Anaerobic Digestion of Coffee Wastewater from a Fast Inoculum Adaptation Stage: Replacement of Complex Substrate

D. Lepe-Cervantes, E. Leon-Becerril, J. Gomez-Romero, O. Garcia-Depraect, A. Lopez-Lopez

Abstract**—**In this study, raw coffee wastewater (CWW) was used as a complex substrate for anaerobic digestion. The inoculum adaptation stage, microbial diversity analysis and biomethane potential (BMP) tests were performed. A fast inoculum adaptation stage was used by the replacement of vinasse to CWW in an anaerobic sequential batch reactor (AnSBR) operated at mesophilic conditions. Illumina MiSeq sequencing was used to analyze the microbial diversity. While, BMP tests using inoculum adapted to CWW were carried out at different inoculum to substrate (I/S) ratios (2:1, 3:1 and 4:1, on a VS basis). Results show that the adaptability percentage was increased gradually until it reaches the highest theoretical value in a short time of 10 d; with a methane yield of 359.10 NmL CH_4/g COD-removed; *Methanobacterium beijingense* was the most abundant microbial (75%) and the greatest specific methane production was achieved at I/S ratio 4:1, whereas the lowest was obtained at 2:1, with BMP values of 320 NmL CH_4/g VS and 151 NmL CH_4/g VS, respectively. In conclusion, gradual replacement of substrate was a feasible method to adapt the inoculum in a short time even using complex raw substrates, whereas in the BMP tests, the specific methane production was proportional to the initial amount of inoculum.

Keywords**—**Fast inoculum adaptation, coffee wastewater, biomethane potential test, anaerobic digestion.

I. Introduction

The coffee industry is one of the biggest industries in the world, which produces a large amount of complex substrates known as CWW, representing production around of 416 tons COD per year with an organic load from 12 to 20 g COD/L [1]. Anaerobic digestion has been considered able to convert the complex substrates of wastes in methane of a way economical and friendly environmental [2]. CWW has significant energy content, due to the high carbohydrates concentration; it is ideal to recover bioenergy by their availability.

BMP test is a suitable method to determine the methane potential production of a substrate. The BMP test is one of the most significant analyses for biogas production from a substrate and has a major impact on the design of an anaerobic digester. An optimal balance to overcome biomass limitation to avoid organic matter overloading is recommended [3]; thus, it is necessary for the assessment of anaerobic biodegradability rate in complex substrates as CWW to find out the BMP, as well as the types of microorganism communities involved in the production of methane.

BMP tests with complex substrates involved many factors such as substrate preparation, type of substrate, inoculum to substrate (I/S) ratio and inoculum adaptation [4], [5]. Among these, inoculum adaptation plays a key role, and is referred to as the ability of inoculum to develop a higher tolerance to the inhibitors founded in complex substrates [2], [6]. Also, the adapted inoculum can accelerate the start-up and improve the efficiency and stability of the digestion process [4], [7]. On the other hand, I/S ratio is recognized as one of the major parameter affecting the BMP tests [8]; therefore, the I/S ratio and adaptation inoculum are two main factors affecting the operating conditions to obtain the optimal methane yield and methane production for a specific inoculum and target substrates.

The aim of this research is to study the anaerobic digestion of CWW by BMP test using an anaerobic granular sludge (AGS) from a fast inoculum adaptation stage. The adaptation process of the inoculum was carried out by gradually replacement of a complex substrate (vinasse) to another (coffee wastewater) during a short time of 10 days (240 hours).

II. Materials and Methods

A. Feedstock and Inoculum Source

This study used raw undiluted wastewater (CWW and vinasse) as complex substrates. CWW was provided from a coffee processing factory located in Veracruz, Mexico, whereas the vinasse was obtained from a local tequila factory in Jalisco, Mexico. Both complex substrates were fresh collected and stored immediately in a cool room at 4°C until use. On the other hand, AGS was used as inoculum; it was obtained from a stable anaerobic digester (Up-flow anaerobic sludge blanket reactor, UASB) for treating vinasse at mesophilic conditions. The volatile suspended solids (VSS), total suspended solids (TSS) to VSS ratio and specific methanogenic activity were 122.8 g/L, 0.79 and 0.37 g COD-CH4/g VS-d, respectively.

