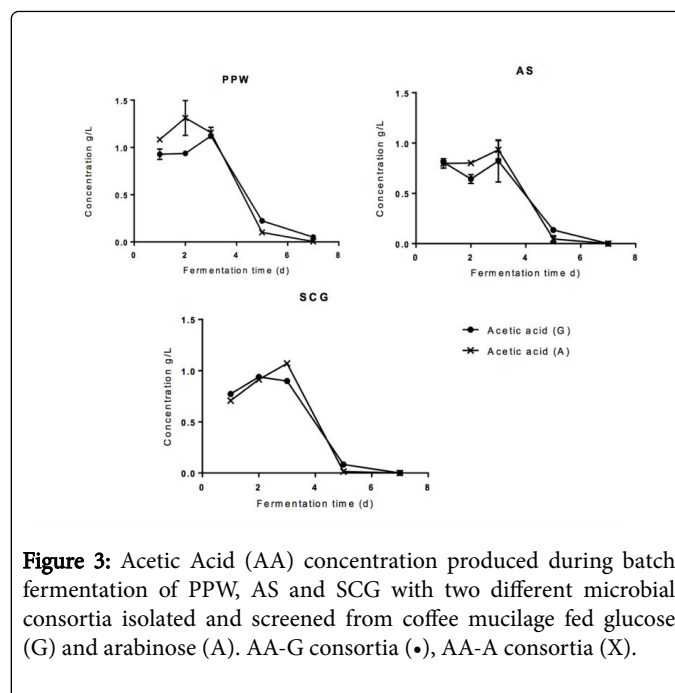


Figure 3: Acetic Acid (AA) concentration produced during batch fermentation of PPW, AS and SCG with two different microbial consortia isolated and screened from coffee mucilage fed glucose (G) and arabinose (A). AA-G consortia (●), AA-A consortia (×).

Effect of biomass hydrothermal and cellulase pretreatment on LA fermentation

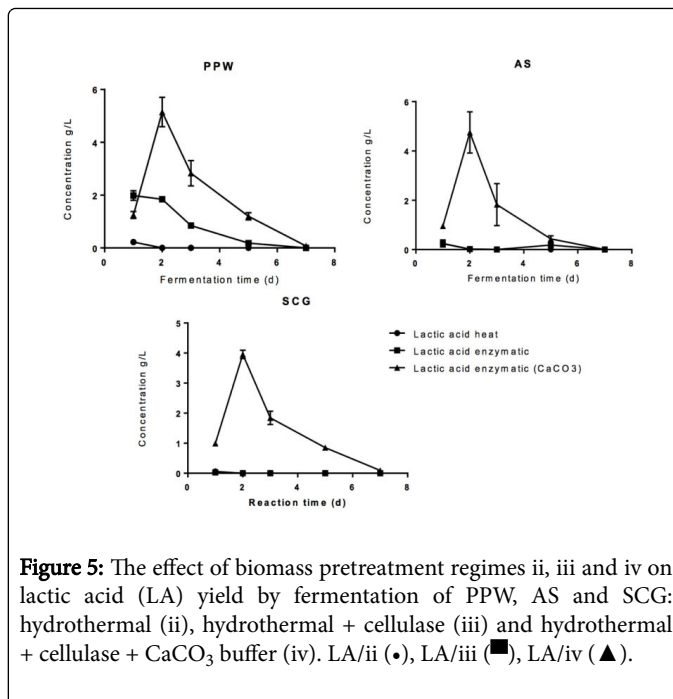
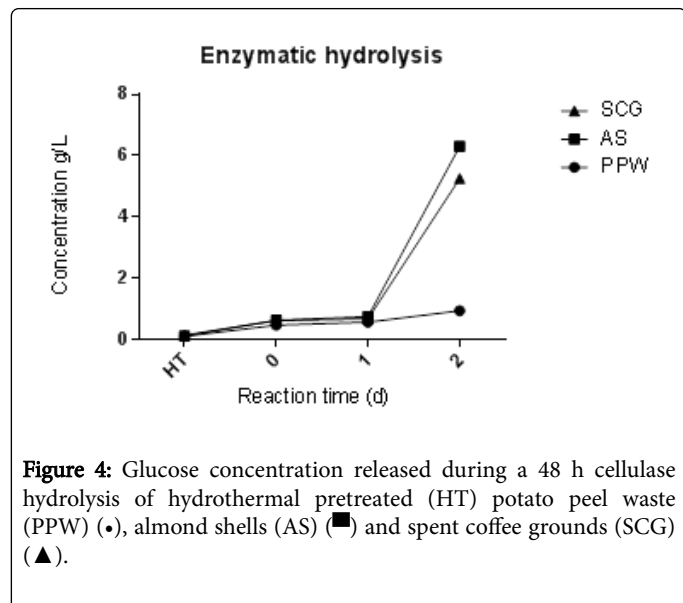
Work by Dai and McDonald [28] had shown a hydrothermal pretreatment of hybrid poplar biomass improved its enzymatic digestability to yield sugars. Pretreatment of PPW, SCG, and AS were based on optimal conditions of 200°C for 20 min [28]. Preliminary studies on direct fermentation of the hydrothermal pretreated biomass gave very low yields of sugars 0.09 g.L⁻¹ (PPW), 0.1 g.L⁻¹ (AS) and 0.136 g.L⁻¹ (SCG). Therefore, an enzymatic hydrolysis step (48 h) using a cellulase cocktail was added after the hydrothermal stage (pretreatment iii) to liberate fermentable sugars. Glucose was the main sugar released during cellulase hydrolysis of hydrothermally treated PPW, SCG and AS (Figure 4). After 48 h significant differences in glucose concentrations of 0.93 g L⁻¹ (PPW), 6.31 g.L⁻¹ (AS) and 5.25 g.L⁻¹ (SCG) were obtained.

