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Effects of coffee processing residues on anaerobic microorganisms and corresponding digestion performance

Juan Pablo Rojas-Sossa[a,h], Mariana Murillo-Roos[b,c], Lidieth Uribe[b], Lorena Uribe-Lorio[g], Terence Marsh[e], Niels Larsen[f], Rui Chen[h], Alberto Miranda[d], Kattia Solís[a], Werner Rodriguez[d], Dana Kirk[h], Wei Liao[h,*]

[a] Agricultural Engineering, University of Costa Rica, San José, Costa Rica
[b] Agronomy Research Center, University of Costa Rica, San José, Costa Rica
[c] National Institute for Innovation and Transfer of Agricultural Technology, Ministry of Agriculture, San José, Costa Rica
[d] Fabio Baudrit Experimental Station, University of Costa Rica, San José, Costa Rica
[e] Microbiology and Molecular Genetics, Michigan State University, MI, USA
[f] Danish Genome Institute, Aarhus, Denmark
[g] Cellular and Molecular Biology Research Center, University of Costa Rica, San José, Costa Rica
[h] Anaerobic Digestion Research and Education Center, Biosystems and Agricultural Engineering, Michigan State University, MI, USA

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ABSTRACT

The objective of this study was to delineate the effects of different coffee processing residues on the anaerobic microbes and corresponding digestion performance. The results elucidated that mucilage-rich feed enhanced the accumulation of methanogens, which consequently led to better digestion performance of biogas production. Fifty percent more methane and up to 3 times more net energy (heat and electricity) output were achieved by the digestion of the mucilage-rich feed (M3). The microbial community and statistical analyses further elucidated that different residues in the feed had significant impact on microbial distribution and correspondingly influenced the digestion performance.

1. Introduction

Coffee is merchandised worldwide as green coffee beans. The beans need to be separated from harvested coffee fruit and dried in order to preserve its quality. Dry and wet processes are the two typical approaches to produce green coffee beans. The wet process is widely used in Central America (Brando and Brando, 2014), it consists of three main steps: 1) removal of the pulp and outer skin of the fruit using a mechanical pulper, 2) removal of the mucilage by fermentation or by mechanical separation, and 3) drying and hulling of beans. The wet processing residues include pulp, mucilage, processing wastewater, and a dry residue known as parchment, which altogether account for more than 50% of the initial weight of fresh coffee fruit (Esquivel and Jiménez, 2012). These residues have high organic contents. Coffee pulp contains 21–32% carbohydrates, 7.5–15% protein and 2–7% fat (Ulloa Rojas et al., 2003); the mucilage is rich in protein (8.9%), sugar (4.1%), and peptic substances (0.91%) (Belitz et al., 2004); whereas the processing wastewater usually has a high organic content as well (Rattan et al., 2015). Costa Rican coffee industry annually produces 250,802, 94,051, and 178,436 tonnes of pulp, mucilage, and wastewater, respectively (Coto, 2013). Without proper management, the residues pose a significant risk to local environment.

In the past decades, numerous studies have been conducted to identify possible applications for these residues. Coffee pulp has been studied for the production of vermicompost (Raphael et al., 2012), isolation of bio-compounds such as anthocyanins (Prata and Oliveira, 2007), ethanol fermentation (Menezes et al., 2013), and enzyme production (Dias et al., 2015). Mucilage has been identified as a good source for hydrogen production (Hernández et al., 2014). Coffee husk has been used for combustion in the drying ovens of the bean (Coto, 2014). Besides these applications, the potential of coffee residues for biogas production has also been intensively studied (Beyene et al., 2014). Low pH, high lignin content, and elevated production of volatile fatty acids in coffee residues have been recognized as the main hindrances to improve the efficiency of these digestion systems (Víquez, 2012). In order to address these issues, co-digestion of coffee residues with other feedstocks, such as chicken litter (Abouelenien et al., 2014) and cow manure (Corro et al., 2013) has been suggested to balance the

* Corresponding author at: 524 S. Shaw Ln. Room 202, East Lansing, MI 48824-1323, USA.
E-mail address: liaow@msu.edu (W. Liao).

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carbon-nitrogen ratio, enhance the microbial growth, and consequently improve the digestion performance. In spite of the good performance of co-digestion, challenges of feedstock availability, transportation, and logistics impede the implementation of such strategy to treat coffee processing residues.

Considering the nutrient conditions of different coffee processing residues and the potential applications of them (i.e., mucilage could be a good feed for digestion; the pulp can be dried and mixed with coffee husk for combustion to dry the coffee beams), anaerobic digestion of different residues was studied in this paper to elucidate their impacts on digestion performance, and conclude a preferred residue combination that maximizes the energy production of coffee residues. In addition, an in-depth microbial community study was carried out to describe the relationship between microbes, processing residues, and operational conditions.

2. Methods

2.1. Feed and inoculum

The fresh samples of wastewater, coffee pulp, and coffee mucilage were collected from San Carlos Coffee Mill owned by Marespi S.A. in Pérez Zeledón Costa Rica (9°14′08.2″N, 83°38′16.1″W) on September of 2014 and stored at 4 °C. The TS contents of wastewater, pulp, and mucilage were 0.34%, 12.21%, and 2.56%, respectively. The seed was obtained from an anaerobic digester at the Fabio Baudrit Experimental Station of the University of Costa Rica, Alajuela, Costa Rica. Three different combinations of residues; M1, M2 and M3 were prepared to feed the digesters. M1 was the actual coffee processing residue without any adjustment, in which volume ratio of wastewater, pulp, and mucilage was 1:0.3:0.1. M1 was served as the control. M2 was the feed with approximately equal TS amounts of pulp and mucilage, in which volume ratio of wastewater, pulp, and mucilage was 1:0.3:1.3. M3 was the mucilage-rich feed, in which volume ratio of wastewater, pulp, and mucilage was 1:0.3:12.9. The characteristics of these mixtures are presented in Table 1.

