The sequential biorefinery approach for biodiesel and glucose production from Spent Coffee Grounds

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ABSTRACT

Spent Coffee Grounds (SCGs) are rich in high-value compounds, such as saturate and unsaturate fatty acids, and in cellulose. These compounds can be exploited for the production of new valuable biomaterials and biofuels through the "cascade biorefinery approach", consisting in the set up of different sequential chemical/physical/biological operations. This work investigated the adoption of SCG for i) biodiesel production from the coffee oil, previusoly extracted by soxhlet apparatus; ii) fermentable sugars production from the SCGs' cellulose and emicellulose and iii) biomethane production from the residual solid fraction after the sugars synthesis. The transesterification process for biodiesel production reached the best performances of 86% w/w of Fatty Acid Methil Esters (FAMEs) using 1:8 coffee oil/methanol ratio and 2% w/w of KOH as catalyst. The organosolv pretreatment, performed with the glycerol derived from biodiesel production, allowed to reduce the acid concentration from 1.0 to 0.5% w/w during the SCG pretreatment. The following saccharification at 10 FPU of enzyme activity, led to a final sugars content of about 45% w/w. Finally, biomethane production by Anaerobic Digestion was conducted. The low content of easily degradable compounds comported a methane production of only 50 L_{CH4}/kg_{TVS}.

KEYWORDS: Spent Coffee Grounds; Biorefinery; Biodiesel; Enzymatic Hydrolysis; Glucose Production; Anaerobic Digestion

1. Introduction

Coffee is one of the most consumed beverages around the world, indeed, its consumption was estimated in 9.3 million tons in 2017 around the world[1], with a consequent annual 6 million tons average generation of Spent Coffee Grounds (SCGs)[2]. SCGs are heterogenous matrixes, which besides cellulose, hemicellulose and lignin, contain also high value-added compounds, such as fatty acids, tocopherols (vitamin E), kahweol and cafestol[3]. Noteworthy, this latters could be recovered from SCGs and exploitated for cosmetical, food and bioenergy applications. However, despite their great potential as source of valuable chemicals, currently SCGs are mainly incinerated or disposed of in landfills. In the best case, SCGs are collected with the Organic Fraction of Municipal Solid Wastes (OFMSW) and used for biogas production via anaerobic digestion[3]. It is worth mentioning that the new EU Waste Framework Directive (2018/851) fosters a better exploitation of the organic wastes, through the adoption of the so called "cascade biorefinery approach" for agri-food residues, for which the extraction of valuable biomolecules (even in small amounts) to produce high value-added bio-materials represents a priority over the current biofuels production[4].

SCGs are promising substrates for biodiesel production because they show a high concentration of long fatty acids, which is comparable with that of conventional 1st generation feedstocks[5–7]. Moreover, SCGs show the additional advantage of having absent or realtivly low costs, being the main leftover of both instant coffee production and coffee brewing[5]. Conversion of oils from SCGs to biodiesel can be performed by acid-, alkali- or enzymatic-catalysed transesterification of coffee oils, usually using methanol as co-substate for the production of fatty acid methyl esters (FAMEs)[8]. The molar ratio of methanol to oils, the reaction temperature and time as well as the concentration of the catalyst are the main operational parameters that influence biodiesel production performances which is measured as yield of FAMEs[9,10]. Theoretically, the increase of solvent to oils ratio affects transesterification in a two-fold manner: a) increasing the efficiency, because the higher solvent concentrations favor biodiesel production (forward reaction) and 2) decreaseing the efficiency, because low oils concentration favor triglycerides production (reverse reaction)[11]. Thus, the setup of an optimal methanol:coffee oils ratio often represents a prerequisite for the efficient production of biodiesel from SCGs. The scientific literature reports FAMEs yields from transesterification process of coffee oils in the range of 55.5-95% w/w, according to the initial coffee oils concentration and the operational parameters[8]. Very high performances were obtained by Najdanovic-Visak et al. (2017)[11] with a FAMEs yield of 96%, corresponding to a methanol:coffee oils ratio of 400, at 60°C, for 90 min. Similarly, Park et al. (2016)[12] reported a 16.75% w/w maximum biodiesel yield (dry basis) at 95°C using 3.33 mL/g_{SCGs} of methanol, 3.33 mL/g_{SCGs} of chloroform and 0.5 mL/g_{SCGs} of H_2SO_4 as the catalyst. In general, it seems that the use of alkaline catalysts ledd to higher biodiesel yields. Optimum results (97%) were achieved when 0.75M of sodium hydroxide was added to coffee oils with a ratio of 1:2 methanol hexane used for fatty acids transesterification[13].

As it concerns polysaccharides in SCGs, these can be used both for the production of biofuels or biochemicals via microbial fermentation. However, SCGs must be pretreated to yield fermentable sugars (FSs), which inconveniently leads to the release and/or generation of toxic chemicals that hamper microbial proliferation and metabolism [14,15]. These chemical inhibitors can be classified into three major groups: i) phenolics (e.g. vanillin, syringaldehyde and coniferyl aldehyde), ii) weak acids (e.g. acetic acid, formic acid and levulinic acid) and iii) furaldehydes (e.g. furfural and 5-hydroxymethylfurfural) [15]. One possible strategy to overcome the inherent toxicity of SCGs-derived sugars

hydrolysates, would consist in performing a detoxification step before microbial fermentation. However, this would lead to the increase of production costs of the final fermented product(s) [16]. What we propose here, in order to mirigate the toxicity of SCGs hydrolysates, is to reduce the concentration of H_2SO_4 used for the chemicl pretreatment of SCGs and, in parallel, recirculate the glycerol leftover from FAME production to perform a mixed acid-catalysed/organosolv pretreatment of SCGs.

