applied sciences

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The Possibility of Using Spent Coffee Grounds to Improve Wastewater Treatment Due to Respiration Activity of Microorganisms

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Received: 10 July 2019; Accepted: 30 July 2019; Published: 2 August 2019

Abstract: Spent coffee ground (SCG) may affect wastewater treatment processes due to high coffee consumption worldwide. The impact of the main chemical compounds present in SCG on respiration activity of sewage sludge was investigated. The results showed approximately two times higher respiration in the samples where various types of SCG were present in comparison with samples without SCG. During intense microbial metabolism, statistically significant ($p < 0.05$) decreases in caffeine, total polyphenols, and chlorogenic acid contents after processing and in filtrate was observed. The monitored compounds (caffeine, polyphenols, and chlorogenic acid) deteriorated due to their probable inclusion in microbiological metabolism. Increase in respiration activity of microorganisms in the presence of cheap waste material such as coffee grounds can help to improve wastewater treatment. The research was focused on spent coffee grounds' impact on the respiratory activity of microorganisms in the activated sludge taken from small and large wastewater treatment plants. The impact was measured in more detail due to the inclusion of different coffee species (Robusta and Arabica) in diverse concentrations. The novelty of the study can also be seen through the literature overview, where information cannot be found about SCG influence on the respiration activity of microbial communities, and data on the possible SCG aerobic degradation or utilization by a sewage sludge bacterial consortium has also never been reported. The study has shown the possibility of improving wastewater treatment due to respiration activity of microorganisms in the presence of cheap waste material such as coffee grounds.

Keywords: wastewater treatment; sewage sludge; microbial respiration; caffeine; chlorogenic acids

1. Introduction

Spent coffee ground (SCG) is the waste that is accumulated after coffee consumption, or it can be described as a byproduct of brewing process. Special attention is given to the accumulation of this food production byproduct due to high coffee consumption worldwide. This statement is supported by the facts: (i) in 2016, 7,200,000 tons of all coffee forms was exported, and from 2000 to 2012, global green

coffee production increased by 17% [1]; (ii) in total, 0.91 g wasted grounds per gram of coffee is produced. Around 6 million tons of spent coffee grounds are produced each year [2]. Consequently, these facts have made many research teams focus on the usage of spent coffee grounds [3]. On the other hand, the release of spent coffee grounds to the environment due to increasing coffee consumption causes environmental contamination due to the fact that its decomposition consumes large quantities of oxygen [3]. All waste disposal issues represent big economic and environmental problems [4]. SCG utilization and management represent a big problem due to the presence of phenols, caffeine, and tannins, which are highly toxic for many life processes. The study showed that the toxicity of SCG could be reduced by warm treatment, microbial biodegradation, and aerobic fermentation [5–9]. SCG is most often part of municipal solid waste, so it can be incinerated or landfilled [10]. SCGs, on average, contain 45.3% (w/w, dry weight) of polysaccharides (mannose, galactose, glucose, and arabinose) bound to cellulose and hemicellulose complexes [11,12]. Mannans are the major component of polysaccharides in SCGs [12]. There are almost no reducing sugars present in coffee [13]. The main phenol presented in coffee is chlorogenic acid (CGA) and accounts for up to 14% (dry matter basis) [14].

