

# THE CASCADE BIOREFINERY APPROACH FOR THE VALORIZATION OF THE SPENT COFFEE GROUNDS

*Federico Battista\**, Giuseppe Strazzera, Serena Zanzoni, Marco Andreolli, David Bolzonella

Department of Biotechnology, University of Verona, Verona, 37134, Italy

\*Corresponding author email: [federico.battista@univr.it](mailto:federico.battista@univr.it)

## ABSTRACT

6 tons of Coffee Spent Grounds (SCG) are produced every year all around the world. Their physical and chemical characterization, rich in organic compounds and in high added value compounds, make SCG ideal for the bioactive molecules recovery and bioenergy production according a cascade biorefinery approach. This work investigates the effect of different polar and no polar solvents for the extraction of the coffee oil by SCG, whose economic value is about two fold the coffee itself. The 50:50 (v/v) ethanol- iso-propanol mixture allowed the reaching a coffee oil recovery of about 16% (w/w). The extracted SCG were performed by solid/liquid separation, after a preliminary acid-enzymatic hydrolysis. The liquid fraction was adopted for the bioethanol production which reached the final value of 15 g/L, while the solid fraction was used for biogas production by Anaerobic Digestion. The final methane yield resulted to about 250 L<sub>CH<sub>4</sub></sub>/kg<sub>VS</sub>.

Keywords: Spent Coffee Grounds, cascade biorefinery, added value compounds, bioethanol, biogas

## 1. Introduction

Coffee is one of the most appreciated beverage around the world with a global consumption of 9.3 tons during the 2016/2017 [1], corresponding to an increase of the 1.3% compared to the 2015/2016. It has been estimated that 1 kg of coffee generates about 2 kg of wet Spent Coffee Grounds (SCG), for an annual amount of 6 tons generated at international level [2].

Currently SCG are mainly incinerated or simply collected with the Organic Fraction of the Municipal Solid Wastes (OFMSW) and disposed in landfill. In the last decade, the emerging of the circular economy concept favoured alternative uses for SCG, encouraging the scientific community to investigate on the bioenergy production, in particular bioethanol and biogas. Recently, Nguyen et al. [3] found that SCGs were excellent for the recovery of oligosaccharides and manno-oligosaccharides through two enzymatic hydrolysis stages followed by ethanol production with a yield of 3.1%. Similar results have previously been reached by Burniol-Figols et al. [4] under an acid hydrolysis stage and after a previous stage of chloridric acid extraction by chloroform as solvent. A volumetric bioethanol concentration of 3.9% v/v was obtained, which is considered very close to the 4%, the limit for an economically feasible distillation. Better yields have been obtained when SCG are adopted exclusively for bioethanol production: between 20-50 w/w of dry SCGs [5, 6]. Biogas production represents an alternative in biofuels production from SCGs. Battista et al. [7] adopted several pretreatments to favour the lignocellulosic materials hydrolysis to improve biogas production from SCGs, adopting a continuous mode in 45 L CSTR. The best performances were obtained after an alkali pretreatment when a biogas production of 1.14 NL/L at steady state conditions was obtained corresponding to a good specific methane yield of 530  $L_{CH_4}/Kg_{TVS}$ . The specific methane production was about 160  $L_{CH_4}/Kg_{TVS}$  without any pretreatment. More recently, Vismara and Marchetti [8] found a methane production of 290  $L_{CH_4}/Kg_{TVS}$  from un-pretreated SCGs through a mesophilic AD process. Some researchers have tested SCG in codigestion mode with other substrates. For example, Hernandez et al. [9] improved the stability of AD for hydrogen production by mixing the coffee wastes with swine manure. In this way, an optimum C/N ratio of about 60 was reached, which in turn led to a high hydrogen generation of about 3.8 L H<sub>2</sub>/L d. Abouelenien et al. [10] also conducted a codigestion process of coffee wastes with chicken manure and other agricultural residues (cassava and coconut wastes). The methane fermentation resulted to be improved two-fold compared to the case of chicken manure fermentation on its own.

Although the good performances, biofuels production does not represent the ideal way for the exploitation of organic substrates. The new EU Waste Framework Directive promotes the investigation of more innovative strategies for the valorisation of the organic wastes to increase the sustainability of their managements, of their market value and to allow the creation of new job opportunities [11]. In particular, it supports the “cascade biorefinery approach” for agrofood wastes, where the extraction of valuable biomolecules (even in small quantities) for pharmaceutical, chemical, cosmetic, agronomic applications and the production of high added value compounds are priority. The European Commission, therefore, specifies that bioenergy production should be approached only after these processes, while the wastes disposition in landfill should be avoided.

Different solid/liquid extraction methods have been tested using polar and non-polar solvents allowing to recover a coffee oil from SCG, whose commercial value is higher than coffee itself. Coffee oil is mainly composed by lipids but contains important bioactive compounds. Among them, tocopherols are the most precious substances being the basic compounds of Vitamin E, useful for the prevention of several healthy diseases, from heart and nervous systems to infertility. Tocopherols are also considered anti-oxidants which can be used to improve the oxidation stability of biofuels. Loyalo et al. [12] adopted different solvents to recover tocopherols finding a maximum tocopherol extraction of about 20 mg/g of dry SCG using ethyl acetate and n-propanol as solvents. Other studies reported tocopherols extraction yields in the range of 6.5-31.7

mg/g dry SCG. The difference depends essentially on sample origin, roasting procedure, storage and processing, but also from coffee particle sizes during tocopherol extraction [13, 14]. Two interesting coffee-specific diterpenes, Cafestol and Kahweol, are particularly important for health, being anticarcinogenic substances. Due to the peculiar taste and flavoring, they are used as fundamental molecules for the production of many food products, such as candies, cakes, and beverages. Consequentially, Cafestol and Kahweol find several applications in pharmaceutical and food sectors [15]. Moreover, SCG have high content of polyphenols compounds, in particular of chlorogenic acid which, with its isomers and derivatives, are known to be antioxidants, to have effects against inflammation, to reduce the risk of cardiovascular and of liver diseases [1]. Linoleic acid is also present in high concentration in SCG. It has an 18-carbon chain with two double bonds in cis configuration, belonging to the polyunsaturated omega-6 fatty acids family. Linolenic acid prevents skin cancer and diseases of the heart and blood vessels [16].

