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Coffee Silverskin as a Renewable Resource to Produce Butanol and Isopropanol

Alessandra Procentese[a,*], Francesca Raganati[b], Luciano Navarini[c], Giuseppe Olivieri[d], Maria Elena Russo[a], Antonio Marzocchella[b].

[a] Istituto di Ricerche sulla Combustione – Consiglio Nazionale delle Ricerche, P.le V. Tecchio 80, 80125 Napoli, Italy
[b] Dipartimento di Ingegneria Chimica, dei Materiali e della Produzione Industriale – Università degli Studi di Napoli Federico II, P.le V. Tecchio 80, 80125 Napoli – Italy
[c] Ilycaffè S.p.A Via Flavia 110 –Trieste 34147 – Italy.
[d] Wageningen University, Bioprocess Engineering, AlgaePARC, P.O. Box 16, 6700 AA Wageningen, The Netherlands.
a.procentese@irc.cnr.it

Coffee is the second largest traded commodity after oil and large amounts of by-products are generated in the coffee industry every year. In particular, coffee silverskin (CS) and spent coffee grounds (SCG) are the main coffee industry residues. CS is about 4.2% (w/w) of coffee beans and the valorisation of this waste through the biorefinery approach may boost the circular economy development.
In the present contribution, CS was pretreated with one of the mainly investigated biomass pretreatment reported in literature: alkaline hydrolysis in NaOH solutions. After enzymatic hydrolysis of the pretreated CS, the obtained sugars were used as carbon source to produce butanol and isopropanol by *Clostridium beijerinckii* DSM 6423. Moreover, fermentation tests were also carried out with synthetic media to investigate the effects of the alkaline pretreatment on the fermentation process. When pretreated CS was used as feedstock, the largest yields were 0.16 and 0.31 $g_{solvent}/g_{sugars}$ isopropanol and butanol, respectively. The reported results foster further studies regarding the reuse of CS for solvents production through fermentation processes.

1. Introduction

The current production of butanol and isopropanol is almost exclusively by petrochemical route. Butanol is used as solvent in the painting and cosmetic industry. Moreover, the butanol features make it an ideal advanced fuel: quite high lower heating value (LHV) - 86 % of that of gasoline and 31 % larger than that of ethanol – low volatility, low hygroscopicity, making it a more desirable liquid fuel in terms of energy density; blending with gasoline at any rate without upgrading of existing internal combustion engines, and being compatible with the current gasoline infrastructure (Procentese *et al.*, 2017a). The market of isopropanol is even larger than that of butanol: solvent in painting and printing industries, fuel additive, and one of the four short chain aliphatic alcohols that may become the major feedstock for the future chemical industry. The worrying environmental impact of the petrochemical route has fuelled the interest in the industrial production of these solvents by fermentation from renewable and sustainable resources. Indeed, both solvents can be produced by *Clostridium beijerinckii* through isopropanol-butanol-ethanol (IBE) fermentation (Yang *et al.*, 2016). The interest in the alternative biotechnological route becomes even more challenging when fermentable resources are waste/residues of anthropic activities (e.g. residues of agro-food industries). Residues of the coffee production – have been proposed as a good candidate as renewable feedstock for fermentation processes (Musatto *et al.*, 2011). As reported by the International Coffee Organization, in 2016 the total coffee production and the world coffee consumption were 8.6 and 9.1Mt respectively (www.ico.org). Every year, large amounts of by-products are generated in the coffee industry. In particular, coffee silverskin (CS) and spent coffee grounds (SCG) are the main coffee industry residues, obtained during the beans roasting, and the process to prepare “instant coffee”, respectively. CS represents about 4.2 % (w/w) of coffee

beans and the valorisation of this waste, using a biorefinery approach, could represent a contribution of many industries for sustainable and competitive development (Procentese *et al.*, 2017b).
The aim of this contribution is to investigate the feasibility of butanol and isopropanol production by fermentation of hydrolyzed CS. The CS powder was pretreated and hydrolyze to produce fermentable sugars. The CS-derived sugars were used as carbon source for the *Clostridium beijerinckii* fermentation. The butanol and isopropanol yields obtained on pretreated CS were assessed.

2. Materials and Methods

2.1 Raw materials

Coffee silverskin (CS) was kindly provided in 2016 by Illy S.p.A. The raw biomass was characterized as follows: total carbohydrates 30.37%, lignin 29.91%, protein 14.43%, fats 4.97%, ash 5.87%, phenolic compounds 0.8%. Before pretreatment, the coffee was oven-dried at 40°C. Then the dried coffee was ground and sieved at 1 mm and stored in sealed plastic bags at room temperature until used (less than two days).