The scope of this study is to demonstrate the possible implementation of sequential processes to create an efficient cascade biorefinery for SCGs valorization (Figure 1). The first operational unit consists in the recovery of coffee oils from SCGs through solid extraction performed in a Soxhlet apparatus. Coffee oils are conv erted, through transesterification reactions, in an environmentally friendly fuel, namely biodiesel. Then, residual solid fraction s produced during oils extraction (defatted SCGs) are converted into FSs by means of a combination of sulfuric acid-catalyzed [17] and glycerol (organolsolv) pretreatments [18,19], followed by enzymatic hydrolysis [20]. SCGs-derived FSs constitute *per se* valuable compounds that can be potentially converted into a wide palette of useful chemicals via microbial fermentation, such as organic acids [21], plastic precursors [22] and biofuels [3]. Moreover, the use of glycerol organosolv pretreatment [19] is particularly appealing in this proposed cascate biorefinery, because glycerol is an internal leftover of the overall procees [23]; which is generated during biodiesel production from transesterification reactions of SCGs oils. The biorefinery cycle is closed by anaerobic digestion (AD) to exploit for biogas production from the more recalcitrant organic matter present in SCGs.

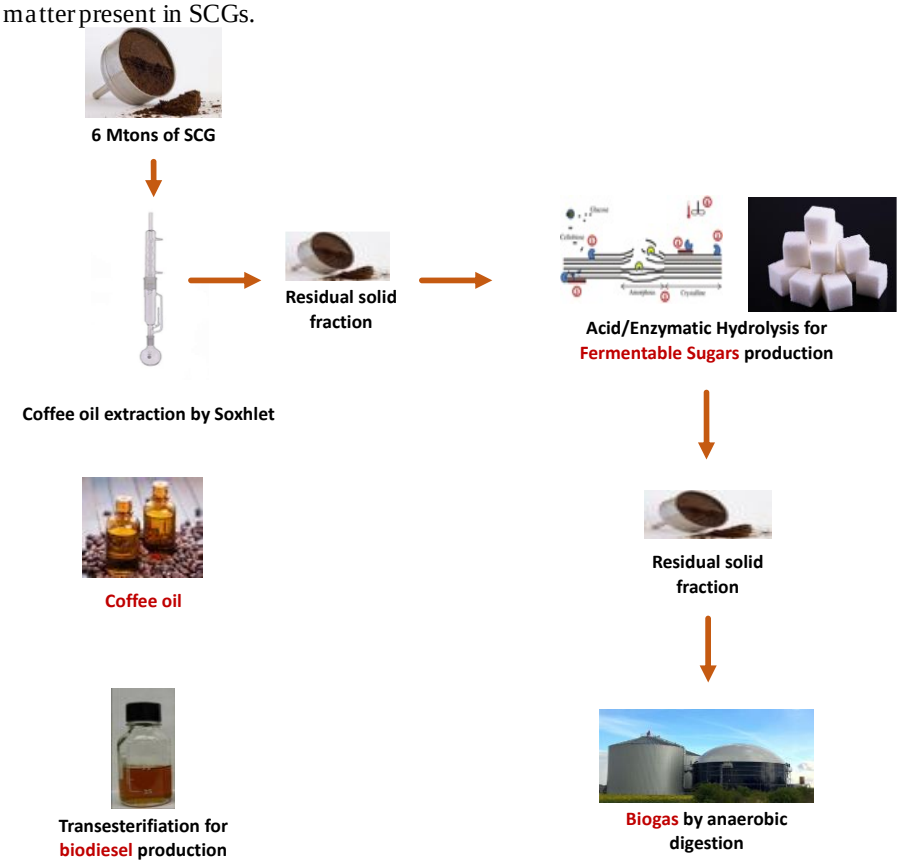

Figure 1. Cascade biorefinery scheme for the valorization of SCGs.

2. Materials and Methods

2.1 Spent Coffee Grounds and microbial inoculum characterizations

SCGs were collected from a coffee machine at University of Verona (Department of Biotechnology) and showed a the total solid (TS) concentration of around 39% w/w, almost completely represented by volatile solids (VS). The COD content was 15 g/kg, while N and P showed average concentrations of 2.5 and 0.6 g/kg, respectively. Table 1 summarizes the chemical characteristics of SGCs immediately after coffee brewing as well as that in the AD inoculum. The microbial inoculum adopted to perform AD tests were obtained from a biogas pilot plant located in Vicenza (Italy), which is regularly fed with OFMSW and operated at mesophilic temperature (35°C).

Table 1. Characteristics of SCG and of the inoculum

	SCG	Inoculum
pH	4.24 ± 0.04	8.29 ± 0.02
TS (% w/w)	42.20 ± 2.95	1.65 ± 0.10
VS/TS (%)	98.31 ± 0.26	68.90 ± 0.61
COD (g/kg)	45.24 ± 2.15	6.30 ± 0.24
TKN (g/kg)	2.54 ± 0.20	1.36 ± 0.19
Cellulose (% w/w dry matter)	20.6 ± 1.62	not measured
Hemicellulose (% w/w dry matter)	25.7 ± 1.86	not measured
Lignin (% w/w dry matter)	12.28 ± 0.83	not measured

2.2. A cascade biorefinery for SCGs valorisation

2.2.1. Coffee oils extraction from SCGs and transesterification reactions

After preliminary evaporation of the moisture content, performed at 60°C for three days, the SCG were subjected to solid-liquid extraction of coffee oils. The operation was previously optimized for the extraction of the long fatty acids, which represent the main substrates involved in the transesterification process for biodiesel production. In particular, n-hexane was used for coffee oils extraction in a Soxhlet apparatus, as described by Battista et al. (2020)[3], which allowed to recover about 10% of coofee oils (w/w) from dried SCGs. Major components were long fatty acids, in particular, palmitic and linoleic acids (Table 2).