This study will investigate on the cascade biorefinery application for the valorization of SCG (Figure 1).

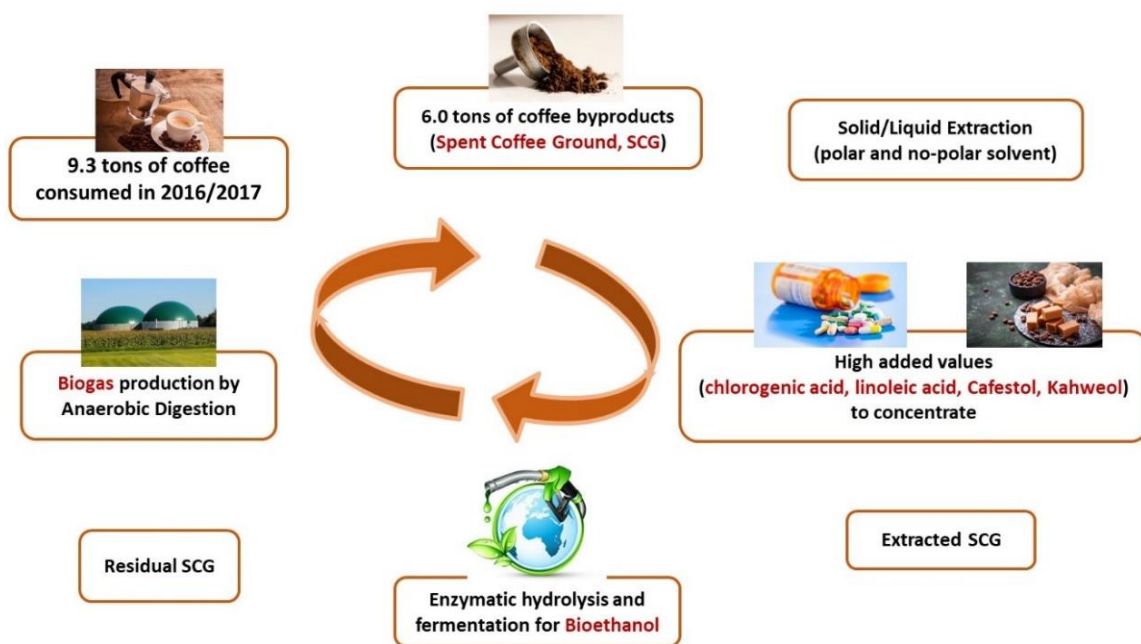


Figure 1. The cascade biorefinery approach for the SCG valorisation

Based on this configuration, SCG will be firstly used for the extraction of high economic value molecules (tocopherols, linoleic acid, chlorogenic acid, Cafestol and Kahweol). Different polar and no polar solvents have been tested in a Soxhlet apparatus and evaluated in terms of coffee oil extracted and of amount of specific high added value molecules recovered. As they still have high content of cellulose and hemicellulose, the extracted SCG have been further used for bioethanol production by *Saccharomyces Cerevisiae* fermentation with a preliminary stage of combined acid-enzymatic hydrolysis for the cellulose conversion in fermentable sugars [17] Lastly, the solid residues from bioethanol production have been exploited for biogas production by AD.

## 2. Materials and Methods

### 2.1 Spent Coffee Grounds and inoculum's characterization

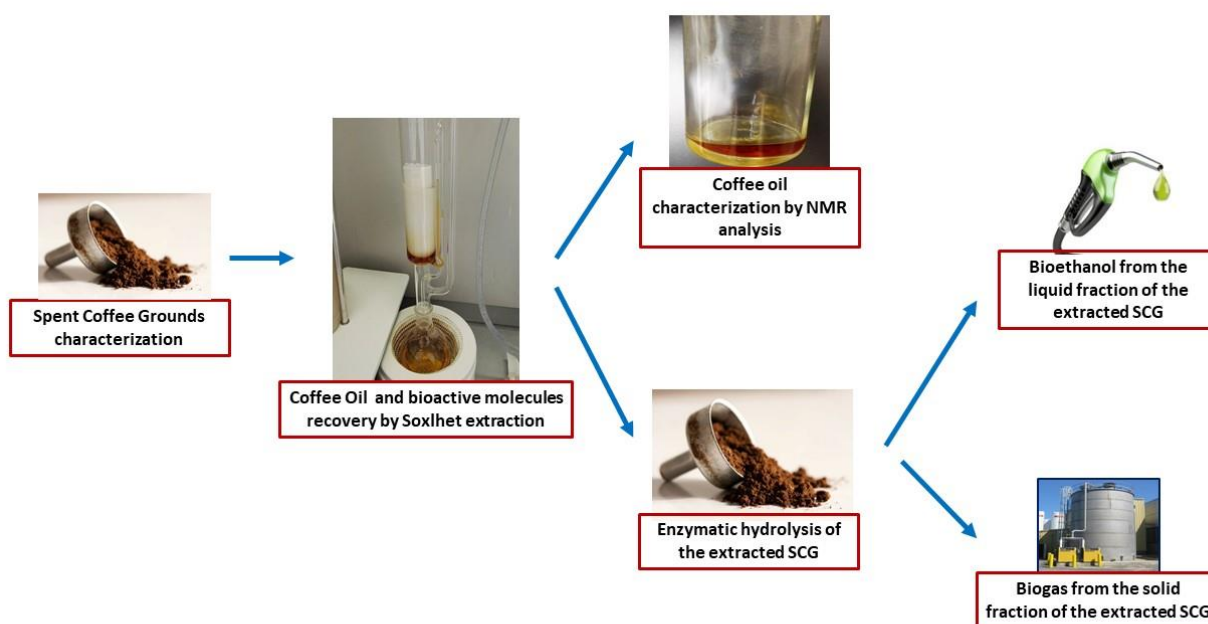
SCG were collected from a coffee machine of the biotechnology department of the University of Verona. Inoculum to assure the microbial presence for the AD tests derived from Isola della Scala biogas plant (Italy) treating a mixture of bovine manure, chicken manure and rice straw and operating at mesophilic conditions (35°C). Table 1 summarizes the chemical characteristics of SGC immediately after the coffee preparation and of the inoculum.