Phenolic compounds, flavonoids, chlorogenic acid, and protocatechuic acid are important for their antioxidant activity [15]. Chlorogenic acid is one of the phenolic compounds found abundantly in SCGs [16,17]. Phenolic compounds can be toxic, but only for some aerobic microorganisms and only in high concentrations [18]. Microorganisms can isomerize, hydrolyze, or degrade them into low-weight molecular compounds. On the other hand, high concentrations of phenolic compounds have an adverse effect on anaerobic microorganisms, which may cause problems at the processing of biodegradable waste in biogas transformation technologies [19–22]. Phenolic compounds are a major cause of digestive failures in anaerobic digestion [23]. The literature also describes the use of SCGs as a cheap raw material for removing dyes and heavy metals from wastewater [24]. There should also be attention focused on the previous research conducted on the valorization of spent coffee grounds as a way to reduce dyes and heavy metals from wastewater, which have reported some yield and the weak points of the proposed processes. Wastewater is polluted by a wide range of pollutants, such as carbonaceous substances or heavy metals, which access wastewater treatment plants (WWTP). The sludge is made up of the entire spectrum of microorganisms and protozoans that make up the community effectively degrading these pollutants. The differences between SCGs are related to how they are prepared, from different ratios of Robusta (*Coffea canephora*) and Arabica (*Coffea arabica*) coffee, as these coffees are commonly labeled [25,26]. The possibility to use of SCGs in wastewater treatment has not been investigated or published yet. There is no information about the influence of SCGs on the respiration activity of microbial communities in the literature. Data on possible SCG aerobic degradation or utilization by a sewage sludge bacterial consortium have never been reported either. The aim of the research was to determine the influence of spent coffee grounds on microbial respiration in the activated sludge and establish differences in the compound profile of unprocessed, processed, and filtrate samples.

2. Materials and Methods

Coffee samples were obtained from three coffee shops (retail shops in Brno, Czech Republic: Three samples from each coffee shop). The samples differ in Arabica/Robusta ratios: Sample No. 1, Arabica 50%/Robusta 50%; sample No. 2, Arabica 80%/Robusta 20%; and sample No. 3, Arabica 100%. The samples of spent coffee grounds were dried at 105 °C to constant weight. Activated sewage sludge used in the experiments was taken from an activation tank at WWTP Moravany 5000 PE, Czech Republic and WWTP Modřice, 480,000 PE, Czech Republic. The cultivation medium was prepared as follows: 1 g of malt extract and 0.5 g of peptone (HiMedia Deutschland AG, Düsseldorf, Germany) dissolved in 1 dm^3 of tapped water, and sterilized in an autoclave. Biological oxygen demand (BOD) values were determined by aerobic system OxiTop® (WTW, Weilheim, Germany). The samples were divided into three groups: Unprocessed (before sludge addition), processed (after sludge addition), and filtrate (the filtrate obtained after sludge addition).

In total, eight bottles (510 mL) were filled up with 15 mL of sewage sludge and 28.5 mL of cultivation media. Consequently, SCG samples (0.7 g) were added. During the experiment, different amounts of SCG were used but the most effectively processed was 0.7 g and the BOD was highest. The amount of CSG first ranged from 0.1 g to 0.5 g by 0.1 g. As the activated sludge was not inhibited and BOD was rising with the amount of SCG, the concentration was risen to 0.5 g, 0.7 g, 1 g, 3 g, 5 g, and 7 g, in order to cover wider range. The 0.7 g provided the highest BOD. We chose this amount and maintained the concentration for our next experiments and analyses to discover what is happening with SCG. The respiratory activity of microorganisms can be an important factor for wastewater treatment, since its increase allows us to achieve the maximum decomposition of complex organic compounds, their involvement in microbial metabolism and, thus, detoxification of the water environment from toxic pollutants.

Two bottles without SCG addition were used as the control. Two NaOH pearls designed to absorb CO_2 were added to the rubber plugs in the bottle opening. Closed bottles were incubated in the dark for 5 days at 20 °C with constant stirring by a magnetic stirrer. Respiratory activity values were recorded with OxiTop® measuring heads. For analyses, the filtration of the mixture through filter paper 2R/60 (Papírna Perštejn s.r.o., Perštejn, Czech Republic) was carried out after five days of incubation; the solid was dried at 60 °C for 24 h. Microbial respiration rates were calculated using the formula described in the paper by Kushkevych et al. (2018) [27].