2.2 Alkaline pretreatment

The dried CS powder was soaked in 0.5 M NaOH aqueous solution: 20 mL of solution was used per gram of solid mass. The suspension was kept in autoclave (VAPORMATIC 770) for 30 min at 121°C according to the procedure suggested by Procentese *et al.* (2017c). The biomass was separated from the liquid phase (black liquor) by centrifugation at 5320 RCF for 10 min. The biomass was washed with distilled water until pH 7 was reached, then it was oven-dried at 40 °C and stored.
The percentage biomass recovery (R) was calculated as the ratio between the dry weight of pre-treated biomass (B_{PT}) and the dry weight of raw material (B_{RAW}) according to Eq. 1.

$$R = \frac{B_{PT}}{B_{Raw}} \quad (1)$$

The dried pretreated biomass was used for the following enzymatic hydrolysis step.

2.3 Enzymatic hydrolysis

The commercial cellulases cocktail Cellic CTec2 was kindly supplied by Novozyme. The hydrolysis was carried out in 0.1 M sodium citrate buffer at pH 4.8 supplemented with 80 µL tetracycline and 60 µL cycloheximide to prevent microbial contamination. 100 mL glass bottles were incubated at 50°C and kept under agitation on a rotary shaker (Minitron Incubator Shaker-Infors HT) at 180 rpm for 72 h. The Cellic CTec2 loading was set at 15 mg_{enzyme}/g_{glucan} according to the glucan content assessed on the raw biomass. The solid loading was set at 10% w/v. At 72 h the hydrolysis liquid medium was sampled, centrifuged, filtered, and analysed to assess sugar concentration. The glucose yield ($Y_{Glucose}$) was calculated as the ration between the amount of glucose obtained after enzymatic hydrolysis and the mass of pretreated biomass (Eq.2)

$$Y_{Glucose} = \frac{g_{Glucose}}{g_{pretreated\ biomass}} \quad (2)$$

2.4 Microorganism and media

Clostridium beijerinckii DSM 6423 was supplied by DSMZ (Germany). The stock cultures were reactivated according to the DSMZ procedure. The reactivated cultures were stored at -80 °C. The thawed cultures were inoculated into 12 mL of pre-fermentation medium containing 30 g L^{-1} glucose and 5 g L^{-1} yeast extract (YE) in 15 mL Hungate tubes (pre-cultures). The cells were grown under anaerobic conditions for 48 h at 37 °C, then they were transferred to fermentation bottles. The fermentation medium consisted of 5 g L^{-1} YE, 2.5 g L^{-1} NH_4Cl, 0.25 g L^{-1} KH_2PO_4, 0.25 g L^{-1} K_2HPO_4 and mineral solution (0.20 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.01 g L^{-1} $MnSO_4 \cdot H_2O$, 0.01 g L^{-1} $FeSO_4 \cdot 7H_2O$). The carbon source was the enzymatic hydrolysate of the NaOH pre-treated CS. Fermentation tests were also carried out with glucose as carbon source (synthetic media) in order to investigate the effects of the alkaline pretreatment on the IBE fermentation process. The glucose concentrations were ranged between 20 and 100 g L^{-1}. Each test was carried out in triplicate. The data reported in tables and figures are the mean values: the standard error was always lower than 5%.

2.5 Analytical methods

The pH was measured off-line by a pH-meter (Hanna Instruments). The cell density was measured as optical absorbance at 600 nm (OD600) using a spectrophotometer (Cary 50Varian). The sugar concentration was determined by high performance liquid chromatography (HPLC) using an Agilent 1100 system (Palo Alto, CA).

The sugars were separated by means of a 8 mm Hi-Plex H 30 cm 7.7 mm column at room temperature and detected with a refractive index detector. Deionized water was used as mobile phase at flow rate of 0.6 mL min^{-1}. The metabolites (acetic acid, butyric acid, acetone, butanol, ethanol and isopropanol) were measured by means of a GC apparatus equipped with a FID and with a capillary column poraplot Q (25 m x 0.32 mm). An internal standard (hexanoic acid) was used to measure acids and alcohols and their concentrations.