	SCG	Inoculum
pH	4,53 ± 0.04	8.53 ± 0.02
TS (% w/w)	39.24 ± 1.31	9.06 ± 0.08
VS (% w/w)	39.09 ± 1.27	6.16 ± 0.57
VS/TS (%)	99.59 ± 0.28	67.99 ± 2.23
COD (g/L)	15.40 ± 0.12	45.24 ± 2.15
TKN (g/kg)	2.54 ± 0.20	7.18 ± 0.40
P (g/kg)	0.60 ± 0.07	2.97 ± 0.10
Cellulose (% w/w dry matter)	20.60 ± 1.62	not measured
Hemicellulose (w/w dry matter)	25.57 ± 1.86	not measured
Lignin (% w/w dry matter)	12.28 ± 0.83	not measured

Table 1. Characteristics of SCG and of the inoculum

## 2.2 A cascade biorefinery approach for the SCG valorization

The SCG, recovered from the coffee machine, followed different biologic processes to create a cascade biorefinery approach, reported in Figure 2.



The SCG were located at 60°C for three days to evaporate water content. 10 g of dried SCG were used for the solid/liquid extraction by Soxhelt apparatus testing different polar and no polar solvents. The solvent was removed from the solvent-oil coffee mixture through a pre-weighted rotary evaporator flask. The extracted oil coffee has been characterized by NMR analysis for the individuation of the most interesting added value molecules: Tocopherols, linoleic acid, Cafestol and Kahweol. These compounds have been quantified by an external chemical laboratory in Bologna.

The extracted SCG were diluted and hydrolysed by a combined acid and enzymatic treatment to desegregate the long cellulose chains and to convert cellulose in glucose, respectively. The hydrolysis was followed by a solid/liquid separation by centrifugation at 5,000 rpm: the liquid fraction rich in soluble sugars, was fermented by *S. Cerevisiae* for bioethanol production; the solid fraction, represented the residual SCG, were mixed with inoculum for biogas production by mesophilic AD. The details of each biological process have been provided in the following paragraphs.

### 2.2.1 Coffee oil extraction and characterization

10 g of dried SCG sample was placed in a cellulose extraction thimble (WHATMAN Cat. No. 2800-373) and plugged with cotton. The thimble was located in a Soxhlet extractor. 300 mL of polar and no-polar solvents, selected based on the oil recovery yields obtained by previous works [12, 18, 19] of acetone, ethanol, iso-propanol and n-hexane were tested in triplicate for the SCG solid/liquid separation. A 50:50 (v/v) mixture of the two best solvents, ethanol and iso-propanol, was tested too. All the solvents were tested at 85°C, value higher than boiling temperature of the different solvents. The Soxhlet extraction was stopped after 3 extraction cycles.

The extracts (coffee oil-solvent mixture), were transferred into a pre-dried and pre-weighted rotatory evaporator flask. The performances of the different solvents were evaluated in terms of coffee oil recovered from SCG and expressed as coffee oil yield (COY), calculated according Eq. (1):

$$\text{COY (\% w/w)} = \frac{M_{oil}}{M_{SCG}} \times 100 \quad \text{/Equation 1/}$$

Where  $M_{oil}$  is the mass of the extracted coffee oil and  $M_{SCG}$  is the mass of the SCG dried sample.

0.025 ml of coffee oil from the different solvents extractions were selected for the  $^1\text{H-NMR}$  analysis. Coffee oils from no-polar solvent (n-hexane) were diluted in 1 ml of chloroform-d ( $\text{CDCl}_3$ ), while the ones extracted from polar solvents (acetone, ethanol, iso-propanol and the 50:50 (v/v) ethanol-isopropanol mixture were diluted in methanol-d [20].

NMR spectroscopic analysis of oil samples was carried out at 298°K using a Bruker Advance III 600 spectrometer, operating at 600.13 MHz, and equipped with a cryoprobe. The  $^1\text{H-NMR}$  spectra were recorded using the Bruker-standard sequence with 8 or 16 scans and a spectral width of 12,019 Hz. The 2D 1H-1H total correlated spectroscopy (TOCSY) spectra were performed with a 60-ms mixing time, using Bruker's "mlevsgpph" pulse sequence. 2D spectra were acquired with 4 total transients, a time domain of 2k points (F2) and 512 experiments (F1) and a spectral window of 10200 Hz in both dimensions. All spectra were manually phased and baseline corrected using TOPSPIN 3.5.6 (Bruker) and were referenced to TSP signal at 0 ppm.  $^1\text{H-NMR}$  signals of identified molecules were assigned based on comparisons with the chemical shifts of standard metabolites in the Biological Magnetic Resonance Data Bank [21].