The total content of phenols was determined using a UV/VIS spectrophotometer CE7210 (Cecil Instruments, Cambridge, UK) at 765 nm according to the method described by Katalinic et al. (2006) [28]. The caffeine content in SCG unprocessed and processed samples was determined by the modified method described by Dobrinas et al. (2013) [29]. The samples were treated with hot water (75–80 °C) and filtrated. Then, 1 mL of filtrate was mixed with 100 mL of distilled water, and absorbance at 272.5 nm was determined. The quantification of the caffeine was done by the usage of calibration curve. Chlorogenic acid was quantitatively determined according to the method described by Naegele (2016) [30]. The extraction of a coffee sample (2 g) was done with 150 mL of methanol/water (50/50, v/v). The samples were left in methanol/water solution for 3 days. The extract was filtered by a syringe filter (LUT Syringe Filter, 0.45 µm, 13 mm), and the filtrate was used directly for injection. The mobile phase consisted of A) water + 1% phosphoric acid and B) acetonitrile. The gradient was adjusted as follows: 0 min—10% B, 20 min—20% B, 25 min—30% B, 35 min—40% B, 40 min—40% B. The duration of analysis was 10 min, and the flow rate was 1 mL/min. The injection of the sample was 10 µL, and retention time was measured at 324 nm for chlorogenic acid. Caffeine and gallic acid were measured at 270 nm. The contents of caffeine and chlorogenic and gallic acids from high-pressure liquid chromatography (HPLC) were calculated by the calibrations of these compounds (Figure 1).

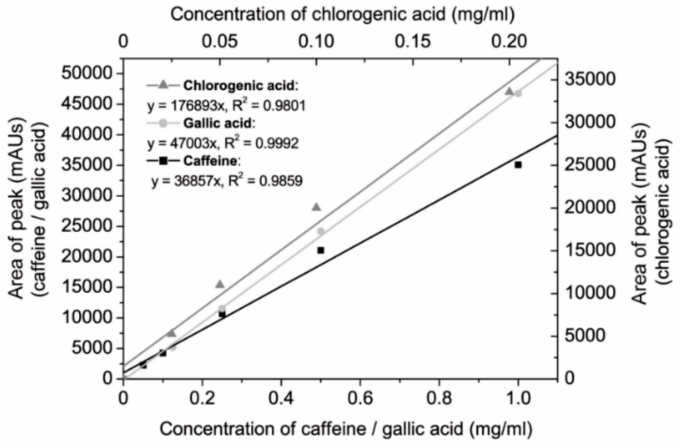

Figure 1. Calibration of caffeine, chlorogenic, and gallic acids for chromatography calculations.

Calibration curves indicated strong correlation between concentrations of measured phenols (caffeine, gallic, and chlorogenic acid) and area of peaks (mAUs). These high correlations confirm the reliability of applied methods since quantification of measured phenols were possible with high accuracy.

Antioxidant activity has the picture about biological activity in SCG and effects of respiration activity of sewage sludge. Since SCG is a good source of antioxidants, antioxidant activity shows its antioxidant reduction during respiratory activity of sewage sludge. It means that biologically active antioxidants present in SCG are used in respiratory processes in sewage sludge. The ferric reducing antioxidant power (FRAP) method was evaluated by a spectrophotometer (CE7210, Cecil Instruments, Cambridge, UK). FRAP reagents (TPTZ, $FeCl_3$, and acetate buffer) were prepared according to the methods described by Rubio et al. (2016) [31]. Then, 0.18 mL of methanolic extracts of the sample and 3.6 mL of FRAP reagent were transferred into a flask, and 0.3 mL of distilled water was added. The obtained blue solutions were kept at room temperature for 8 min, and the absorbance was measured at 593 nm. All analyses were done in three replicates.

Statistical significance at $p < 0.05$ was evaluated by the *t*-test (one sample), one-way ANOVA analysis of variance, and the parametric Tukey post hoc test (in the case when Levene's test showed equal variances $p > 0.05$) or the nonparametric Games–Howel post hoc test (in the case when Levene's test showed unequal variances $p < 0.05$) for finding differences within groups (control, processed, and filtrate samples). Overall differences between the respiration of the control samples (without spent coffee) and other samples that included spent coffee were checked by principal component analysis (PCA). The matrix for PCA was constructed based on microbial respiration data. SPSS 20 statistical software (IBM Corporation, Armonk, NY, USA) was used.