B. Experimental Set-Up

An adaptation stage was carried out in order to adapt the AGS to the CWW. The experimental setup is shown in Fig. 1. It was performed using an AnSBR with a total cycle time of 2

Daniela Lepe-Cervantes, Elizabeth Leon-Becerril, Jacob Gomez-Romero, Octavio Garcia-Depraect, Alberto Lopez-Lopez * are with the Department of Environmental Technology, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, Normalistas 800, Colinas de la Normal, 44270 Guadalajara, Jalisco, México (* corresponding autor; phone: 01 33 3345 5200; e-mail: allopez@ciatej.mx; alopez103@yahoo.com).

d and a working volume of 4 L. Temperature was maintained at 30 ± 2°C using a water bath; pH was adjusted at 7 ± 0.2 with NaOH (5M), while a full scale operation approach was taken into account to set low and intermittent mixing inside the reactor (three times per day).

The AGS was washed with distilled water and then it was employed to inoculate the AnSBR with 64 g VSS/L. Different mixtures of vinasse and CWW were tested. The amount of CWW was increased from 20% to 100% (w/w) with increments of 20% each cycle. Therefore, the AnSBR was operated at organic loads of 16.4 to 10.78 g/L. Influent and effluent COD and biogas production/composition were monitored continuously.

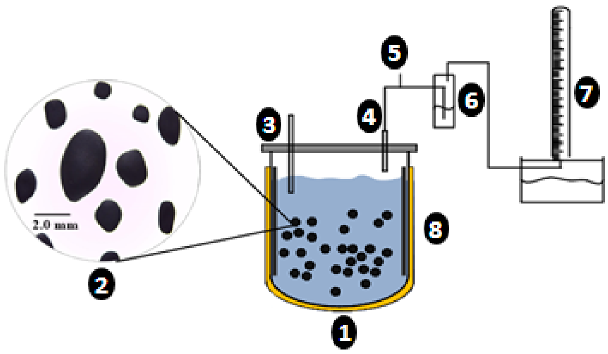

Fig. 1 Schematic diagram of the adaptation stage: (1) AnSBR; (2) AGS; (3) liquid sampling port; (4) biogas outlet; (5) biogas sampling port; (6) CO_2 fixing trap; (7) methane measurement system and (8) water bath

The effect of three different I/S ratios were assessed on the BMP test of CWW. I/S values were 2:1, 3:1 and 4:1 (on a VS basis). The BMP assays were performed during 16 d using an automatic methane potential test system (AMTPS) supplied by Bioprocess Control. The AMPTS consists of four major parts as can be seen in Fig. 2. A total of 12 reactors including controls and triplicates were run with the following conditions: working volume, 300 mL; temperature, 37°C, mixing speed, 112 rpm, mixer on time, 60 s and mixer off time 180 s. Anaerobic conditions were assured by flushing nitrogen gas into the liquid for 3 minutes; pH was controlled at 7.0 ± 0.2 by addition of NaOH (5M) or H_2SO_4 (3.5 N). The biogas passed through a fixing carbon unit with NaOH (3M). Then, methane passed through individual flow cells which automatically measured and recorded gas flow.

C. Analytical Techniques

The parameters BOD, COD, total nitrogen, total phosphorus, TS, VS, TSS, alkalinity and pH were analyzed according to standard methods [9]. Biogas composition was monitored with a Perkin-Elmer Clarus 580 gas chromatograph (Perking Elmer Inc., NY, USA) equipped with a thermal conductivity detector and Hayesep D column (10 ft x 1/8 in., 100/120 mesh). The oven, injector and detector temperatures were 20, 120 and 75 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 30 mL/min.

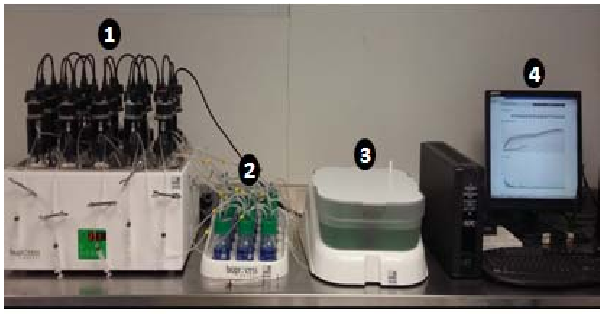

Fig. 2 Schematic diagram of the adaptation stage BMP test system: (1) Bioreactors with control temperature and stirring; (2) CO2 fixing trap, (3) gas flow meter and (4) data acquisition system

D. DNA Extraction and Illumina MiSeq Sequencing of AGR

DNA extraction and Illumina Miseq sequencing were performed, as previously described [10]. DNA was extracted using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Carsbad, CA, USA). Microbial ecology was performed via Illumina Miseq sequencing at the Research and Testing Laboratory (RTL, Lubbock, TX, USA).