The quantification of the tocopherols, linoleic acid, Cafestol and Kahweol were performed by an external laboratory by HPLC.

### 2.2.2 Acid – Enzymatic hydrolysis

The extracted SCG from the test having the higher coffee oil extraction, the 50:50 (v/v) ethanol-iso-propanol mixture, were prepared for bioethanol production according the Know et al. method [5]: they were diluted at a TS content of 15.00 % w/v and then performed through an acid pretreatment by 1%  $\text{H}_2\text{SO}_4$  addition. By this way, long cellulose and hemicellulose chains were induced to smaller molecules. After 1 hour, the pH was adjusted at 5.5 for the enzymatic hydrolysis adding a 2% w/w of Cellic CTec-2 (Novozymes) cellulase. The enzymatic hydrolysis was performed at 55°C and had a duration of 48 h, which was found as the ideal duration for an adequate cellulose conversion into glucose [22]. The glucose concentration was measured before acid pretreatment and at the end of the enzymatic hydrolysis by colorimetric method provided by Sigma-Aldrich assay kit [23].

### 2.2.3 Ethanol fermentation of the liquid fraction

At the end of the enzymatic hydrolysis the SCG were centrifuged at 5,000 rpm for 20 minute. The liquid fraction, rich in soluble sugars, have been fermented by *Saccharomyces Cerevisiae* anaerobic batch fermentation at 37°C (L-EtOH tests). In particular, the *S. cerevisiae* strain (24860 ATCC) at the concentration 2% (w/w<sub>dry SCG</sub>) was used for the fermentation [5]. *S. cerevisiae* yeast was considered for the ethanol production from lignocellulosic materials because of its successful exploitation in the fermentation industry, its proven ability to produce high ethanol concentrations and its high level of resistance against the inhibitors found in lignocellulose hydrolysates [24]. During the batch tests, the biogas production,

constituted by the CO<sub>2</sub> fermentation byproducts, was measured by the water replacement method [7]. Ethanol fermentation was considered concluded when no biogas production was produced. Ethanol fermentation tests were conducted in triplicate.

L-EtOH tests have been compared with i) SCG which were not previously performed by oil coffee recovery by Soxhlet solid/liquid separation (SCG EtOH tests) and ii) by SCG which were performed by both Soxhlet separation and acid-enzymatic hydrolysis but were not centrifuged. Consequentially, the ethanol production was conducted keeping Solid and Liquid phase (SL-EtOH).

At the end of the fermentation, samples were analysed for the determination of ethanol concentration, following the procedure described by Megazyme booklet [25].

#### **2.2.4 Biogas production from the solid fraction**

Solid fraction (SF) from acid-enzymatic hydrolysis was performed for the biogas production by AD (SF-AD tests). SF-AD tests were compared with the AD of SCG coming directly from coffee (without no further biological process or pretreatment) (SCG-AD test) and with the AD of SCG's solid phase coming from Soxhlet Extraction (SE) but not processed by an acid-enzymatic pretreatment (SE-AD tests), conducted according the method by [24]. By this way, it was possible to estimate how each bio-chemical stage influenced the potential of methane production from SCG.

The methane yield was evaluated through biomethane potential tests (BMP) which were carried out following the methodology suggested by Angelidaki et al. [26]. Trials were carried out in 0.5 L reactors, with 150 mL working volume, sealed with chloro-butyl caps. Each trial was performed in triplicate. The duration of the tests have been determined following the protocol by Holliger et al. [27], which established that BMP tests have to be stopped when the daily biogas production is lower than 1% of the cumulative one for three consecutive days.

Inoculum (Table 1) was incubated at the test temperature (37°C) for one week to reach the endogenous methane production, while microcrystalline cellulose BMP tests were used as positive control [26, 27]. All batch digesters were manually stirred once a day. The volume of biogas generated during the batch trials was determined by water displacement method, while the methane content was determined using a portable biogas analyser (Geotech Biogas 5000 by GeoTech, United Kingdom).

To evaluate BMP parameters, a regression curve was calculated to describe the biogas produced in our experimental set up. The Gompertz model was employed as its sigmoidal function is one of the most useful for characterizing saturation kinetics [28]. During saturation, biogas production proceeds slowly at early time points and continues for a long time, until the end of the reaction is reached (producing an asymptotic value), it is possible to describe exponential growth using the following Gompertz equation:

$$G = G_{\max} \exp \left\{ -\exp \left[ \frac{R_{\max} + e}{G_{\max}} (\lambda - t) + 1 \right] \right\} \quad \text{/Equation 2/}$$

where G (NL/L) represents the cumulative volume of biogas produced during time t, R<sub>max</sub> (NL/L day) represents the maximum value of the biogas production rate, G<sub>max</sub> (NL/L) represents the total quantity of the biogas produced as an asymptotic value and λ (day) represents the duration of the lag phase.

### **2.3 Analytical methods**

Each substrate was analysed in terms of dry matter (TS), volatile solids (TVS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total phosphorus (TP) according to the Standard Methods [29]. In particular, TKN and TP have been processed through a high performance Ethos-One microwave digestion system by Milestone (Italy) and the UDK 129 distillation unit by Velp Scientifica (Italy).

### 3. Results and discussions

#### 3.1 Coffee oil extraction

Figure 3 shows the coffee oil extraction yields (COY) obtained from the different solvents.