Table 2. Most abundant long fatty acids from coffee oils extracted by n-hexane

Fatty acids in coffee oil (%)		
Palmitic Acid	$C_{16}H_{32}O_2$	34.78
Stearic Acid	$C_{18}H_{36}O_2$	8.58
Oleic Acid	$C_{18}H_{34}O_2$	10.07
Linoleic Acid	$C_{18}H_{32}O_2$	37.06
Linolenic Acid	$C_{18}H_{30}O_2$	0.83
Arachidonic Acid	$C_{20}H_{40}O_2$	3.49
Other		< 0.50

After extraction, coffee oils were used as substrates for transesterification tests at different molar ratios of coffee oils:methanol, using potassium hydroxide (KOH) at two different concentrations (1 and 2% w/w, referred to the coffee oils content) as alkaline catalyst. Transesterification tests were carried out in 50 mL tubes, filled with 5 g of SCGs, 0.05g of potassium hydroxide (1% of the coffee oils, w/w) and different methanol volumes, according to the following coffee oils:methanol molar ratios of 1:4; 1:8; 1:12; 1:16; 1:20. Another round of the tests was performed at the same coffee oils:methanol ratios, but with a double amount of alkaline catalyst (2% w/w). After its individuation, the best coffee oils:methanol molar ratio was adopted also for a transesterification tests at 3% w/w of KOH. Reaction tubes were placed in a thermostatic bath at 60°C for 2 hours, which was identified as the optimal conditions in terms of FAME yields[8,24,25]. To guarantee that the reaction had stopped, potassium hydroxide was neutralized by adding acetic acid (mole ratio KOH:CH_3COOH = 1:1). A laboratory distillation apparatus was used to evaporate the residual methanol from the biodiesel-glycerol mixture. Further separation of FAMEs and glycerol was performed by liquid–liquid extraction using n-hexane as the solvent. In particular, the mixture resulted in phase separation: i.e., a glycerol-rich bottom layer, and hexane-FAME-rich upper layer[11]. Determination of the total biodiesel yield as well as of the content of the different esters in biodiesel were achieved through Gas Chromatography/Mass Spectroscopy analysis of the organic liquid sample by an external laboratory.

2.2.2. Acid-catalysed and organolsolv pretreatments of defatted SCGs

Solid residue of SCGs from solid-liquid extraction by Soxhlet apparatus (defatted SCGs, D- SCGs) were first dried overnight in an oven at 105°C, and then were further dried by placing them into a silica gel dryer for 30 min to completely remove the moisture. Then, 15 g of SCGs were placed into a 250-ml glass bottle (Duran GL45) and diluted at a solid content of 15% w/v by adding 100 ml of solvent (Table 3). In particular, we tested a total of twelve pretreatments, using different concentrations of either sulfuric acid and/or glycerol, as listed in table 3 below:

Table 3. D-SCGs chemical pretreatments

Sample name	H_2SO_4 % (v/v)	Glycerol % (v/v)
D-SCGs-H_2O	-	-
D-CH-SCGs-0.25	0.25	-
D-CH-SCGs-0.5	0.50	-
D-CH-SCGs-1	1.00	-
D-CH-SCGs-10	-	10
D-CH-SCGs-20	-	20
D-CH-SCGs-40	-	40
D-CH-SCGs-80	-	80
D-CH-SCGs-0.25-10	0.25	10
D-CH-SCGs-0.25-20	0.25	20
D-CH-SCGs-0.5-10	0.50	10
D-CH-SCGs-0.5-20	0.50	20

Filled bottles were placed in an autoclave and a cycle at 121° for 60 minutes was run to promote: i) dissolution of hemicellulose as well as breaking of lignin and cellulose bonding (by sulfuric acid-catalysed hydrolysis), to increase cellulose digestibility by enzymes [26,27], and/or ii) selective removal of some barrier components (by glycerol organolsolv), such as lignin, hemicellulose and acetyl group [18].

After allowing the sample to cool down at room temperature, pH was measured using a pH meter (HI 2211, Hanna Instruments) and adjusted to 5.0 by adding NaOH. Pretreated samples were then centrifuged at 8000 x g for 10 min. The supernatant (black liquor) was stored at -20 °C before further analyses. To remove all the solvent's residues, solid fractions, i.e., the deffatted and chemical hydrolised (D-CH-SCGs) were washed three times with 100 ml of deionized water (dH_2O). D-CH-SCGs were then transferred to a glass becher and dried overnight at 105°C in an oven. Eventually, D-CH-SCGs were further dried by placing them into a silica gel dryer for 30 minutes and stored at room temperature into tight-sealed plastic tubes.

2.2.3. Enzyme hydrolysis of deffatted and chemical hydrolised SCGs

For all D-CH-SCGs samples, enzyme hydrolysis (saccharification) was carried out using the commercial enzyme cocktail Cellic CTec 2 (Novozymes). Enzyme activity, expressed as filter paper units (FPUs), was determined according to the NREL protocol TP-510-42628 [28]. A 50mM citrate buffer was used as the reaction medium, keeping a solid to liquid ratio of 15% w/v, and hydrolysis reactions were carried using 5 or 10 FPUs/g_{SCG}. Before incubating the saccharification reactions for 24 h at 50 °C and 180 rpm in an incubator shaker (Innova 42, New Brunswick), a time-zero sample was collected, centrifuged (3000 x *g* for 3 min) and the supernatant was stored at -20°C. Control samples, without enzyme, were incubated in the same buffer and for the same time frame. Eventually, after 24 h of incuvation, all samples were centrifuged and supernatants stored at -20°C, before further analyses.