E. Data Processing

Methanogenic activity was measured by the composition and volume of methane produced over time. During the adaptation stage, methane yield and adaptability percentage were calculated according to (1) and (2), respectively. On the other hand, to evaluate the BMP of CWW, the methane produced per gram VS added was calculated according to (3). The mean value of methane produced by three blanks was withdrawn from assays. Methane volume for all calculations was normalized at STP (1 atm, 0°C).

$$\text{Methane yield} = \frac{\text{NmL } CH_4}{\text{g COD-removed}} \quad (1)$$

$$\text{Adaptability (\%)} = \frac{\text{Methane yield}}{\text{Theoretical methane yield}} * 100 \quad (2)$$

$$BMP = \frac{(V_S - V_B)\frac{m_{IS}}{m_{IB}}}{m_{VS}} \quad (3)$$

where: BMP is the normalized volume of methane produced per gram VS of substrate added (NL/g VS), VS is the mean value of the accumulated volume of methane produced from sample (NL), VB is the mean value of the accumulated volume of methane produced by three blanks (NL), mIS is the total amount of inoculum in the sample (g VS), mB is the total amount of inoculum in the blank (g VS), and mVS is the total amount of organic material (g VS).

III. Results and Discussion

A. Physicochemical Characterization of CWW and Vinasse

Table I summarizes the physicochemical characterization of

CWW and vinasse. Organic matter content as COD was 60 g/L for vinasse and 30 g/L for CWW. Organic matter of both substrates had an average biodegradability showing a COD/BOD ratio of 0.44 and 0.51 for CWW and vinasse, respectively. According to this, CWW and vinasse are potential substrates for biogas production. On the other hand, pH values were 3.83 and 3.6 for CWW and vinasse, respectively. Furthermore, a concomitant effect of null alkalinity in substrates was detected. There is a well-established understanding of the effects produced by a lack of alkalinity on the performance of AD [11]. Therefore, in order to achieve a steady process with high efficiency, the addition of an external alkalinity source is an important aspect that must be considered during the anaerobic treatment of CWW and vinasse.

Additionally, another key point to take into consideration is to balance correctly the nutrients due to both substrates showed a not well-balanced C:N:P ratio. Respect to solids concentration, TS was higher in vinasse with 34.55 g/L than in CWW with 25.57 g/L. For CWW about 95% of TS are VS whereas vinasse showed 92%. Therefore, for physicochemical characterization of CWW and vinasse, similar results are reported on several works reported previously [12]-[15].

B. Microbial Ecology

The community of AGS was characterized via Illumina MiSeq sequencing; the result showed that the major microorganisms were methanogenic archaea-microbes (Fig. 3). Such as *Methanobacterium beijingense, Methanobacterium* sp. and *Methanolinea* sp. with abundances of 75%, 25% and 5%, respectively. Methanogenic archaea-microbes i.e. *Methanobacterium beijingense, Methanobacterium* sp., and *Methanolinea* sp. are clustered within five orders of the *Euryarchaeota* and constitute the last step in the trophic chain of decomposers degrading organic matter in oxygen-free environments [16].

TABLE I
PHYSICOCHEMICAL CHARACTERIZATION OF CWW AND VINASSE

Parameter	CWW	SD	Vinasse	SD
pH	3.83	0.01	3.60	0.01
BOD (mg/L)	13,248.21	25.10	30,625.00	4,207.80
COD (mg/L)	30,278.20	4.84	59,687.50	2,251.00
TS (g/L)	25.57	0.81	34.55	0.54
VS (g/L)	24.20	0.44	30.93	0.40
Total nitrogen (mg /L)	412.19	2.19	188.33	24.74
Total phosphorous (mg/L)	106.43	0.11	341.25	19.80