3. Results

Microbial respiration in coffee samples with different Arabica/Robusta proportions was determined (Figure 2). The respiration in the first ratio sample (Arabica/Robusta: 50%/50%) slightly differed, but was not statistically significant ($p > 0.05$), from other investigated samples (Arabica 80% and Arabica 100%). There was a 10% reduction in respiration in the sample containing 50% Robusta. Oppositely, statistically significant ($p < 0.05$) respiration differences were observed for all investigated samples in comparison with control samples. After 50 h, the respiration in the SCG samples (1, 2, and 3) was 215%, 206%, and 198%, respectively; after 100 min: 219%, 236%, and 235%; at the end: 202%, 220%, and 219% compared to the control samples (without SCG).

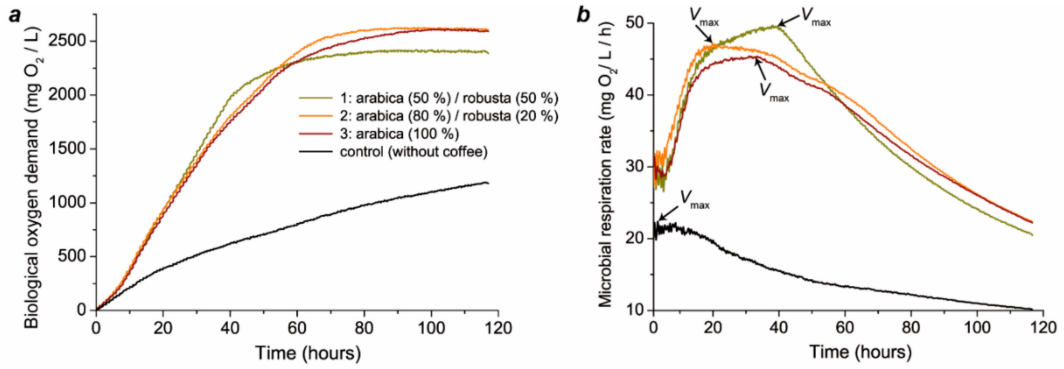

Figure 2. Microbial respiration and its rate in activated sludge depending on Arabica and Robusta content: (**a**) Respiration; (**b**) rate, V_{max} is maximal rate.

The microbial respiration rates were calculated and are presented in Figure 2 and Table 1. The main kinetic parameters (V_0, $V_{average}$, and V_{max}) indicated significant differences between the control and other investigated samples.

SCG represent a source of nutrient for community of microorganisms, due to content of polysaccharides, proteins, lipids, and phenolic compounds, which serves as substrate for various species of microorganisms. It is still speculated if the phenolic compounds can stimulate growth another way than as a substrate, although inhibitory effect on some microorganisms is known. Our results imply the possibility of carbon source, since the content of phenolic compounds significantly decreased, and this decrease could not happen without activity of microorganisms. Another hypothetical effect, although not verified, is the sorption ability of SCG particles that could protect microorganisms in consortia against harmful substances such as heavy metals. These are positive results for WWTP, as the communities of microorganisms are protected from inhibitory effects contrary to pure cultures alone and even degrading SCG, which arrive to the WWTP.

Thus, the biggest differences were observed in samples with a higher portion (50%) of Robusta coffee cultivar. The results shown in Table 1 correspond to the obtained data in Figure 2.

Table 1. Kinetic parameters of microbial respiration of spent coffee grounds (SCG) samples (n = 3, mean ± SD).