2.6 Fermentation tests

The batch fermentations were characterized in terms of cell growth, pH, sugar conversion, and metabolites (acids and solvents) production. In particular, the measured data were worked out to assess the following parameter:

- Glucose conversion (%), the ratio between the converted sugar and the initial sugar
  $X_S=(S_0 - S)/S_0\ 100$ (3)
- The solvents yields ($Y_{solvent}/_{Sugars}$, $g_{solvent}\ g^{-1}_{sugars}$) were calculated as the concentration of solvent (g L^{-1}) produced divided by the concentration of used fermentable sugar ($g_{sugars}\ L^{-1}$).

3. Results

3.1 NaOH Pretreatment and enzymatic hydrolysis

After NaOH pretreatment the biomass was deeply washed and dried. After this process a recovery of 40% (Eq.1) was reported. The recovered biomass was used for the enzymatic hydrolysis step and 20 g L^{-1} of glucose were obtained in the liquid phase. Glucose yield of 0.2 $g_{glucose}/g_{pretreated\ biomasss}$ was obtained.

3.2 Synthetic media

Figure 1 shows the time resolved concentration of glucose, isopropanol, butanol and butyric acid measured during *C. beijerinckii* fermentation on synthetic media. In particular, three different glucose concentration were investigated namely 20, 60 and 100 g L^{-1} (Figure 1A - C). As reported, for all investigated conditions about 10 g L^{-1} of glucose were consumed after 24h. During this phase 0.5 g L^{-1} of butyric acid was produced, no acetic acid was detected. After 15h the expected solventogenic phase occurred and isopropanol and butanol production was observed. No ethanol was detected. As reported in Figure1 the same amount of isopropanol (about 1 g L^{-1}) was obtained for all investigated conditions. As regards the butanol production, the higher butanol concentration 3 g L^{-1} was reached when the lower glucose concentration (20 g L^{-1}) was used as carbon source (Figure 1A). It seems that, if on one hand the glucose concentration in the fermentation medium did not affect the isopropanol production, on the contrary, the concentration of the carbon source in the medium affected the butanol production.

3.3 Fermentation on Coffee Silverskin

Time resolved concentration of glucose, isopropanol, butanol and butyric acid measured during *C. beijerinckii* fermentation on hydrolysed medium from NaOH pretreated CS is reported in Figure 2. Results shows that 15 g L^{-1} of glucose were consumed after 30h. In less than 40h, 4.3 and 2.2 g L^{-1} of butanol and isopropanol were obtained. This behaviour confirms the trend obtained with synthetic medium: when the glucose concentration in the fermentation medium decreases, the butanol production increases. As regards the isopropanol production, the higher solvent concentration (2.2 g L^{-1}) was reported only when sugars from hydrolysis of pretreated CS were used as carbon source. This behaviour could be probably explained by the nature of the used biomass and by the release of antioxidant molecules which characterize the CS (Ballesteros *et al.*, 2014; Costa *et al.*, 2014). Table 1 shows the main data regarding fermentation of *C. beijerinckii* on synthetic medium and hydrolysate of pretreated CS. The glucose conversion increases when the glucose concentration in the fermentation medium decreases. The butanol and isopropanol concentrations obtained from hydrolysate of pretreated CS are twice than the solvent concentrations obtained from synthetic medium. In particular, yields of 0.16 and 0.31 were obtained for isopropanol and butanol respectively. Youn and co-worker (2016), carried out IB fermentation at different glucose concentration and on glycerol as carbon source. The best performance in alcohol production was obtained after 32 h (9.43 g/L butanol and 4.49 g/L isopropanol) from 46.35 g/L glucose. Within 24h, 9.86 g/L butanol and 2.88 g/L isopropanol were produced when 23 g/L glucose and 13 g/L of crude glycerol were used as carbon source. However, these results cannot be directly compared due to different employed bacteria (*Clostridium beijerinckii* DSM 6423 and *Clostridium* sp. A1424) and medium(CS and glucose/glycerol mixture).