2.2.4. Fermentable sugars measurement by DNSA assay and HPLC

3,5-dinitrosalicylic acid (DNS) assay is a well-established quantitative method to detect and quantify reducing sugars (RSs) within a sample. This method involves the oxidation reaction between aldehyde functional groups of RSs present in the sample and the DNS reagent, leading to the production of 3-amino,5-nitrosalicylic acid under alkaline conditions[29]. To determinate the RSs concentration (in terms of glucose equivalens), a series of glucose standard solutions, with a concentration spanning from 0.5 to 4 mg/ml, were prepared. Each samples or standard solution (40 µl) were mixed with 160 µl of DNS reagent into the wells of a 96-well plate (Sarstedt) and incubated at 100°C for 20 minutes using a block heater (LLG-uniTHERMIX 1, LLG Labware). Water (40 µl) was used for negative control samples. The plate was cooled down, centrifuge and placed into a multi-mode reader Synergy Neo2 (BioTek) to measure the absorbance of each sample at 570 nm. Each absorbance value was normalized by subtracting the absorbance of the negative control (water sample) as well as that of the corresponding time-zero. The concentration of RSs was detrmined as mg/ml of glucose equivalents and expressed as weight by weight percentage of obtained RSs over total treated D-SCGs ($g_{RSs}/g_{D\text{-}SCGs}$). Moreover, in order to separately quantify each fermenatable sugar (FS) released from SCGs during saccharification, HPLC analysis was performed using the Extrema LC-4000 system (Jasco), equipped with a Rezex ROA H+ column (Phenomenex). The mobile phase consisted of 5mM H_2SO_4 and the flow rate was set at 0.8 ml/min. The column oven was kept at a constant temperature of 80 °C. All the samples were first filtered with 0.22 µm syringe filters (SPHEROS, LLG Labware) and then mixed with 5mM H_2SO_4. After separation, analytes (glucose, mannose, galactose and arabinose)

were identified with a refractive index detector RI-4030 (Jasco) set to 35 °C. All the analytes were then quantified using standard curves generate with appropriate standard samples.

2.2.5 Biogas production from SCG

After chemo-enzymatic hydrolysis to gain FSs from the lignocellulose fraction of D-SCGs, solid residues (i.e., the deffatted and chemo-enzymatic hydrolised SCGs, D-CEH-SCGs,) were subjected to biomethane tests (BMP) by AD, in order to close the biorefinery cycle with the biogas production. The D-CEH-SCGs tests were compared with the AD of SCGs from coffee brewing, (SCGs-AD test) and with: i) the AD of D-SCGs obtained after coffee oils extraction and ii) the AD of D-CEH-SCGs-1 obtained after chemo-enymatic hydrolysis (see, Table 3 and Fig. 4 and 5). By doing so, it was possible to estimate how each stage of the cascade biorefinery influenced the potential of methane production from SCGs.

BMP tests were performed according the protocols redacted by Angelidaki et al. (2009)[30] and Holliger et al. (2016)[31]. 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, the Gompertz curve was calculated to describe the biogas trend from the different BMP tests[3].

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) according to the Standard Methods[32]. In particular, TKN was 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 Transesterification for biodiesel production

The transesterification process (Figure 2) consists in the conversion of fatty acid moieties from triglycerides into fatty acid methyl esters (FAMEs); i.e., the major components of biodiesel. Another product of the transesterification reaction is glycerol that is an undesired compound in the final product; therefore, it reduces biodiesel quality[33]. Triglycerides conversion to FAMEs can be carried out by means of several solvents; however, methanol is preferred because it was demonstrated that it allows a better separation between lipidic and hydrophilic phases of the triglycerides as well as increasing the synthesis of FAMEs. Other solvents, such as ethanol or n-hexane, lead to worse performances[8,34,35].

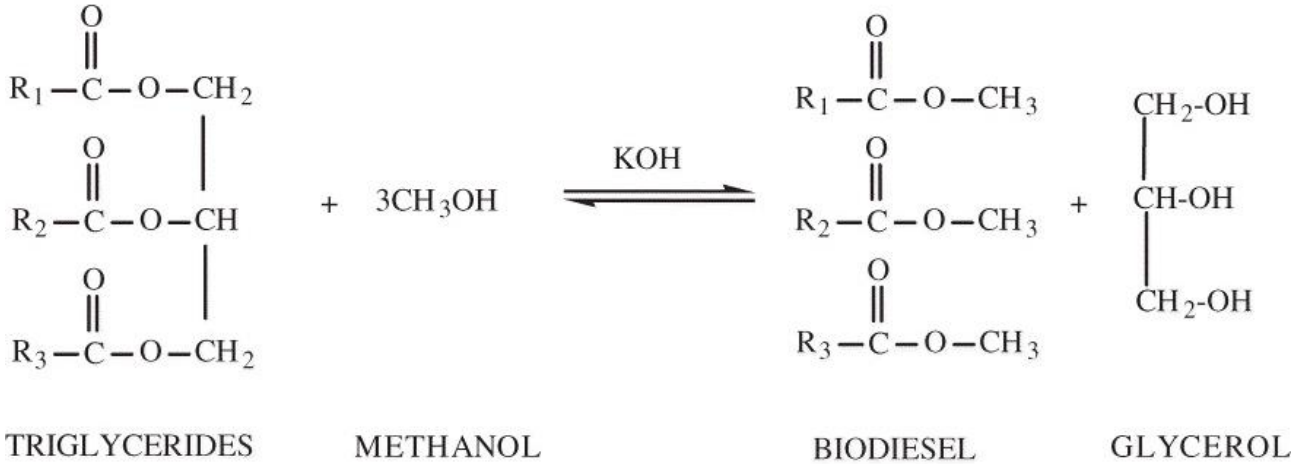

Figure 2. Scheme of the transesterification reactions[33].

