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Optimization of Biogas Production from Organic Municipal Waste: Development of Activated Sludge as Digesters Inoculum

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Abstract

This study is a contribution to the optimization of organic fraction of municipal waste bioconversion into biomethane, by activated sludge production as inoculum for digesters. The wastewater (WW) and cow dung (CD) samples were taken from the slaughterhouse of Ouagadougou town, Burkina Faso. Different mixtures were made, enriched with mineral solution and cellulose at 5% (w/v) as: 10% CD + 90% WW (C7), 30% CD + 70% WW (C6), 50% CD + 50% WW (C5), 70% CD + 30% WW (C4), 90% CD + 10% WW (C3), 100% CD (C2) and 100% WW (C1). The pH evolution and biogas (CH_4 and CO_2) production were followed for 25 days. Cultures tend to acidify with increase in cow dung proportion. Biogas production was significantly higher ($p < 0.05$) in C5 (880.0 mL), C6 (862.0 10 mL) and C7 (772.0 mL). Mixture C5 had a highest level of CO_2 production (40%). Also C7 and C6 retained in the experiment contained respectively organic matter, volatile fatty acids (VFA) and total alkalinity (TAC) as 41.06%, 47.02%, 1320 mg acetic acid/L, 3036 mg Acetic acid/L and 520 mg $CaCO_3$/L, 1310 mg $CaCO_3$/L. Mixture C6 was the best medium for microorganism proliferation stability with 3.5×10^5 UFC/ml of methanogens bacteria. It also possessed buffering capacity, which prevents acidification of medium during VFAs production.

Keywords

Bioconversion, Municipal Waste, Activated Sludge, Biomethane, Burkina Faso

1. Introduction

Demographic and economic growth, rapid urbanization and rising living standards lead to an increase in quantity and quality of solid waste production [1] [2] [3]. This phenomenon creates enormous risks to the environment and consequently to population health [4] [5]. The management of solid waste has become a major problem today, especially in developing countries (DCs), due to the lack of resources and difficulty to develop an approach adapted to their context. Despite recommendations and regulatory measures to reduce waste generation at source and ultimate waste, promoting eco-design, sorting, recycling and incineration, landfill remains a significant part of waste. In order to protect environment, the controlled methanization process in sealed enclosed spaces has been developed as opposed to landfill centers [6]. Indeed, the advantages of this technique are multiple. Methanization requires less space and considerably reduces volume and weight of waste to be buried. It permits significant reductions in greenhouse gas emissions (CO_2 and CH_4), and odors elimination. The digestate issued can be used to reinforce agriculture. The biogas (renewable fuel) generated can be used in several final applications. Therefore, methanization is more profitable than all other forms of waste treatment [7] [8] [9] [10] [11]. Biogas containing methane was recognized as a new renewable energy source according European Directive 2001/77/EC. Also, it added that 20% of energy consumed in EU in 2020 will coming from renewable sources. Thus, studies have been carried out in a context of anaerobic digestion process optimization. The different stages of anaerobic digestion process were dissected [12]-[17]. Many studies are interested in substrates diversification and their biomethanogenic potential [18] [19] [20] [21]. Co-digestion systems that treat a mixture of different king of waste, including animal, food and organic household waste have been developed [22] [23] [24]. Nations have embarked on energy crops to increase renewable energy production more rapidly [25] [26]. Amarante [27] reported several commercialized technologies worldwide for municipal organic residues treatment. The use of adequate inoculums is an important parameter for anaerobic digestion. It must be adapted to the substrate. Most digesters are inoculated with old sludges from other mesophilic or thermophiles anaerobic digesters [28] [29] [30]. In other studies a commercial solution containing some microorganisms was involved in anaerobic degradation [31]. Nikièma *et al.* [32] and Traoré *et al.* [33] showed the possibility of using wastewater and bovine dung from slaughterhouse as a source of inoculum for biogas production. Nature and proportion of inoculums used have potentially a significant impact on methane production [34] [35] [36] [37] [32]. The aim of this study was to develop activated sludge to inoculate municipal organic solid waste digesters.