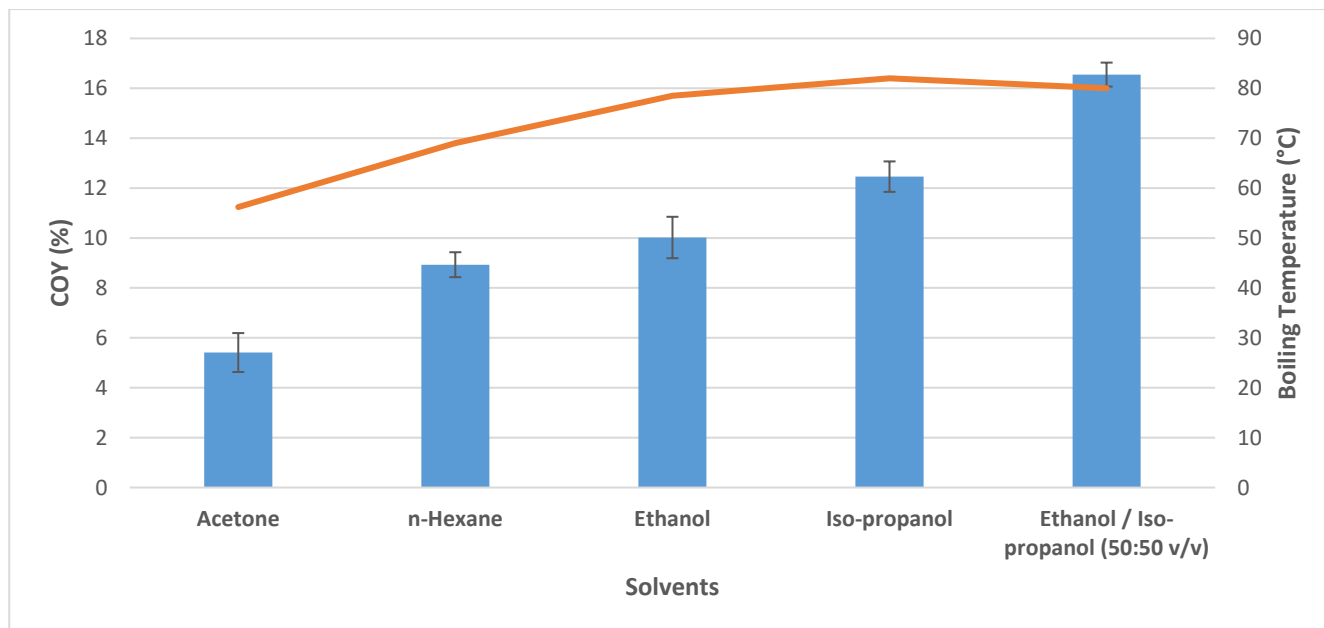


Figure 3. Coffee oil yield obtained from different solvents.

Acetone obtained the lowest COY performances with the 5.41%. It was followed by n-hexane with 8.93%. Ethanol and iso-propanol had better yields: the 10.02% and 12.46%, respectively. Considering the better performance of these two polar solvents, a mixture of ethanol and iso-propanol (50:50 % v/v) was considered to evaluate their combined effect. The mixture increased COY which reached the 16.55%. The oil extraction trends of the different solvents were coherent with data from Loyao et al. [12] where no polar solvents have lower yields than polar ones, with the only exception of acetone. On the contrary, Al-Hamamre et al. [18] achieved better performances with n-hexane and acetone (15.28% and 12.92%, respectively), than ethanol and iso-propanol, which had both a COY of about 11.50%. This behaviour can be explained considering the limited extraction time of 0.5 h adopted by Al-Hamamre et al. [18]. This time was too short for a complete oil extraction from SCG, especially with solvents having higher boiling temperatures, such as ethanol and iso-propanol having 78.5°C and 82°C against the boiling temperature of acetone and n-hexane of 56.2°C and 69°C. The boiling temperature has a direct effect on the extraction oil. By one hand, lower is the boiling temperature, quicker is the solvent evaporation. By this way, the re-condensed solvent meets the SCG before than the solvents having higher boiling temperatures, spending more time in contact with SCG. Anyway, this effect is valid when extraction time is limited, as in the case of Al-Hamamre et al. [18]. Instead, with the increasing of the extraction time the behaviour is opposite: higher boiling temperatures solvent favoured the oil extraction as the time between the cycle in Soxhlet apparatus is higher, allowing a more efficacy extraction action by the solvents. This theory explained results obtained by this work, where 3 Soxhlet extraction cycles were made. The relation between the boiling temperature and COY has been evidenced in Figure 3. Acetone was the first to complete the first cycle within 1.0 hour and the all-3 cycles in less than 2.5 hours. Iso-propanol, having the higher boiling temperature among the selected solvents, completed the first cycle in about 3.0 hours and the all process in 5.5 hours. Thus, it was evident that 0.5 h was inadequate for the solid/liquid extraction, especially with high boiling temperature solvents, which required longer contact time with the substrates to express their solubilisation potential.

The boiling temperature is clearly just one factor affecting COY. The chemical symbiosis between solvent and SCG is fundamental. The solvent-SCG chemical interaction can be expressed by the water-octanol partition coefficients ( $K_{ow}$ ), defined as a particular ratio of the concentrations of a solute between two solvents: a hydrophobic solvent (octanol) and a hydrophilic one (water). Consequentially,  $K_{ow}$  expresses a measure of lipophilicity or hydrophobicity of a compound [30].