Community composition of AGS was similar to that previously reported by the reference [17] with the existence and high predominance of *Methanobacterium beijingense* from an UASB reactor. As well as, the genus Methanobacterium is essential to the methane production from complex substrates, it has been identified as the principal hydrogen scavenger in methanogenesis, which can use H_2/CO_2 for its growth. Furthermore, Methanobacterium plays an important role in the anaerobic digestion of complex substrates constituting the main microbial flora. On the other hand, despite the low abundance of *Methanolinea* sp., its presence is important to the overall process. *Methanolinea* sp. hydrogenotrophic pathway is coupled with syntrophic bacterial oxidation of volatile fatty acids to H2/CO2 [18].

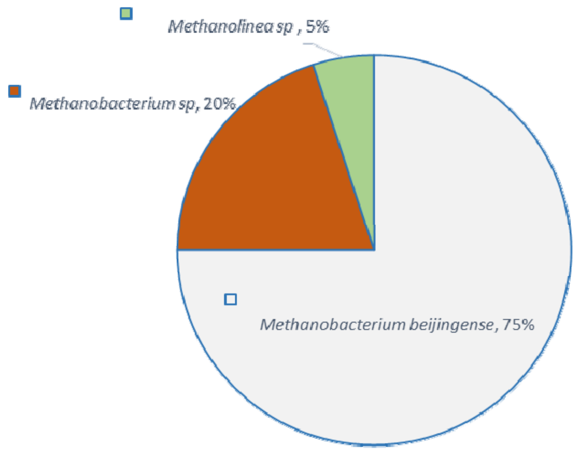

Fig. 3 Result of 455-pyrosequencing analysis at specie level distribution of the AGS

C. Inoculum Adaptation Stage by Replacement of Complex Substrate

The adaptation of inoculum exposed to either environmental conditions or different substrates is an important factor influencing both the rate and the extent of biodegradation [19]. As is shown in Fig. 4, a short lag phase was observed at start-up methane production, with an average of 2 h in all proportions tested. Table II shows the methane yield, adaptability and the maximum removal of organic matter. Methane yield was low at first CWW/vinasse proportions, 277.75 and 260.77 NmL CH_4/g COD-removed for 20 CWW/80 vinasse and 40 CWW/60 vinasse, respectively. However, the highest methane yield was reached with a value of 359.16 NmL CH_4/g COD-removed at 100% CWW. This result is consistent to the theoretical methane yield of 350 NmL CH_4/g COD-removed [14]. According to others reports, high methane yields from CWW have also been reported.

Adaptability of the granular sludge to the CWW was increased from 79.35% to 102.6%, which indicates that bacterial community was able to rapidly adapt to a new complex substrate.

The high adaptability reached in all proportions in a short time, can be correlated with the organic matter removal from 46.88 to 54.52%. Studies by [20] with fresh CWW showed that bacterium consortium needed 150 days to acclimate themselves. They concluded that values lower than 0.3 g COD CH_4/g VSS-d could be explained by the lack of the acclimation period of the bacteria to CWW.

TABLE II
GLOBAL YIELD METHANE, ADAPTATION INOCULUM AND ORGANIC MATTER REMOVAL IN ASBR AT DIFFERENT PROPORTIONS OF CWW AND VINASSE

Period	CWW/Vinasse Proportion (% w/w)	Organic Load (g COD/L)	Methane Yield (NmL CH_4/g COD)	COD Removal (%)	Adaptability (%)
I	100/0	16.40	277.75	46.88	79.35
II	40/60	15.28	260.77	54.22	74.50
III	60/40	15.13	310.94	55.18	88.84
IV	80/20	11.88	331.30	48.42	94.64
V	100/0	10.78	359.10	54.52	102.60