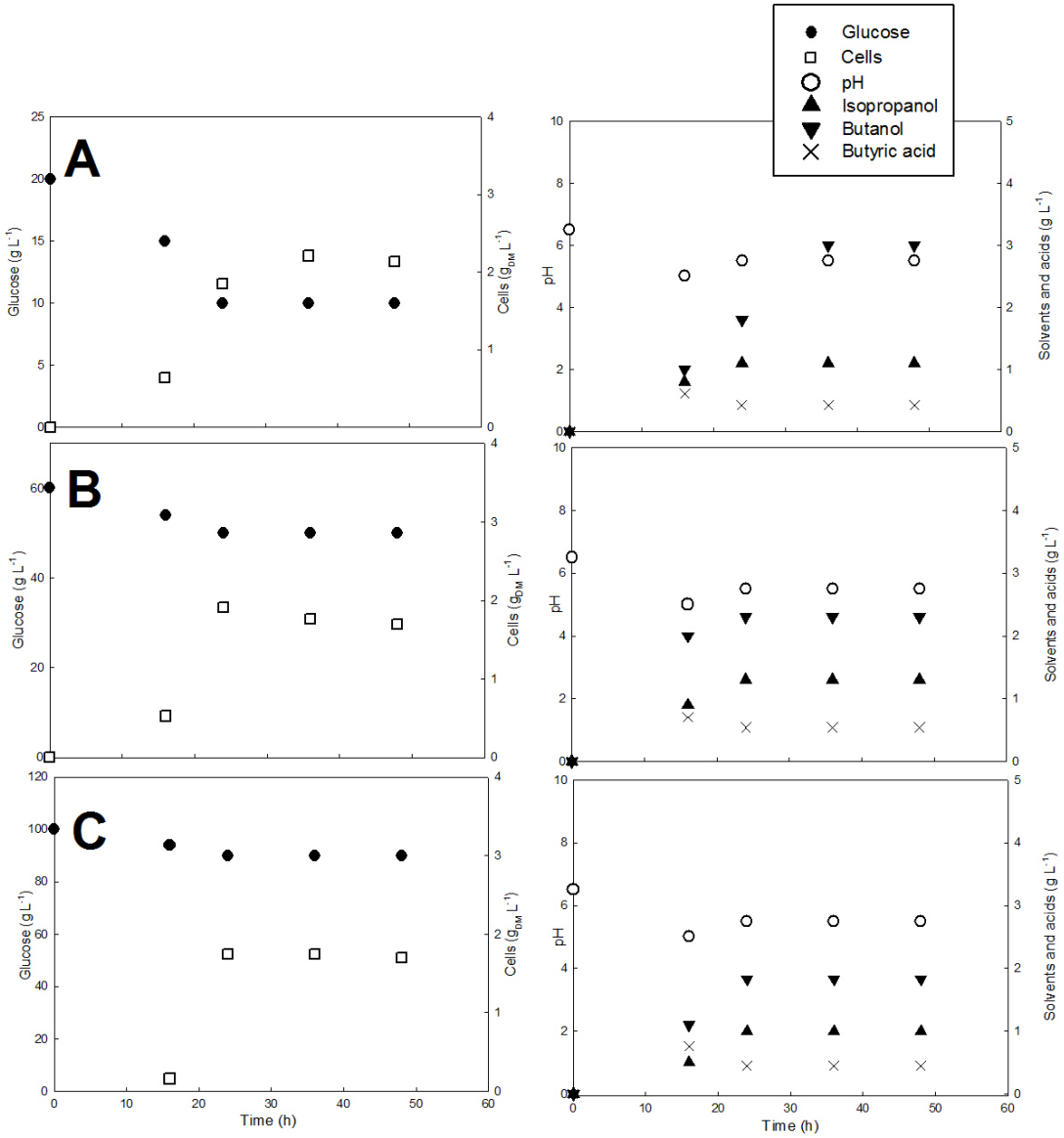

Figure 1: Time resolved concentration of glucose, isopropanol, butanol and butyric acid measured during C. beijerinckii *fermentation on synthetic media at different glucose concentrations: 20g L^{-1} (A), 60 g L^{-1} (B) 100 g L^{-1} (C).*

Table 1: Main data regarding fermentation of C. beijerinckii *on synthetic medium and enzymatic hydrolysis of NaOH pretreated coffee silverskin CS.*

Medium	Glucose (g/L)	Glucose conversion (%)	Isopropanol (g/L)	Butanol (g/L)	Yg_{IP}/g_G	Yg_B/g_G
Synthetic	20	50	1.1	3.0	0.11	0.30
	60	17	1.3	2.4	0.13	0.24
	100	10	1	1.8	0.10	0.18
Coffee Silverskin	21	67	2.2	4.4	0.16	0.31