Considering the  $K_{ow}$  of the solvents adopted by this work, used in this study, like n-hexane is characterized by high value,  $K_{ow} = 4.5 \times 10^4$ , revealing its hydrophobic nature. Instead, iso-propanol ( $K_{ow} = 0.64$ ) and ethanol ( $K_{ow} = 0.54$ ) have partition coefficients close to one, which means that these solvents, even if are more hydrophilic, are also able to extract hydrophobic components during Soxhlet extraction, especially iso-propanol. Consequentially, the higher COY reached by ethanol and mainly iso-propanol explainable by the extraction of both hydrophilic and hydrophobic molecules, instead of the only hydrophobic compounds by n-hexane, characterized by very high  $K_{ow}$ . This trend found confirmation also through Polarity index (P) which are 0.10, 3.90 and 5.20 for n-hexane, iso-propanol and ethanol, respectively [31].

### 3.2 Enzymatic hydrolysis and Ethanol fermentation

Table 2 reports the final glucose and bioethanol concentrations after the acid-enzymatic and fermentation stages, respectively.

	Glucose (g/L)	Ethanol (g/L)
SCG EtOH tests	31.86 ± 2.60	14.66 ± 0.52
L-EtOH tests	33.29 ± 0.84	15.12 ± 2.20
SL-EtOH tests	32.27 ± 0.91	12.99 ± 0.28

Table 2. Glucose and ethanol concentrations

As commented, SCG have high content of cellulose and hemicellulose (about 20% and 25%, respectively, Table 1), potentially degradable in pentose and hexoses sugars. These latter, and in particular glucose, are the main indispensable substrates for ethanol production by *S. Cerevisiae*. Lignocellulosic matter derives essentially from skin and pulp of coffee seeds. They are high molecular weight and insoluble polymers and, consequentially, are recognized to be highly recalcitrant by the microorganisms action [7].

The first acid pretreatment on SCG helped the solubilisation of cellulose and hemicellulose, reducing the crystallization degree and increasing porosity and specific surface of SCG. Moreover, it allowed the hydrolysis of lignin, the most recalcitrant between lignocellulosic materials [32]. The following enzymatic hydrolysis was the responsible of the conversion of the cellulose fragments from acid stage into hexose sugars, and in particular glucose. All the considered tests allowed the reaching of the same glucose concentrations (around 30 g/L), with no difference for SCG without previous coffee oil extraction by Soxhlet, or solid/liquid phase separation after acid-enzymatic stages. Consequentially, the resulting bioethanol production was also similar for SCG-EtOH, L-EtOH and SL-EtOH tests between 13 and 15 g/L, close to the theoretical glucose-ethanol conversion of the 50%.

These results allowed achieving two important conclusions. The first is that bioethanol production was not influenced by coffee oil extraction. Long fatty acids are the main molecules constituting coffee oil. In particular, palmitic acid (C16:0), stearic acid (C18:0) and linoleic acid (C18:2) counting for the 90% of the total fatty acids [33]. Glycerides unsaponifiable matter are other important constituents of the coffee oil [34]. All these compounds are not involved in bioethanol production, thus, their extraction from SCG did not affected the bioethanol fermentation yield. The second conclusion is deductible by the performances of L-EtOH and SL-EtOH tests. It was demonstrated that liquid phase after acid-enzymatic stage contained the soluble molecules, hexose sugars, exploitable by *S. Cerevisiae* for bioethanol production. Instead,



solid phase brought to a slight reduction of bioethanol production (Table 2), probably as effect of a minimal glucose absorption of solid phase.

### 3.3 Biogas production from the solid phase

The methane trends along time for SF-AD, SCG-AD and SE-AD tests were reported in the following Gompertz curves (Figure 4).