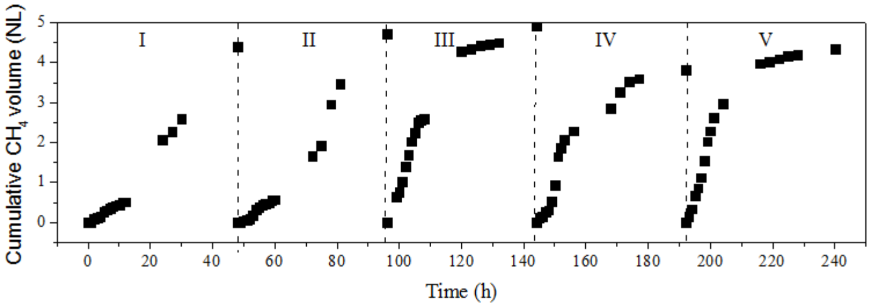

Fig. 4 Accumulated methane volume at different proportions of CWW and vinasse

Finally, the results demonstrated that the adaptation of the inoculum to CWW was necessary and important, at first the CWW/vinasse proportions of the methane yield was slightly low compared to the methane yield obtained at CWW 100%. In other words, the adaptability percentage was gradually increased until the highest theoretical value was reached. Thus, the organisms could be adapted in a short time.

D. BMP Test of CWW

In this study, BMP tests were carried out in order to estimate biogas potential from CWW. The inoculum from the fast adaptation stage of 10 days (240 hours) was used. I/S ratios 2:1, 3:1 and 4:1 were tested. Gas methane production was decreased after approximately 12, 10 and 9 d for 2:1, 3:1 and 4:1 ratios, respectively (Fig. 5). It can be due to either the exhaustion of substrate or by the developing of inhibitory conditions. These results showed that the higher amount of granular sludge in the reactors produced a higher methane volume. Thus, the greatest specific methane production was achieved at I/S ratio 4:1 (320 NmL CH4/g VS), whereas the lower was obtained at I/S ratio 2:1 (151 NmL CH4/g VS). For I/S ratio 2:1, the biomass activity could be affected due to either low substrate concentration or the organic overloading. In contrast, I/S ratio 4:1 had the optimal balance of inoculum to substrate; it could be inhibited by higher concentrations of volatile fatty acids. It is important to remark that was necessary to control pH during the first eight hours. On the other hand, behavior of BMP tests with different I/S ratios helped to validate the results from the adaptation stage. Reference [12] assessed the production of methane from Robusta coffee solid waste (mixture of pulp and husk), coffee pulp and CWW, obtaining 650, 730 and 300 mL CH4/g VS, respectively. These BMP results are compared to other reported results with complex substrates. Reference [4] showed the importance of inoculum adaptation to substrate at micro BMP assays. However, reported biomethane yields from complex substrates can vary due to the heterogeneous nature of the material and differences in composition and toxic components. Also operating temperature, bioreactor design and organic load can significantly affect results.

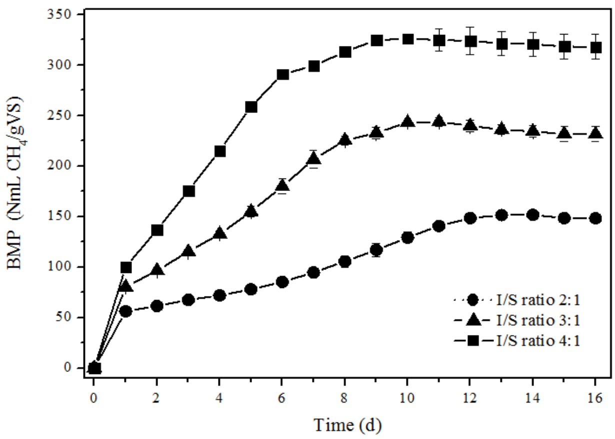

Fig. 5 Specific methane production at different I/S ratios

IV. CONCLUSIONS

Results show that CWW and vinasse are considered as complex substrates with an acid pH, high amount of COD and total solids. The best adaptation results were achieved when CWW concentration was 100% with the highest methane yield and adaptability percentage of 359.10 NmL CH_4/g COD-removed and 102.6% for AGS. Adaptation of AGS to a new complex substrate was achieved in a relatively short time of 10 d. The microbial analysis of the AGS showed the high predominance of *Methanobacterium beijingense* with 75%, which is associated to methane production. Finally, the BMP test of adapted AGS to CWW revealed that the greatest specific methane production was achieved at I/S ratio 4:1 (320

NmL CH_4/g VS), whereas the lower was obtained at I/S ratio 2:1 (151 NmL CH_4/g VS).

Acknowledgment

The authors wish to express their gratitude to the National Council of Science and Technology (CONACYT) for supported this research (Grant 23288) through the program "Programa de Estímulos a la Investigación, Desarrollo Tecnológico e Innovación 2015."