As above-mentioned, transesterification reactions are reversible; thus, the setup of optimal coffee oils:methanol ratio is a prerequisete to yield the highest possible biodiesel production. Figure 3 shows the total FAMEs content at different coffee oils:methanol molar ratio as well as of catalyst (KOH) concentrations at the end of the transesterification reactions. These tests allowed the identification of 1:8 as the optimal coffee oil:methanol molar ratio. Indeed, below this value, methanol is not enough to assure a complete transesterification of triglycerides into FAMEs, whereas above this value, much methanol remains unreacted and contridutes to a dilution effect which reduced the quality of the produced biodiesel. Some authors explained the reduction of biodiesel production in presence of a solvent excess as consequence of the instauration of reverse reactions which favour the triglycerides synthesis[11].

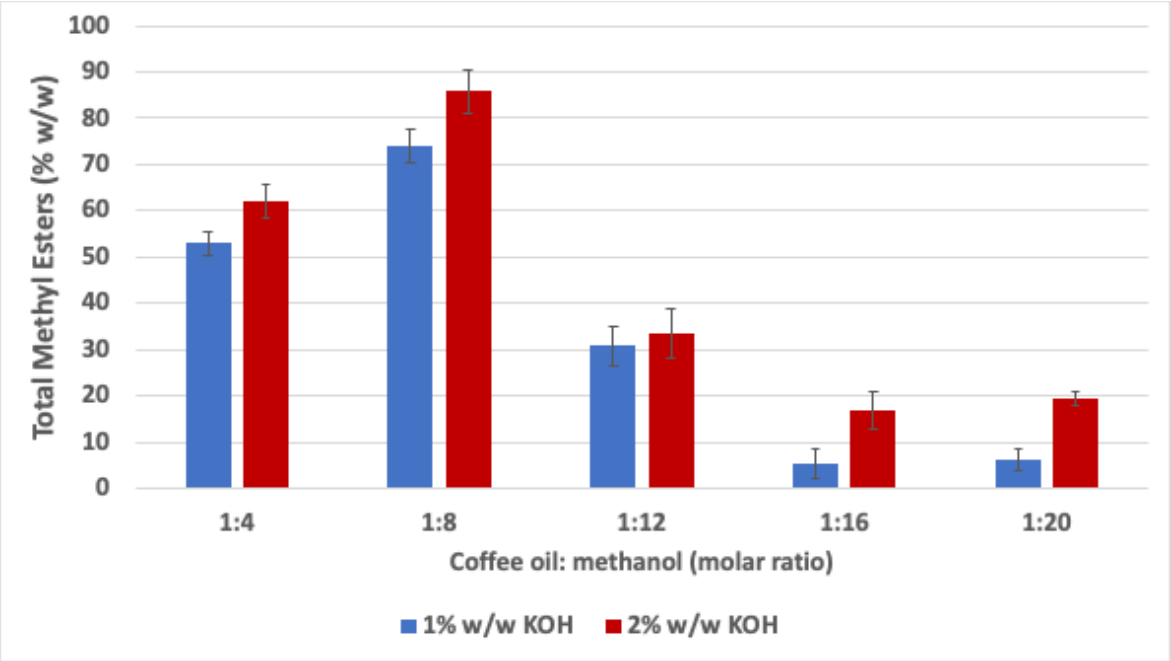

Figure 3. Methyl esters content after the transesterification reactions for the biodiesel production.

Several previous works reported an optimal coffee oils:methanol ratio of 1:5 and 1:6, which are close to the 1:8 shown in the present work, with methyl ester yield varying from 60 to 90% w/w. This wide range can be explainable to the choice of the operational temperature of the tests. 60-65°C had better performances, while temperatures higher than 75°C were associated to the evaporation of methanol, and consequently to a reduction of the transesterification performances[36].

Another important consideration is related to the alkaline catalyst, indeed, by doubling KOH concentraiton, conversion of the long fatty acids into FAMEs increased of about 15-20% at 1:4 and 1:8 coffee oils:methanol molar rations. The highest FAMEs yield of 86% w/w was obtained with a coffee oils:methanol ratio of 1:8 and a KOH concentration of of 2% w/w. The positive effect of the increased KOH concentration is also detectable at higher coffee oils:methanol ratio, even if the final FAMEs yields are lower as consequence of the methanol excess, as above-mentioned . These results are not completely consistent with previous researches, in which alkaline catalysts are recommended only after an acid-catalysed pretreatment step, in order to reduce the free fatty acids content. These latters, indeed, are known to interact with the alkaline catalyst and lead to saponification reactions, which increase glycerol production, instead of FAMEs[8,10,37]. However, Al-Hamamre et al. (2012)[5] and Najdanovic-Visak et al. (2017)[11] achieved an yield of FAMEs of about the 80% w/w, using 2.5% w/w of KOH and operational conditions (T = 65°C and duration of 4 h) similar to those adopetd in the present work (T = 60°C and duration of 2 h). Thus, good performances reported in this work are not unique and can be probably explained taking into account the mitigate operational condition, in particular of temperature, which contribute to keep tricglycerids in stable form, avoiding its dissosiation in free fatty acids.

The alkaline catalyst was also tested at 3% w/w under the best coffee oils:methanol molar ratio of 1:8. The esters production was almost the same (82% w/w) of the one achieved at 2% w/w. Thus, the molar ratio of 1:8 of coffee oils:methanol and the concentration of 2% w/w of KOH catalyst, can be selected as the optimal operational conditions for the FAMEs synthesys under a tempearture of 60°C and a test duration of 2 h.