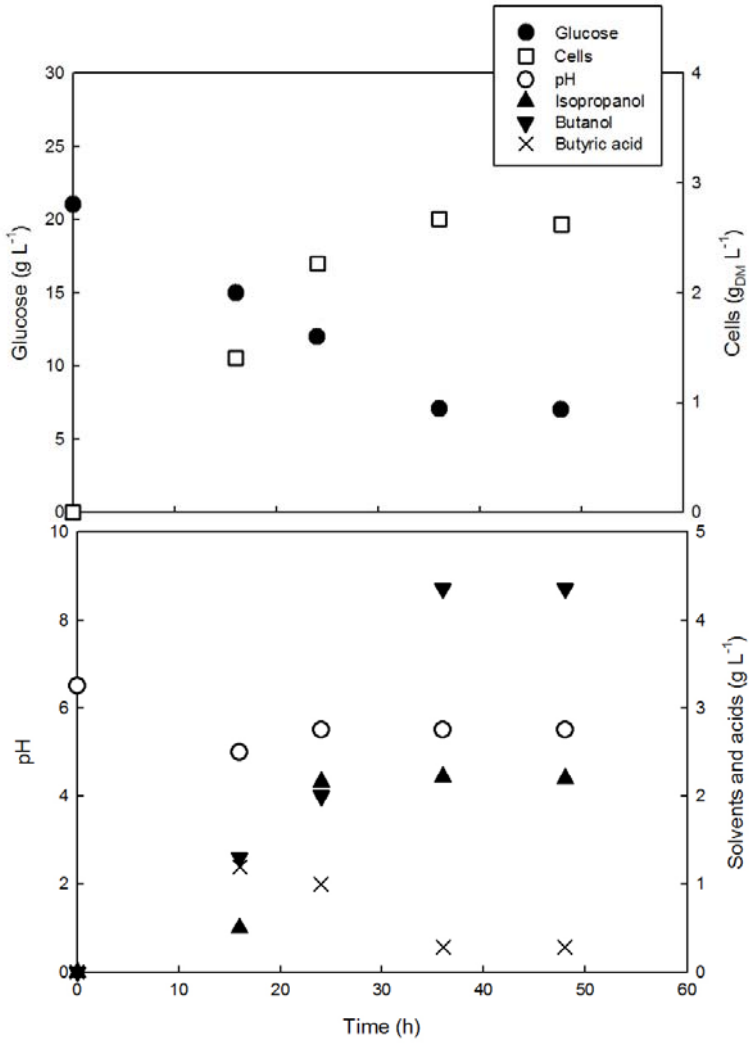

Figure 2: Time resolved concentration of glucose, isopropanol, butanol and butyric acid measured during C. beijerinckii *fermentation on sugars from enzymatic hydrolysis of NaOH pretreated CS.*

3.4 Consideration on process design

On the basis of the obtained experimental results, a mass flow of the potential biorefinery of CS for butanol and isopropanol production has been reported in Figure 3. After biomass pretreatment and enzymatic hydrolysis a glucose yield of 0.2 $g_{glucose}/g_{pretreated\ biomasss}$ was obtained (see section 3.1). Moreover, if the glucose conversion and the solvent yields reported in Table 1 are taken into account, 0.86g and 1.66g of isopropanol and butanol respectively are expected from 100g of dry coffee silverskin. Both solvents are released in the aqueous fermentation broth thus, products recovery values should be eventually assessed depending on the separation processes selected for the downstream operations.

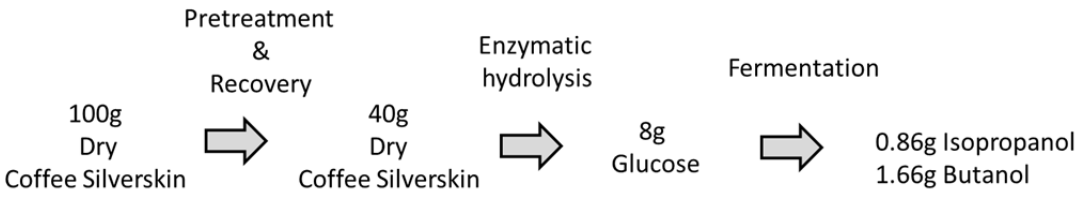

Figure 3: Mass flows of CS biorefinery for butanol and isopropanol production

4. Conclusions

The experimental study proved that CS is as good candidate as renewable feedstock for IBE production. The reported process can be considered as part of a biorefinery process that might contribute to the development of circular economy in the fields of agro-food industry and solvents/biofuels sector.

Acknowledgments

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