Sample	Kinetic Parameters			
	Initial Rate V_0 **(mg O_2/L/h)**	**Average Rate** $V_{average}$ **(mg O_2/L/h)**	**Maximal Rate** V_{max} **(mg O_2/L/h)**	**Time** t **(hours)**
Control (without coffee ground)	15.93 ± 1.45	14.73 ± 1.34	25.54 ± 2.32	2
Arabica (50%)/Robusta (50%)	15.94 ± 1.44	35.95 ± 3.27	49.99 ± 4.54	38
Arabica (80%)/Robusta (20%)	26.54 ± 2.41	36.47 ± 3.32	47.51 ± 4.32	21
Arabica (100%)	26.55 ± 2.43	35.46 ± 3.22	45.56 ± 4.14	32

The differences were confirmed by PCA (Figure 3); factorial analysis distinguishes three samples with spent coffee as one component and the control (composed without spent coffee) as another component. PCA is a powerful method to reduce the complexity of a dataset and highlight relations between the variables. SCG samples are one cluster and control samples are another cluster.

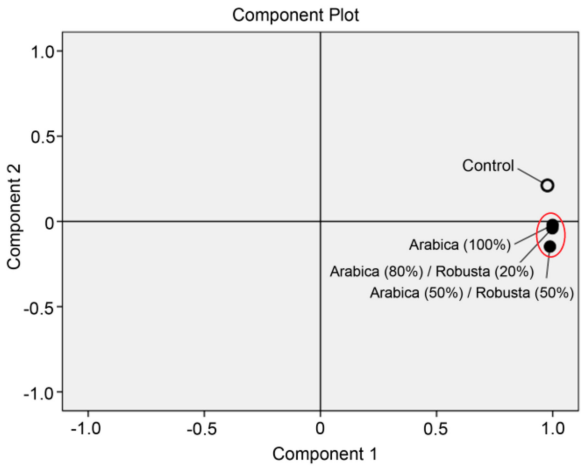

Figure 3. Principal component analysis of microbial respiration: The dots in the figure correspond with samples marking explained in Table 1.

Based on eigenvalue (>1), two components were formed since eigenvalue distinguishes clusters of variances [32]. The first component consisted of three samples with spent coffee and the second component was only the control sample without spent coffee. Consequently, PCA results clearly and unambiguously indicate the influence of spent coffee addition on microbial respiration. Figure 3 represents an individual plot.

The contents of caffeine and polyphenols (gallic acid and chlorogenic acid) are represented as mean values of three investigated samples included in respiration and evaluated on the spectrophotometer in the control and processed samples, and are shown in Figure 4. Caffeine and total polyphenols contents decreased statistically significantly ($p < 0.05$) after processing; especially significant decreases were observed in the filtrate samples. Caffeine contents decreased (compared to the control samples) by 82% and 99% in the processed and filtrate samples, respectively. Relatively the same total polyphenol contents decreased by 92% and 99% in the processed and filtrate samples, respectively.

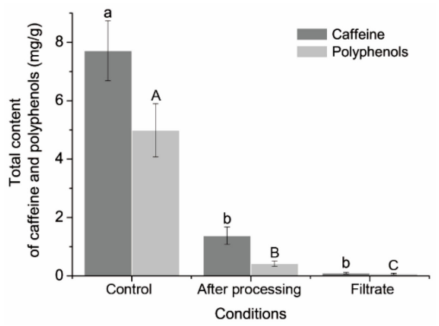

Figure 4. Caffeine and polyphenol contents in coffee samples evaluated on spectrophotometer: Lowercase letters (**a**,**b**) indicate statistically significant difference ($p < 0.05$) between coffee samples in caffeine content; uppercase letters (**A**,**B**,**C**), in total polyphenols content. Data are expressed as mean ± SD of triplicate determinations.

The HPLC method unambiguously showed complete deterioration of caffeine, gallic acid, and chlorogenic acid in the processed and filtrate samples, as can be seen in chromatograms obtained at 270 nm and 324 nm signals (Figure 5).

The results gained by HPLC had the same trend as the results obtained by the spectrophotometer; the loss of properties of the measured samples was so high that quantification was under a detectable level. Caffeine, polyphenols, and chlorogenic and gallic acids were detected only in the control samples (Figure 6). The antioxidant capacity (measured by the FRAP method) of the unprocessed samples was statistically significant ($p < 0.05$), while the antioxidant capacity of the filtrate samples was undetectable.