As it cocnerns the FAMEs profile of the produced biodiesel samples, the two most predominant FAMEs are those rerived from the two most abundant fatty acids in coffee oils, i.e., palmitic and linoleic acids. Table 4 shows that their concentrations account for 30-37% w/w, respectively. Comparing the palmitic and linoleic esters concentrations with those of the corresponding fatty acids in Table 2, it is possible to verify the very high conversion degree of these fatty acids into biodiesel. This is a further confirmation of the good performance of the transesterification process which led to a low glycerol production of 12% w/w.

Table 4. The profile of the biodiesel from the coffee oil transesterification.

Content of Methyl ester from the transesterification of	% w/w
Myristic acid	0.1
Palmitic acid	30.0
Palmitoleic Acid	0.1
Heptadecanoic acid	0.2
Stearic acid	5.8
Oleic acid	8.7
Linoleic acid	37.6
Arachidic acid	1.8
Gadoleic acid	0.2
Behenic acid	0.3

3.2 Recovery of fermentable sugars from defatted SCGs

After chemical pretreatment of defatted SCGs to yield D-CH-SCGs samples, these were subjected to enzyme hydrolysis (saccharification) using two different ratios of Cellic CTec 2 enzyme cocktail: 5 and 10 FPUs/$g_{D\text{-}CH\text{-}SCGs}$. Total reducing sugars (RSs) released from solid fractions of D-CH-SCGs samples as well as those in the liquid phase from chemical pretreatment (black liquor, see above) were quantified by DNS assay. Figure 4 shows that increasing concentration of H_2SO_4 led to an augmented release of total RSs during saccharification. Either 38,9 or 47,89 $g_{RSs}/g_{D\text{-}CH\text{-}SCGs}$ were released from sample D-CH-SCGs-1 using 5 or 10 FPU/$g_{D\text{-}CH\text{-}SCGs}$, respectively. These values are close or above the maximal yield of RSs achievable from SCGs (i.e., 46,3 g_{RSs}/g_{SCGs}), probably because the DNS assay slightly overestimate the concentration of RSs in such complex samples. To replace the use of H_2SO_4 for chemical pretreatments with a less toxic and environmental-friendly chemical, we resolved to test an organolsolv pretreatment using glycerol. However, glycerol alone did not positively influence saccharification, indeed, in all conditions tested yields of RSs were extremely low (Fig. 4). Nevertheless, when glycerol at 10 or 20% was coupled to H_2SO_4, it was possible to halve the concentration of the acid, still achieving yields of RSs (48.28 and 45.24 g_{RSs}/g_{SCGs}) which are close or above the maximal yield. Higher concentrations of glycerol negatively affected the yields of RSs (data not shown).

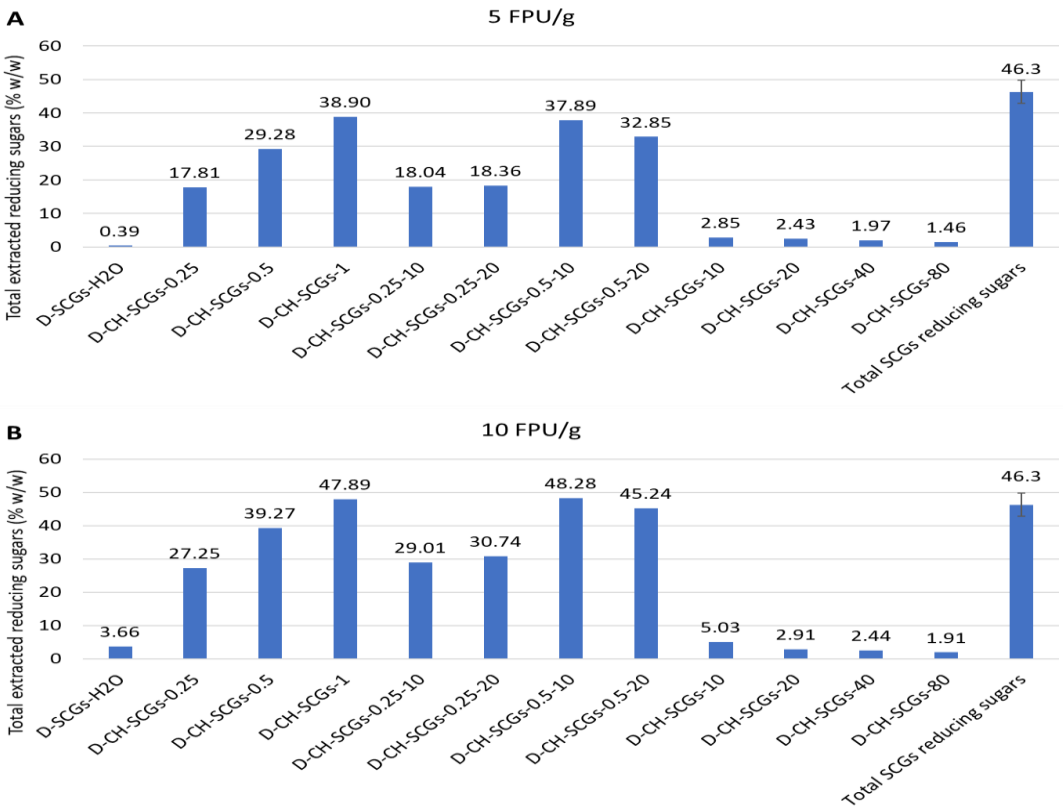

Figure 4. Reducing sugar analysis of chemo-enzymatic hydrolysed SCGs saamples.