References

[1] Pineda, C., Reyes, C. and Oseguera, A. (2003) Beneficiado y calidad del café. Manual del beneficiado del café, Instituto Hondureño del Café (IHCAFE), 212- 241.
[2] Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, L., Guwy, A. and Van Lier, J. (2007) Anaerobic Biodegradation, Activity and Inhibition (ABAI) Task Group Meeting 9th to 10th October 2006, in Prague. Kgs. Lyngby: Institute of Environment & Resources, Technical University of Denmark.
[3] González, C., and García, P. A. (2009) Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry. Biomass Bioenergy. 33(8), 1065-1069.
[4] Nizami, A.S., Korres, N.E. and Murphy, J. D. (2009) Review of the Integrated Process for the Production of Grass Biomethane, Environ Sci Technol. 43(22), 8496-8508.
[5] Singh, A., Smyth, B.M. and Murphy, J.D. (2010) A biofuel strategy for Ireland with an emphasis on production of biomethane and minimization of land-take. Renew Sust Energ Rev. 14, 277-288.
[6] Raposo, F., De la Rubia, M. A., Fernández, V., and Borja, R. (2012) Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. Renew Sust Energ Rev. 16(1), 861-877.
[7] Gonçalves, M., Costa, J., Marques, I., and Alves, M. (2011) Inoculum acclimation to oleate promotes the conversion of olive mill wastewater to methane. Energy. 36(4), 2138-2141.
[8] Neves, L., Oliveira, R., and Alves, M. M. (2004) Influence of inoculum activity on the bio-methanization of a kitchen waste under different waste/inoculum ratios. Process Biochem. 39(12), 2019-2024.
[9] APHA. Standard Methods for the Examination of Water and Wastewater. 20th ed. J. American Public Health Association/American Water Works Association/Water Environment Federation: Washington, DC, 1999.
[10] Valdez, I., Torres, G. J., Molina, C., and Ruiz, G. M. (2016) Characterization of a Lignocellulolytic Consortium and Methane Production from Untreated Wheat Straw: Dependence on Nitrogen and Phosphorous Content. Bioresour Technol. 11(2), 4237-4251.
[11] Van Lier, J. B., Mahmoud, N., and Zeeman, G. (2008) Anaerobic wastewater treatment. Biological Wastewater Treatment: Principle, Modelling and Design. IWA Publishing, London, 415-456.
[12] Kivaisi, A.K. and Rubindamayugi, M.S.T. (1996) The potential of agro-industrial residues for production of biogas and electricity in Tanzania. Renew Energ. 9(4), 917-921.
[13] Hernandez, M. A., Rodríguez, M. and Andres Y. (2014) Use of coffee mucilage as a new substrate for hydrogen production in anaerobic co-digestion with swine manure. Bioresour Technol. 168, 112-118.
[14] López, A., León, E., Rosales, M. E., and Villegas, E. (2015) Influence of alkalinity and VFAs on the performance of an UASB reactor with recirculation for the treatment of Tequila vinasses. Environ Technol. 36(19), 2468-2476.
[15] Novita E. (2016) Biodegradability simulation of coffee wastewater using instant coffee. Agric Sci Procedia. 9, 217-229.
[16] Thauer, R. K., Kaster, A.K., Seedorf, H., Buckel, W. and Hedderich, R. (2008) Methanogenic archaea: ecologically relevant differences in energy conservation. Nat Rev Microbiol. 6, 579-591.
[17] Ma, K. (2005) Methanobacterium beijingense sp. nov., a novel methanogen isolated from anaerobic digesters. Int J Syst Evol Microbiol. 55(1), 325-329.
[18] Wilkins, D., Rao, S., Lu, X., and Lee, P. K. (2015) Effects of sludge inoculum and organic feedstock on active microbial communities and methane yield during anaerobic digestion. Front. Microbiol. Frontiers in Microbiology. 6, 1-11.
[19] Whatson, H. M. (2009) A comparison of the effects of two methods of acclimation on aerobic biodegradability. Environ Toxicol Chem. 12(11), 2023-2030.
[20] Houbron, E., Larrinaga, A, and Rustrian, E. (2003) Liquefaction and methanization of solid and liquid coffee wastes by two phase anaerobic digestion process. Water Sci Technol. 48(6), 255-62.