2.2. Anaerobic digestion of coffee residues

M1, M2, and M3 were used as the feeds to evaluate digestion performance. Nine reactors (three replicates for each mixture) were used in the study, each of which contained a working volume of 0.5 L. The culture temperature was 35 °C. The hydraulic retention time (HRT) was 30 days and the experiment lasted for 90 days. At the beginning of the experiment, all reactors were inoculated with 1:1 vol of the stabilized seed (2.5% TS). The reactors were then fed daily with 17 mL of the feed inside a Siplfyter Hands-In-Bag (NPScorp, WI) purged with nitrogen to maintain anaerobic condition. An equal volume (17 mL) of digestate was removed from the digesters prior to the feeding. The feeds were prepared a few days before the feeding, according to the mixing ratios and stored at 4 °C. The reactors were manually shaken twice a day. The daily biogas production was measured using a water displacement unit. The pH was maintained above 6.7 by dosing 20% sodium hydroxide (NaOH) when necessary. Twelve mL of the digestate samples were taken and stored at −20 °C for total solids (TS) and volatile solids (VS) determination, and 5 mL were stored at −80 °C for microbial community analysis. At the end of the experiment, the remaining samples were processed and dried for fiber analysis (cellulose, xylan and lignin).

Table 1
Characteristics of different feeds.

Parameters	M1	M2	M3
TS (wt%)[a]	2.38	2.99	3.45
VS (wt%)[a]	2.12	2.67	2.96
C/N	15.0	16.3	16.5
Cellulose (wt%, dry basis)[b]	16.6 ± 0.2	12.4 ± 0.2	10.8 ± 0.1
Xylan (wt%, dry basis)[b]	9.7 ± 0.1	6.7 ± 0.8	5.4 ± 0.1
Lignin (wt%, dry basis)[b]	32.5 ± 0.4	33.8 ± 0.5	35.8 ± 0.6

[a] The data are average of two replicates.
[b] The data are average of three replicates with standard deviation.

2.3. DNA Extraction PCR and sequencing procedures

A PowerLyzer® PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad) was used to extract genomic DNA from the initial seed and digestate from the second HRT. The DNA concentrations were quantified using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA). The extracted DNA was concentrated to 100–150 ng/μL using 5 M NaCl and cold ethanol (200 proof).

Pilot-scale polymerase chain reactions (PCR) were performed to test the capability of amplification using the universal primers, the forward primer Pro 341 F (5′-CCTACGGGNGGCWGCAG-3′) and the reverse primer Pro 805 R (3′-GACTACNVGGGTATCTAATCC-5′), to amplify both archaeal and bacterial DNAs (Takahashi et al., 2014). A 25 μL reaction solution containing 12.5 μL GoTaq® Green Master Mix (PromegaTM, Madison, WI), 1 μL forward primer, 1 μL reverse primer, 0.5 μL BSA, and 1 μL of extracted DNA, was mixed with 9 μL DNase and RNase free water for PCR reaction. The amplification included an initial denaturing step at 95 °C for 5 min, followed by 30 cycles of the touchdown steps (denaturing at 95 °C for 2 min, annealing at 58 °C for 5 s, and elongation at 48 °C for 5 s using 30 cycles), and a final extension at 72 °C for 5 min. PCR products were visualized in a 1% agarose gel.

After pilot PCR showed positive results on the electrophoresis gel (1% agarose, dyed with ethidium bromide) for all samples, samples containing original DNA template were analyzed at the Research Technology Support Facility at Michigan State University. The V3-V4 region (341–806) of the 16S rRNA gene was amplified using a set of primers designed to capture a wider range of targets from both bacteria and archaea (Takahashi et al., 2014). Primary PCR was performed using fusion primers with target specific portions as described in Takahashi's report and Fluidigm CS oligos at their 5′ ends. Secondary PCR targeting the CS oligos was then used to add sequences necessary for Illumina sequencing and unique indexes. The PCR products were normalized using Invitrogen SequalPrep DNA normalization plates, and normalized eluates form the plates were pooled. After validation and quantification, the pool was sequenced on an Illumina MiSeq flow cell (v2) using a 500 cycle reagent kit (2 × 250 bp paired end reads). Custom sequencing primers matching the Fluidigm CS1 and CS2 oligos were used. Base calling was done by Illumina Real Time Analysis (RTA) v1.18.54 and output of RTA was de-multiplexed and converted to FASTQ format by Illumina Bcl2fastq v1.8.4.

2.4. Bioinformatics

Fastq files from Illumina sequencing were analyzed with BION, a semi-commercial open-source package for microbial community analysis from the Danish Genomic Institute, Aarhus, Denmark. Primer sequences were used to extract the paired sequences from the raw reads and a minimum quality of 99% was required for at least 14 of 15 bases for forward reads and 28 of 30 for reverse reads. A minimum length of 50 was imposed. Paired reads were joined where there was at least 25 base overlap and 85% similarity. Sequences were then filtered for length (250 minimum) and quality (99.6%), dereplicated, preclustered at 99% and checked for chimeras using an algorithm unique to BION. Non-chimeric sequences were clustered at 99% stringency and a minimum length of 300. Sequences were then matched to reference sequences using a K-mer length of 8 with a step size of 4 and compared against the 340–807 region in RDP 11.04. The sequence similarities of each sample were converted to a taxonomic profile, using the RDP taxonomy and the profiles were combined into abundance tables.