In order to gain a broader picture about the kind of fermentable sugars (FSs) obtained from saccharification of D-CH-SCGs samples, HPLC analyses were carried out to quantify glucose (released from cellulose) as well as the sum of mannose, galactose and arabinose (released from hemicellulose) (Fig. 5). Once again, it is confirmed that increasing concentrations of H_2SO_4 allow releasing higher amounts of total RSs during saccharification, regardless of the quantity of enzymes used (either 5 or 10 FPUs/$g_{D\text{-}CH\text{-}SCGs}$, see Fig. 5 A and C). Moreover, according with the lignocellulose composition of SCGs (see Table 1, 20.6% of cellulose and 25.7% of hemicellulose), sugars from hemicellulose were released in higher amount in all conditions tested. Noteworthy, combining 0.5% of H_2SO_4 with 10% glycerol allowed to extract almost all FSs releasable from D-CH-SCGs, using 10 FPU/$g_{D\text{-}CH\text{-}SCGs}$ (Fig. 5D); whereas higher concentrations of glycerol were detrimental (data not shown). Altogether these data prove that not only it is possible to reduce the amount of acid used for pretreatments of D-SCGs, but also that recirculation of glycerol leftover from the transesterification reaction is a good strategy. Indeed, it is worth mentioning that the proposed strategy allow the valorisation of an internal waste streams. Moreover, since the glycerol used for pretreatments is still present in the enzymatic hydrolysate from D-CH-SCGs samples, this chemical can represents an additional carbon source for the microorganisms used to ferment the FSs releasable from D-CH-SCGs; thus, increasing the final yield of the targeted fermented product(s).

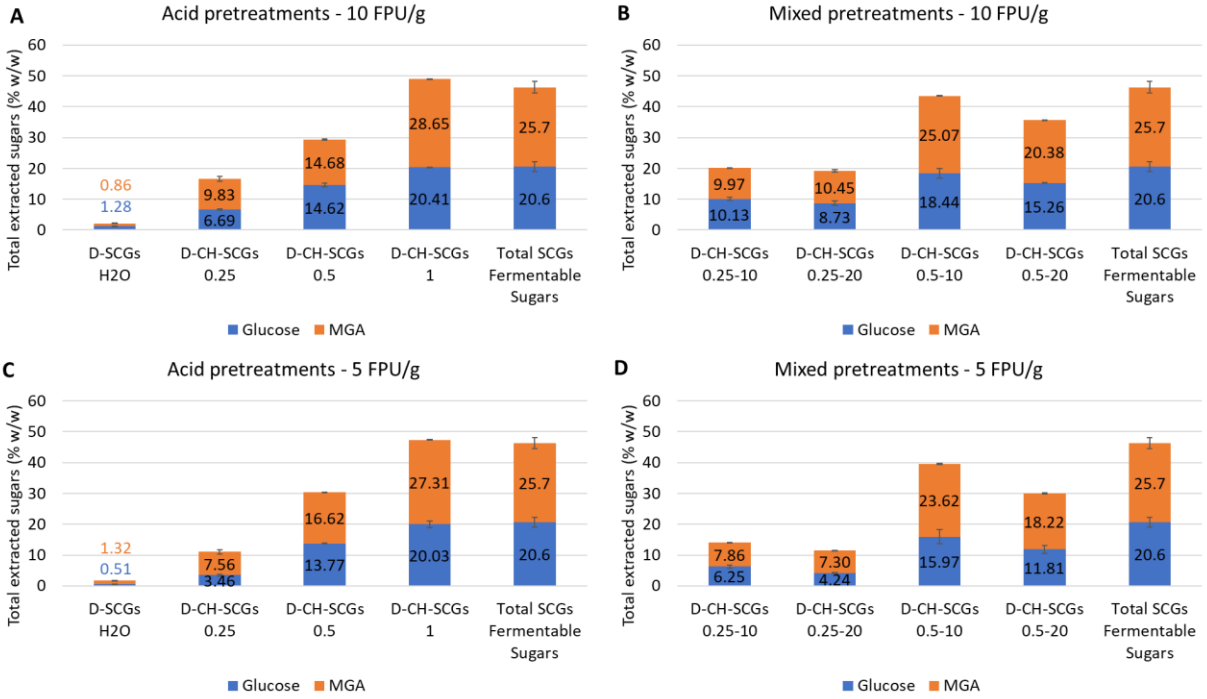

Figure 5. Fermentable sugar analysis of chemo-enzymatic hydrolysed SCGs saamples.

3.3 Biogas production from SCG

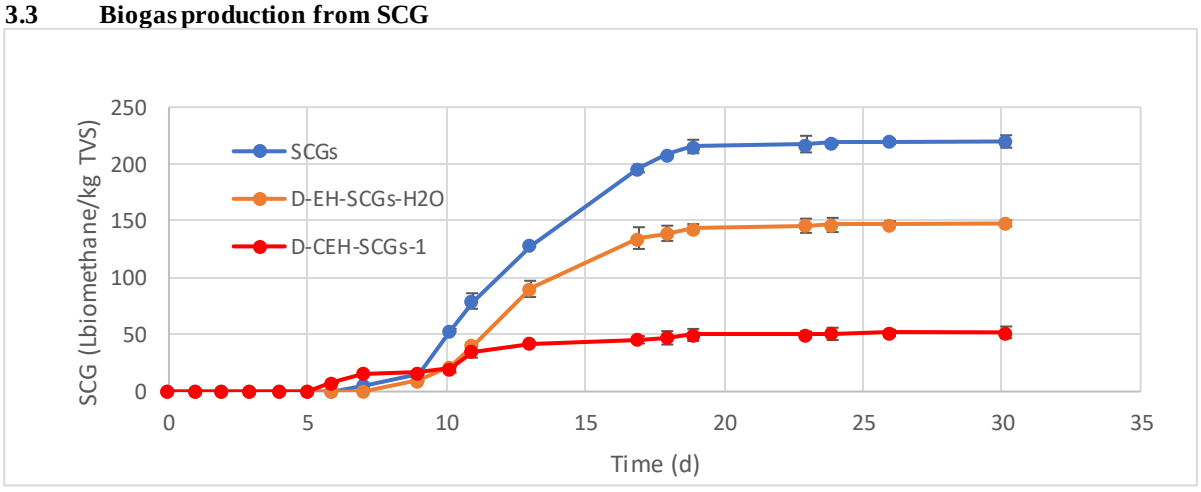

Figure 6. Cumulative biomethane production from SCGs assays.