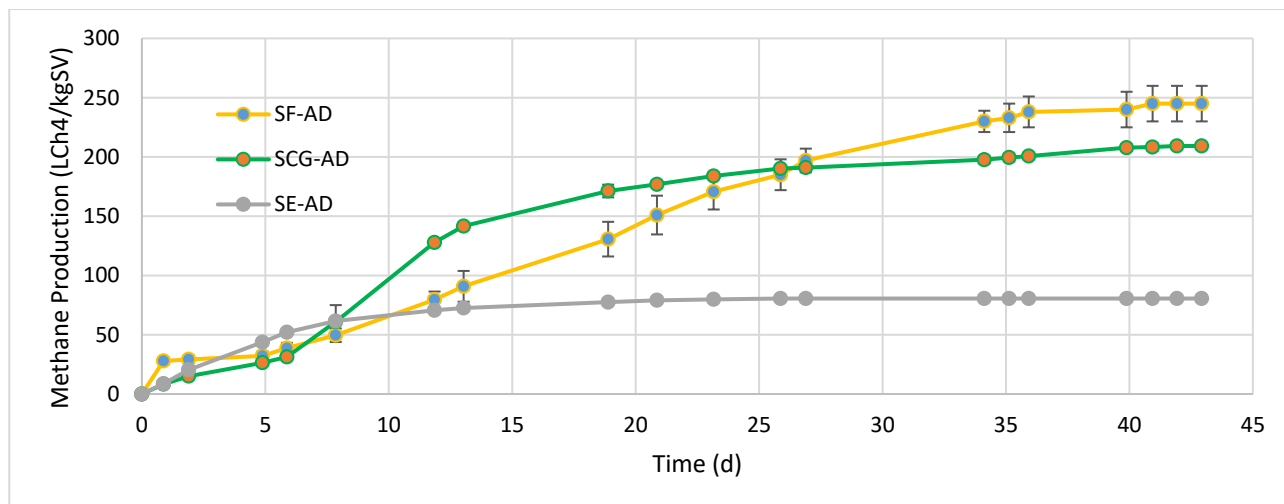


Figure 4. Methane production over time

SCG-AD tests, performed on SCG directly taken from coffee machine, were characterized by a long lag phase,  $\lambda = 4.50$  days, demonstrating that without employing pretreatment the kinetics of the bioreactions are slow, likely because of the presence of complex molecular structures within the reaction environment that inhibit the microorganisms activity [35]. The final methane production achieved 208.35 L<sub>CH<sub>4</sub></sub>/kg<sub>VS</sub>, coherent with the performances obtained by previous research works, founding a yield between 150-250 L<sub>CH<sub>4</sub></sub>/kg<sub>VS</sub> [7, 36]. SE-AD tests, where the SCG were coffee oil was extracted, but the solid particles were not treated by acid-enzymatic process, obtained lower yield: 80.54 L<sub>CH<sub>4</sub></sub>/kg<sub>VS</sub>. It represents a fundamental result to take into account in the design of the biorefinery concept for SCG valorisation. In fact, it showed that coffee oil recovery, even if it did not influence bioethanol production, had consequence on methane production. As previously reported, coffee oil is mainly composed by lipids and in particular by fatty acids, which remained just in small amounts in solid phase of the SCG and which are among the main substrates for the biogas production by AD [37].

SF-AD tests were performed on solid phase from acid-enzymatic hydrolysis, preceded by coffee oil recovery by Soxhlet extraction. The performances in terms of methane yield were the best with 245.10 L<sub>CH<sub>4</sub></sub>/kg<sub>VS</sub>. It was an unexpected result as the molecules, precursors of the biogas, the lipids, fatty acids and sugars, have been already removed by Soxhlet extraction and solid/liquid separation after acid-enzymatic stages. Probably, acid-enzymatic hydrolysis contributed to desegregate the lignocellulosic materials which were exploited by microorganisms after a long lag phase (7 days), necessary to methanogens to reach a stable kinetic. It is important to remember that previous considered SE-AD and SCG tests were not representing the proposed cascade biorefinery approach, proposed by this work (Figure 2). They served just as comparisons with the SF-AD tests, constituting the last phase of our cascade biorefinery process.

### Conclusions

A cascade biorefinery approach for the simultaneous production of high added values compounds and biofuels has been applied to SCG, one of the most abundant food wastes produced in the world. Ethanol-iso-propanol (50/50 v/v) mixture presented a boiling temperature, among the higher than the proposed solvents, which favored a longer contact time with SCG. Moreover its water-octanol partition coefficient, close to the unit, allowed the extraction of both hydrophilic and hydrophobic compounds. By this way, ethanol-iso-propanol mixture had the best coffee oil recovery yield of more than

16% (w/w). The extracted SCG were treated by acid-enzymatic hydrolysis to allow the cellulose desegregation and conversion into glucose. After the solid/liquid separation, the liquid phase was fermented by *S. Cerevisiae*, obtaining an ethanol production of 15 g/L. Instead, the solid fraction, were used for biogas production by AD with a methane yield of about 250 L<sub>CH<sub>4</sub></sub>/Kg<sub>vs</sub>.

## References

- [1] Pettinato M., Casazza A.A., Ferrari P.F., Palombo D., Perego P.: Eco-sustainable recovery of antioxidants from spent coffee grounds by microwave-assisted extraction: Process optimization, kinetic modeling and biological validation. *Food and Bioproducts Processing* 114, 31–42 (2019).
- [2] Mata T.M., Martins A.A., Caetano N.S.: Bio-refinery approach for spent coffee grounds valorization. *Bioresource Technology* 247, 1077-1084 (2018).
- [3] Nguyen, Q.A., Cho, E.J., Lee, D.-S., Bae, H.-J.: Development of an advanced integrative process to create valuable biosugars including manno-oligosaccharides and mannose from spent coffee grounds. *Bioresource Technology* 272, 209-216 (2019).
- [4] Burniol-Figols A., Cenian K., Skiadas I.V., Gaval H.N.: Integration of chlorogenic acid recovery and bioethanol production from spent coffee grounds. *Biochemical Engineering Journal* 116, 54–64 (2016).
- [5] Kwon E.E., Yi H., Jeon Y.J.: Sequential co-production of biodiesel and bioethanol with spent coffee grounds. *Bioresource Technology* 136, 475-480 (2013).
- [6] Mussatto S.I., Machado E.M.S., Martin S., Teixeira J.A.: Production, composition and application of coffee and its industrial residues. *Food Bioprocess Tech.* 4, 661-672 (2011).
- [7] Battista F., Fino D., Mancini G.: Optimization of Biogas production from coffee production waste. *Bioresource Technology* 200, 884-890 (2016).
- [8] Vasmara C., Marchetti R: Spent coffee grounds from coffee vending machines as feedstock for biogas production. *Environmental Engineering and Management Journal* 17(10), 2401-2408 (2018).
- [9] Henandez, M.A., Susa, M.R., Andres, Y.: Use of coffee mucilage as a new substrate for hydrogen production in anaerobic co-digestion with swine manure. *Bioresour. Technol.* 168, 112–118 (2014).
- [10] Abouelenien, F., Namba, Y., Kosseva, M.R., Nishio, N., Nakashimada, Y.: Enhancement of methane production from co-digestion of chicken manure with agricultural wastes. *Bioresour. Technol.* 159, 80–87 (2014).
- [11] Directive (EU) 2018/851 of the european parliament and of the council of 30 May 2018 amending Directive 2008/98/EC on waste. *Official Journal of the European Union* L150/140 (2018).
- [12] Loyao A.S., Villasica S.L.G., Dela Peña P.L.L., Go A.W.: Extraction of lipids from spent coffee grounds with non-polar renewable solvents as alternative. *Industrial Crops & Products* 119, 152–161 (2018).
- [13] Akgun, N.A., Bulut, H., Kikic, I., Solinas, D.: Extraction behavior of lipids obtained from spent coffee grounds using supercritical carbon dioxide. *Chem. Eng. Technol.* 37, 1975–1981 (2014).
- [14] Tavares, K.M., Lima, A.R., Nunes, C.A., Silva, V.A., Mendes, E., Casal, S., Pereira, R.G.F.A.: Free tocopherols as chemical markers for Arabica coffee adulteration with maize and coffee by-products. *Food Control* 70, 318–324 (2016).
- [15] Williamson, K., Hatzakis, E.: NMR analysis of roasted coffee lipids and development of a spent ground coffee application for the production of bioplastic precursors. *Food Research International* (2019). <https://doi.org/10.1016/j.foodres.2018.10.046>.
- [16] <https://www.webmd.com/vitamins/ai/ingredientmono-1035/alpha-linolenic-acid>.