2. Material and Methods

2.1. Inoculum Sampling

Wastewater (WW) and bovine dung (BD) were sampled at slaughterhouse in

Ouagadougou, Burkina Faso (12˚25'5.87"N, 1˚28'29.23"O). The cow dung was freshly removed, placed in a sterile stomacher plastic bag and transferred to the Laboratory. The wastewater was removed at depth (1 meter) and all samples were stored at 4˚C.

2.2. Experimental Conditions

The experimental conditions for the production of activated sludge described was adapted from the process of Angelidaki [38]. The experimental conditions are presented in Table 1.

Experiments were carried out in 300 mL flask filled with 20 ml of mineral solution supplemented with 29 mL cellulose 5% (w/v). Combinations of different proportions of bovine dung and wastewater were used as inoculum: 100% WW, 100% CD, 90% CD + 10% WW, 70% CD + 30% WW, 50% CD + 50% WW, 30% CD + 70% WW and 10% CD + 90% WW. 0.4 ml of a buffer solution ($NaHCO_3$, 10%) and 0.6 ml of a reducing solution (Na_2S_9, H_2O, 2.4%) were added in a sterile manner. The tests were carried out in duplicate. The cow dung was washed in distilled water (2% w/v) prior to combinations.

The mineral solution contained 10 mL of phosphate buffer 10 mL (2 g/L K_2HPO_4 and 2 g/L NH_4Cl), 8 mL of Balch's trace mineral solution [39]; 1 mL of Widdel trace element solution [40] and 1 mL of vitamin solution.

2.3. Evaluation of Methanogenic Activities

The evaluation of activity of the different microbial groups was followed by the measurement of methane production [41]. Estimation of biogas production was carried out using liquid raising method. The gaseous products (CO_2 and CH_4) were analyzed using a gas chromatograph (Girdel series 30 catharometer equipped with a thermal conductivity detector [TCD] associated to SERVOTRACE potentiometer recorder type Sefram Paris of 1 mV). CH_4 and CO_2 were analyzed under following: Injector temperature 90˚C, column temperature 60˚C, detector temperature 100˚C, filament current 150 mA, carrier gas pressure 1 bar, and attenuation 32, Paper flow rate 10 mm/min. 0.5 ml of the flask gas phase was taken

Table 1. Experimental design matrix.

Combination	MS (mL)	WW (mL)	BD (mL)	Cellulose 5% (mL)
100% WW (C1)	20	100	0	29
100% CD (C2)	20	0	100	29
90% CD + 10% WW (C3)	20	90	10	29
70% CD + 30% WW (C4)	20	70	30	29
50% CD + 50% WW (C5)	20	50	50	29
30% CD + 70% WW (C6)	20	30	70	29
10% CD + 90% WW (C7)	20	10	90	29

MS: Mineral solution, WW: Wastewater, CD: Cow dung.

and then injected into the chromatograph using a 1 ml waterproof syringe. The CH_4 and CO_2 content were determined using a standard curve based on CH_4 and CO_2.

2.4. Monitoring for the Selection of Microbial Complex

The microbial complex that gave more production of biogas was chosen as the best activated sludge. For this complex, the physicochemical parameters were determined. PH, salinity, Total dissolved solutes (TDS), electrical conductivity (EC) and resistivity were measured with a multi-parameter analyzer (9420 WTW). Dry matter (DM), total ash (Ct), organic matter (MO), total organic carbon (TOC) in the sample was obtained according to the protocol described by Sakaki [42]. Total alkalinity (TAC) and volatile fatty acids (VFAs) content were determined using the method described by Dilallo and Albertson [43]. An amount of 25 mL of the sample was titrated with H_2SO_4 (0.1 M) at pH 4.0 and the amount of titration was noted. The sample was then boiled lightly for 3 minutes, cooled, titrated with Na_2CO_3 (0.05 M), and the titration amount was noted from pH 4.0 to 7.0. Viable methanogenic bacteria were enumerated by the three-tube most probable number (MPN) technique (8-fold dilutions) using a medium adapted from Dianou [44] and a modified medium of Zhilina [45]. The composition of medium for serial dilution was respectively: NH_4Cl 2 g/L, K_2HPO4 0.33 g/L, KH_2PO_4 0.33 g/L, methanol 10 mM, sodium acetate 20 mM, sodium formate 20 mM, yeast extract 1 g/L, oligoelements solution 1 mL, resazurin solution (0.1%: v/v) 1 mL. Also 1 mL reducing solution was added to the medium and the headspace was filled with N_2 gas.