Caffeine is a purine alkaloid that is mostly recognized as the main compound in coffee products and consequently in spent coffee grounds. The caffeine content is reduced in spent coffee grounds in comparison with coffee beans, but a large amount of caffeine still remains [1]. Caffeine content in spent coffee grounds ranges from 0.734 µg/mg to 41.3 µg/mg, which is in accordance with our results [1]. Coffee extract was found to be an effective bacterial inhibitor (against Gram-negative bacteria *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* and Gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Lactobacillus bulgaricus*, *Streptococcus lactis*, and *Streptococcus faecalis*) but not against molds and yeasts. *L. bulgaricus*, *E. coli*, *S. typhi*, and *S. faecalis* were found to be sensitive differently to caffeine concentrations; the most sensitive of them was *L. bulgaricus*, while *S. faecalis* was the most resistant [18]. As shown by the results, not all microorganisms are sensitive to higher amounts of caffeine. The concentration of caffeine in our samples was 0.18 mg/mL, which is in accordance with the work of Ibrahim et al. (2014) [33], where authors describe concentration higher than 2.5 mg/mL as an inhibiting concentration for the growth of many bacterial species. Caffeine in our samples did not affect the viability of microorganisms in the activated sludge. Contrarily, the microorganisms and protozoans were stimulated in the environment with SCG addition. The decomposition of caffeine by bacterial strains of *Pseudomonas*, *Serratia*, *Rhodococcus*, and *Klebsiella* has been described [33]. By means of bacterial enzymes, caffeine is decomposed to 3-methylxanthine, uric acid, and allantoin, and the final products are CO_2 and NH_4 (Figure 7).

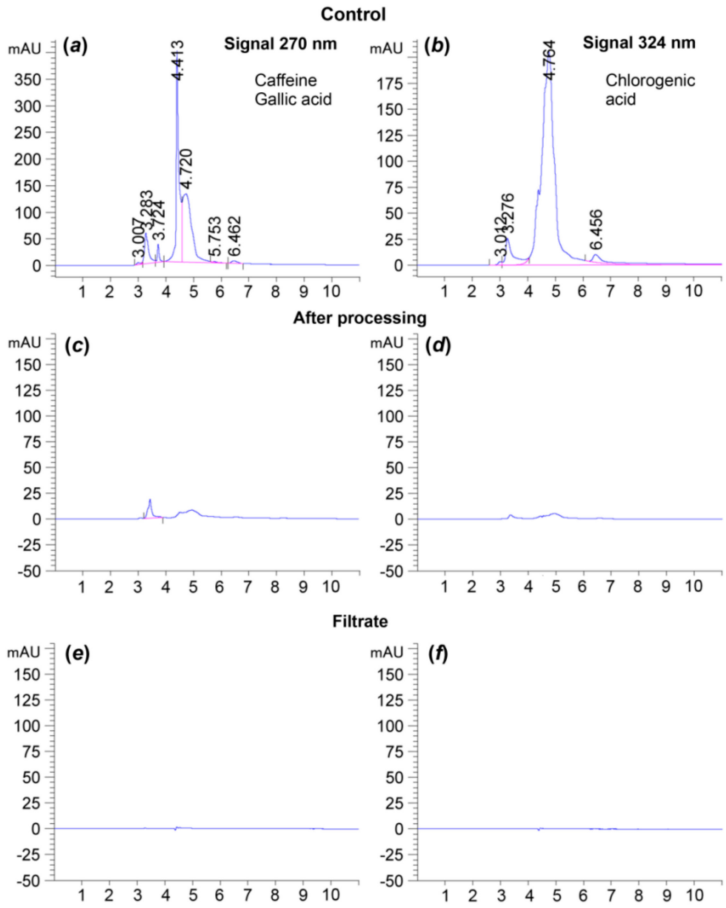

Figure 5. Caffeine, gallic acid, and chlorogenic acid contents determined by chromatography method: (**a**,**b**) Control; (**c**,**d**) processed samples; (**e**,**f**) filtrate samples.