Figure 6 compares the cumulative methane production from the SCGs after coffee brewing (control sample) and without any pretreatments, with that from D-SCGs after different chemo-enzymatic treatments (see table X, la tabella dei pretrattamenti) . After an initial lag-phase of about 6-7 days, biomethane production started, following an exponential

growth for 10 days. Then, the methane production rate decreased until reaching a plateau at ~220 L_{CH4}/kg_{TVS} for the untreated SCGs. Lower performances (~150 L_{CH4}/kg_{TVS}) were achieved for the D-EH-SCGs-H_2O sample, which was previously autoclaved in H_2O (a cycle at 121° for 60 minutes) and enzymatically hydrolysed. Only 50 L_{CH4}/kg_{TVS} were achieved with the chemo-enzymatic treated sample D-CEH-SCGs-1.

The initial long lag phase can be explained by low concentration of the inoculum. Which had a very low TS content of 1.65 %w/w. As consequence, microorganisms are diluted, and thus more time is needed to reach optimal biomass concentration to assure efficient substrates degradation. In fact, recent works on AD of SCGs have shorter lag phases in presence of higher concentrations of inoculum. For istance, Battista et al. (2020)[3] used an inoculum with 9.08 %w/w TS, reporting no lag phase and an instant start of the AD; Akyol (2020)[38], with an inoculum of 6.55 %w/w TS, registered very short lag phases, ranging from 1 to 3 days; Atelge et al. (2021)[39], who adopted an inoculum at 4.25 %w/w TS, reported longer lag phases of ~5 days. As shown in figure x, the chemical composition of the different SCGs samples influenced methane production.thethe control BMP assay, the untreated SCGs, is rich in long chain fatty acids, proteins and simple carbohydrates[8,40] that can be immediately used by the microbial inoculum, thus leading to the higher biomethane production observed. These results are similar to those achieved recently from untreated SCGs: Oliva et al. (2021)[41] and Atelge et al. (2021)[42] reported a BMP of 293 and 310 L_{CH4}/kg_{TVS} respectively; while Battista et al. (2020)[3] reported 217 L_{CH4}/kg_{TVS}. In the assays performed with pretreated SCGs, the coffee oils were extracted (D-EH-SCGs) and a fraction of the lignocellulosic materials was converted into sugars by the acid/enzymatic pretreatment (D-CEH-SCGs-1). In the case of D-EH-SCGs sample, the BMP production was slower because of the absence of nutrients like fatty acids, proteins and oligosaccharides; thus forcing the microbial inoculum to use the lignocellulose residue.[43] However, digestion of cellulose is cumbersome because it is still embedded into a hemicellulose and lignin matrix, which renders it difficult to access[43,44]. As it concerns the D-CEH-SCGs-1 sample, the solid residues is composed mainly of lignin with only residual (hemi)cellulose available to be hydrolyzed and converted to methane during AD; thus explaining the further lower methane productions.

Given the above considerations, it is clear that the BMP is directly linked to the previous steps of the SCGs valorization process: lower yields of coffee oils and fermentable sugars will lead to a higher biomethane production and vice versa. This correlates with the experimental data: the assay with 0% H_2SO_4 added has a decent BMP due to a poor yield of the sugar extraction process, where the lack of acid pretreatment denied cellulose and hemicellulose solubilization and thus extraction[8,45], leaving those polymers not available for BMP. The difference between the 0% H_2SO_4 assay and the control SCG no pretreated assay is thus only caused by the AD of coffee oil removal by Soxhlet. When the previous steps gave higher yields of oils and/or sugars, the BMP is very poor and not economically sustainable. Indeed, for the D-CEH-SCGs-1 sample, the solubilization of cellulose and hemicellulose fractions and the subsequent saccharification left a by-product with almost only lignin. The AD is then severely hindered by nutrient scarcity and inhibitory effects, that originated by the lignin itself [45,46] or by acidic pretreatment, that can leave inhibitory molecules like furfurals and hydroxyfurfurals as products of lignin degradation.[43,47]

4 Conclusions

SCGs is one of the most abundant urban waste and are usually recollected with the Organic Fraction of Municipal Solid Wastes and disposed in landfill or sent to the anaerobic digestion for biogas production. Being rich in high added value compounds, SCGs can be better valorized and used in a cascade biorefinery process for production of biodiesel and fermentable sugars. A FAME yield of 86% was achieved when transesterification was conducted with a coffee oil/methanol molar ratio of 1:8 and a KOH concentration of 2% w/w. The fermentable sugars production was optimized recycling glycerol from the transesterification step, which allow to reduce the sulfuric acid content from 1 to 0.5% for the pretreatment of SCG. The following saccharification, conducted at 10 FPU, led to total sugars production of about 45% w/w. The residual SCG have been anaerobically digested to produce biomethane. As they are low in easily degradable compounds, the methane production was low (50 L_{CH4}/kg_{TVS}) and low with a lag phase of about 7 days.

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