- [17] Battista, F., Bolzonella, D.: Some critical aspects of the enzymatic hydrolysis at high dry-matter content: A review. *Biofuels, Bioproducts and Biorefining* 12 (4), 711-723 (2018).
- [18] Al-Hamamre, Z., Foerster, S., Hartmann, F., Kroger, M., Kaltschmitt, M.: Oil extracted from spent coffee grounds as a renewable source for fatty acid methyl ester manufacturing. *Fuel* 96, 70-76 (2012).
- [19] Karmee, S.K.: A spent coffee grounds based biorefinery for the production of biofuels, biopolymers, antioxidants and biocomposites. *Waste management* 72: 240-254 (2018).
- [20] Efthymiopoulos, I., Hellier, P., Ladommatos, N., Russo-Profilo, A., Eveleigh, A., Aliev, A., Kay, A., Mills-Lamptey, B.: Influence of solvent selection and extraction temperature on yield and composition of lipids extracted from spent coffee grounds. *Industrial Crops & Products* 119, 49–56 (2018).
- [21] Biological Magnetic Resonance Data Bank. A Repository for Data from NMR Spectroscopy on Proteins, Peptides, Nucleic Acids, and other Biomolecules. <http://www.bmrb.wisc.edu/>
- [22] Battista, F., Gomez Almendros, M., Rousset, R., Bouillon, P.A. : Enzymatic hydrolysis at high lignocellulosic content: Optimization of the mixing system geometry and of a fed-batch strategy to increase glucose concentration. *Renewable Energy* 131, 152-158 (2019).
- [23] Sigma-Aldrich, 2014. Glucose and sucrose colorimetric/fluorometric assay kit. Catalog number MAK03. <https://www.sigmaaldrich.com/catalog/product/sigma/mak013?lang=it&region=IT> .
- [24] Battista, F., Mancini, G., Ruggeri, B., Fino, D.: Selection of the best pretreatment for hydrogen and bioethanol production from olive oil waste products. *Renewable Energy* 88, 401-407 (2016).
- [25] Megazyme, 2018. Ethanol assay kit. [https://secure.megazyme.com/files/Booklet/K-ETOH\\_DATA.pdf](https://secure.megazyme.com/files/Booklet/K-ETOH_DATA.pdf)
- [26] Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B.: Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays, *Water Sci Technol*; 59 (5), 927-934 (2009).
- [27] Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S. et al : Towards a standardization of biomethane potential tests. *Water Sci Technol* 74, 2515-2522 (2016).
- [28] Lay, J., Lee, Y.J., Noike, T.: Feasibility of biological hydrogen production from organic fraction of municipal solid waste, *Water Res.* 33 (11), 2579- 2586 (1999).
- [29] APHA/AWWA/WEF, Standards Methods for the Examination of Water and Wastewater, United Book Press Inc., Baltimore, Maryland (1998).
- [30] Sangster, J: Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry. Wiley Series in Solution Chemistry. 2. Chichester: John Wiley & Sons Ltd. p. 178. ISBN 978-0-471-97397-3 (1997).
- [31] Linstrom, P.J., Mallard, W.G.: NIST Chemistry Webbook: Standard Reference Database No. 69 (2001).
- [32] Vismara, R., Canziani, R., Malpei, F., Piccinini, S.: Biogas da agrozootecnica e agroindustria. Dario Flaccovio Editore, Palermo (2011).
- [33] Ahangari B., Sargolazei J.: Extraction of lipids from spent coffee grounds using organic solvents and supercritical carbon dioxide. *J. Food Process.* 37, 1014-1021 (2013).
- [34] Jenkins, R.W., Stageman, N.E., Fortune, C.M., Chuck, C.J.: Effect of the type of bean, processing, and geographical location on the biodiesel produced from waste coffee grounds. *Energy Fuels* 28, 1166–1174 (2014).
- [35] Ruggeri, B., Battista, F., Bernardi, M., Fino, D., Mancini, G.: The selection of pretreatment options for anaerobic digestion (AD): A case study in olive oil waste production. *Chemical Engineering Journal* 259, 630–639 (2015).
- [36] Houbroun, E., Larrinaga, A., Rustrian, E.: Liquefaction and methanization of solid and liquid coffee wastes by two phases anaerobic digestion process. *Water Sci. Technol.* 48, 255–262 (2003).

[37] Bolzonella, D., Battista, F., Cavinato, C., Gottardo, M., Micolucci, F., Lyberatos, G., Pavan, P., Recent developments in biohythane production from household food wastes: A review. *Bioresource Technology* 257, 311-319 (2018).