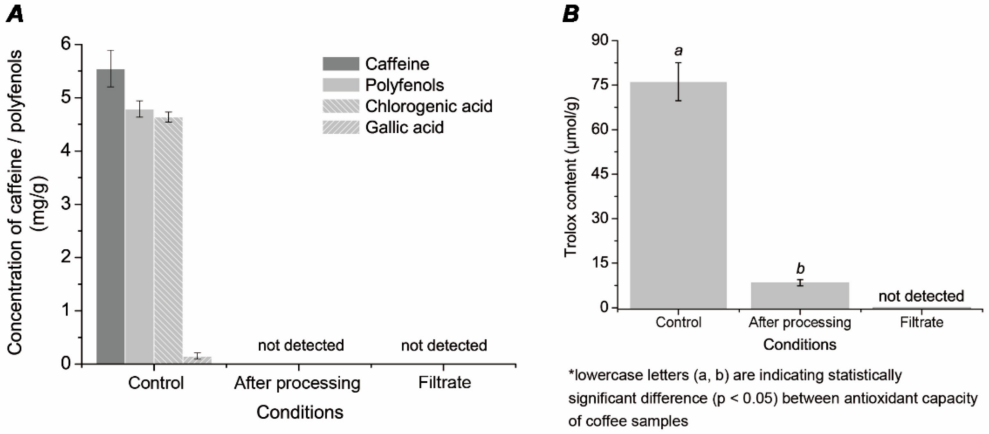

Figure 6. Caffeine and polyphenol contents in coffee samples evaluated by chromatography (**A**) and antioxidant capacity of spent coffee ground samples: Lowercase letters (**a**,**b**) indicate statistically significant difference ($p < 0.05$) between antioxidant capacity of coffee samples (**B**). Data are expressed as mean ± SD of triplicate determinations.

Therefore, caffeine may serve as a source of carbon, nitrogen, and energy for microorganisms. The bacterial degradation of caffeine is influenced by many factors: Temperature, pH, caffeine concentration, and the presence of other sources of carbon and nitrogen [33]. Also, the presence of other substances in the activation tank together with caffeine can be used by microorganisms for growth and respiration. SCGs contain a large number of polysaccharides and monosaccharides,

of which glucose is best utilized by conventional aerobic microorganisms. There is approximately 9% (w/w, dry weight) glucose in SCGs [11], and it can be used as a readily available carbon source. Our previous non-published research also showed high content of lipids (10–12%, w/w, dry weight) and proteins (12–14%, w/w, dry weight) in SCGs, which can also be used as substrate for microorganisms. On the other hand, there was a high content of hardly biodegradable lignin (15%–20%, w/w, dry weight) in SCGs. As it can be seen from the respiration test results, the samples with different Arabica/Robusta content did not affect the respiration activity of activated sludge microorganisms. Taking into account the different caffeine contents in the SCG samples (different Arabica/Robusta ratio), not only caffeine plays a key role in the microbial respiration activity.