The three biomass pretreatment regimes (ii, iii, and iv) were evaluated for LA and AA production by fermentation (Figures 5 and 6). Addition of a commercial cellulase to the hydrothermally treated biomass increased sugar yields and therefore LA yield was expected to increase. The pretreatment showed an increase in glucose yield for all the raw materials.

However, the LA concentrations for pretreatment ii were lower at 0.2 g.L⁻¹ (PPW), 0.2 g.L⁻¹ (AS) and 0.06 g.L⁻¹ (SCG), than that produced with gelatinized biomass (pretreatment i). Using pretreatment iii resulted in a significant LA increase for PPW to 1.9 g.L⁻¹, however no increase was observed for AS and SCG (0.2 g.L⁻¹ and 0.02 g.L⁻¹, respectively).

To mitigate the issue of low LA production calcium carbonate was added (pretreatment iv) to buffer the pH of the broth. This resulted in a significant LA yield increase [15], during fermentation on the second day to 5.14 g.L⁻¹ (PPW), 4.74 g.L⁻¹ (AS) and 3.95 g.L⁻¹ (SCG).

Sequencing batch fermentation could not be sustained mainly because of the low solids loading during the study (2.85 g.L^{-1}), increasing the solids may improve the LA yield [27].



Biomass type (pretreatment regime)	Lactic acid concentration (g.L ⁻¹)	Acetic acid concentration (g.L ⁻¹)	Day maximum LA concentration obtained	Yield ((g LA g ⁻¹ biomass)
PPW (ii)	0.2 ± 0	0.3 ± 0	1	0.007
PPW (iii)	1.9 ± 0.1	0.2 ± 0	1	0.069
PPW (iv)	5.1 ± 0.4	0.4 ± 0	2	0.179
AS (ii)	0.2 ± 0.1	0.7 ± 0.2	1	0.008
AS (iii)	0.2 ± 0	0.6 ± 0	1	0.008
AS (iv)	4.7 ± 0.5	0.8 ± 0.1	2	0.166
SCG (ii)	0.06 ± 0.02	0.3 ± 0	1	0.002
SCG (iii)	0.02 ± 0.02	0.2 ± 0	1	0.0008
SCG (iv)	3.9 ± 0	0.3 ± 0	2	0.138

Table 4: The effect of biomass (SCG, AS, PPW) pretreatment regimes ii (hydrothermal), iii (hydrothermal + cellulase) and iv (hydrothermal + cellulase + CaCO₃) on lactic acid (LA) and acetic acid (AA) yields.

During the fermentations of pretreatment regimes ii and iii, respectively, the highest LA yield occurred within the first 24 h for PPW (0.2 g.L^{-1} and 1.9 g.L^{-1}), AS (0.2 g.L^{-1} and 0.2 g.L^{-1}) and SCG (0.06 g.L^{-1} and 0.02 g.L^{-1}) then decreased rapidly (Figure 5).

However, the highest yield of LA was obtained using pretreatment iv with 0.179 g.g^{-1} (PPW), 0.166 g.g^{-1} (AS) and 0.138 g.g^{-1} (SCG). The LA concentrations were comparable to the other experiments on day 2 ($\sim 5 \text{ g.L}^{-1}$) and decreased on the third day to 2.82 g.L^{-1} (PPW), 1.82 g.L^{-1} (AS) and 1.84 g.L^{-1} (SCG).

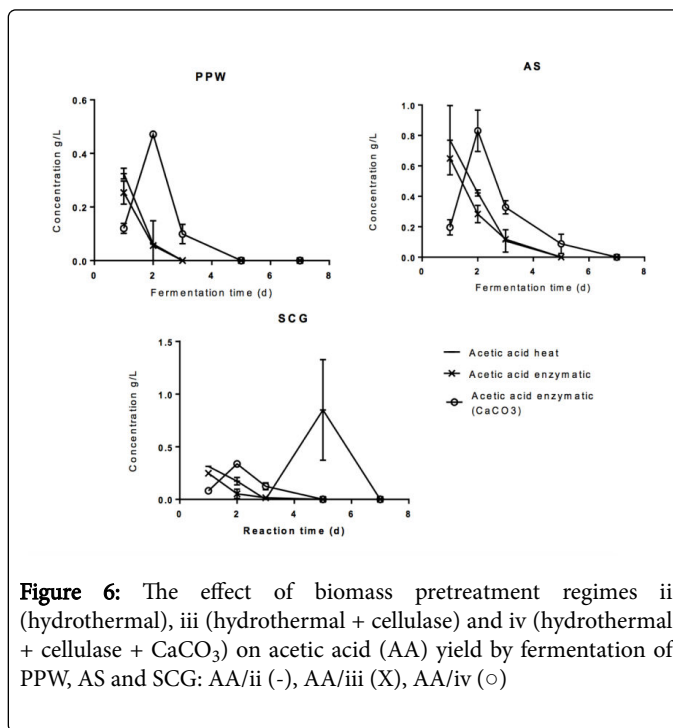


Figure 6: The effect of biomass pretreatment regimes ii (hydrothermal), iii (hydrothermal + cellulase) and iv (hydrothermal + cellulase + CaCO₃) on acetic acid (AA) yield by fermentation of PPW, AS and SCG: AA/ii (-), AA/iii (X), AA/iv (o)

This drop in LA could be explained as conversion of LA to other products by secondary fermentation [36]. The LA fermentation yields for PPW, SCG and AS using pretreatments ii, iii and iv are given in Table 4. The addition of calcium carbonate significantly improved LA production by acting as a buffer and forming calcium lactate and avoiding a decrease in pH. Calcium carbonate is widely used rather than NaOH or NH₄OH because it's easier to treat after fermentation to release LA [15].

Conclusion

The lactic acid bacterial consortia (G and A) isolated from coffee mucilage produced lactic acid (LA) from spent coffee grounds, almond shells and potato peel. Consortia G produced a higher yield of LA than consortia A. Several biomass pretreatment regimes were employed (hydrothermal treatment, addition of cellulose, and addition of calcium carbonate) to increase the release fermentable carbohydrates from the waste. The hydrothermal + cellulase treatment gave low LA yields during fermentation due to a low pH and cell death. The addition of calcium carbonate as a buffer to the fermentation media of pretreated substrates significantly improved LA production.

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