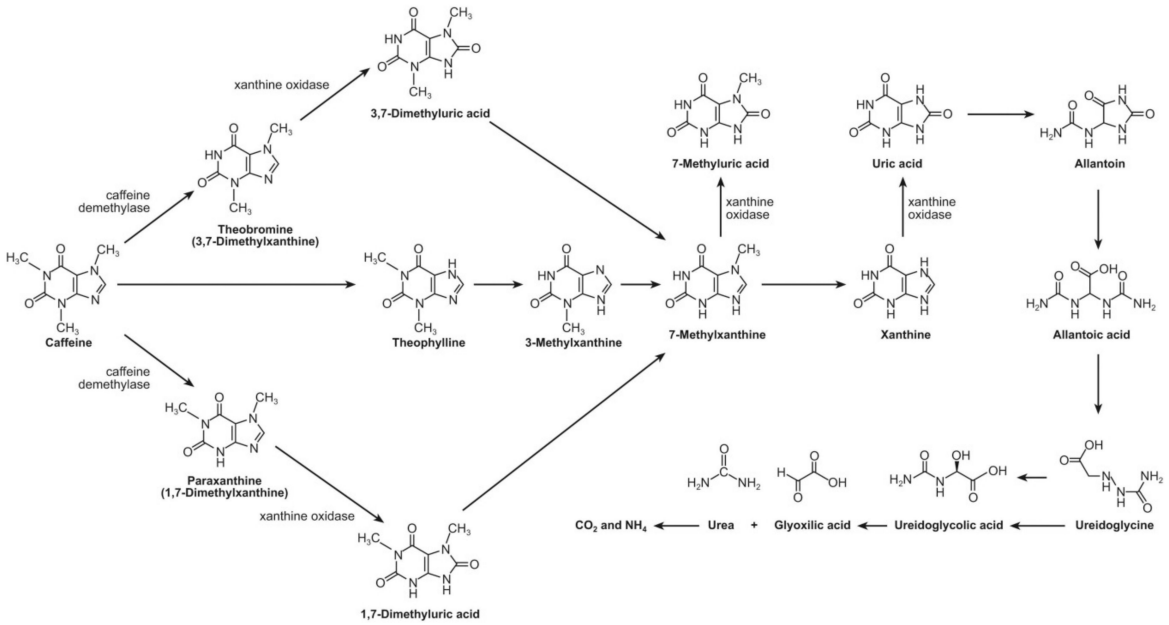

Figure 7. Caffeine degradation by bacteria (modified from Ibrahim et al. 2014) [33] and Mazzafera (2002) [34].

The high oxidant stability of SCG was proved by our results gained for antioxidant activity [10,34–38]. The phenolic content in spent coffee grounds represents a valuable source that can be used and applied in different food, pharmaceutical, or technological processes [11]. The method used for the extraction of phenolic compounds from spent coffee grounds is a big issue if phenolic compounds have to be used in the food and pharmaceutical industries, because extraction by methanol was found to be the most efficient method. Consequently, new methods for phenolic compounds extraction are in demand, or other possibilities for spent coffee ground application/usage have to be investigated [11]. Phenolic compounds in plants serve as response to environmental stresses. Chlorogenic acid is the main phenol in green coffee beans [14]. Chlorogenic acid undergoes changes during coffee processing and can be isomerized, hydrolyzed, and degraded into low molecular weight compounds. Especially high temperatures affect the transformation of chlorogenic acid into quinolactones and melanoidins [14,33]. This study showed a rapid decrease of chlorogenic acid content by an aerobic microbial consortium in the activated sludge, though it was found that in the presence of *Aspergillus alutaceus* fungus, the content of chlorogenic acid increased, while it did not have effect on caffeine content [13,33].

4. Conclusions

Spent coffee ground is a very promising waste material that can be used to stimulate microorganisms in wastewater treatment plants, which was confirmed in our work. Our results did not confirm differences in the influence of SCGs with different Arabica and Robusta ratios on

the respiration activity of microorganisms in sewage sludge. For higher SCG utilization, it would be necessary to create a SCG collection network in cafes and restaurants, regardless of the coffee mix used. Our research gave promising results for using a waste material such as SCGs in water purification with no extra cost. Further research should be focused on experiments with pure bacterial cultures and detailed chemical analysis of degradation products after decomposition of caffeine.

Author Contributions: Methodology, M.V., T.V., J.E., and D.D.; software, S.J.; validation, M.V., J.J., J.E., and I.K.; formal analysis, D.D. and S.J.; investigation, N.H. and S.J.; resources, M.V., J.E., and N.H.; data curation, M.V.; writing—original draft preparation, I.K., D.D., and S.J.

Funding: This study was supported by Grant Agency of the Masaryk University (MUNI/A/0902/2018).

Acknowledgments: The authors express their gratitude to Igor Starunko, Head of Editorial Office of Studia Biologica Journal at Ivan Franko National University of L'viv (Ukraine) for his help in the graphical preparation of the scheme of the caffeine degradation pathway by bacteria.

Conflicts of Interest: The authors declare no conflict of interest.

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