2.5. Statistical Analysis

The XLSAT software was used for statistical analysis of the data. The analysis of variance (ANOVA) was carried out to compare the mean values of biogas production obtained from different combinations using Fisher's tests at the probability threshold p = 5%. Principal Component Analysis (PCA) was performed to reduce geometric space and visualize data, using a linear combination of variables that maximizes variance. This method allowed to visualize the typology of the different combinations and to avoid the redundancy of the variables by considering the study in the reduced space of uncorrelated.

3. Results and Discussion

3.1. Evolution of pH

Figure 1 shows the evolution of pH according to the combinations of inoculums during the anaerobic digestion. The optimum pH during the anaerobic digestion is around neutrality and varied from, 6.5 to 8. pH value is less than 6.5 or greater than 8, a malfunction of the process [46] [47]. The analytical follow-up concerned the control of pH by adding NaOH (5N), in order to correct it. The pH of mixture C1 (100% WW) was increased to around 9. Parawina *et al.* [48] have

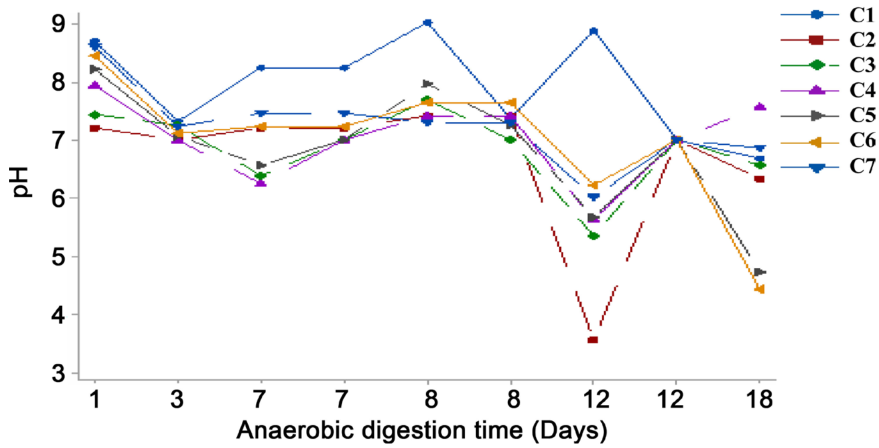

Figure 1. Evolution of the pH as a function of time of different inoculums.

found similar results with pH evolving from 6 to 9 until the 20th of anaerobic digestion. These results are in agreement with those previously reported by Cuetos *et al.* [49] and [50]. According to Bechir [51], wastewaters from the slaughterhouse of Ouagadougou are loaded with solid waste, organic material (residue of rumen) and concentrated by blood, flesh, fat, haires, excrement and urine of slaughtered animals. These waters have COD and BOD in order 2827, 1620 mg O_2/L, respectively [52]. This is confirmed by [53] [54]. Study of Cuetos *et al.* [49] was showed that the first 10 days, nitrogen compounds are degraded and ammonia nitrogen is released into the environment. Indeed, anaerobic bacteria mainly needed in organic form, nitrogen source (ammonia or urea nitrogen) and a source of phosphorus (orthophosphates). These nutrients are used for synthesis of molecule and energy during biological reactions. In the case of bovine dung, an increase of proportion causes acidification of environment. As also underlined some studies, the anaerobic digestion of bovine dung causes acidification of environment [55] [56]. Acidification is due to the presence of macromolecules in rapidly decomposing bovine dung [8] [57]. A weak variation in pH around neutrality (pH 7) was observed with the combinations C7 and C6.

3.2. Microbial Activities

Microbial activities were correlated by gas production during anaerobic digestion [41]. Studies showed that animal manure as co-substrate allows optimization of biomethane production, thus improving the anaerobic digestion system [58] [59]. These conditions permit a good development of microbial communities (bacteria and archaea) in anaerobic digestion process. Microbial community plays a role in process performance and stability [60].

3.3. Assessment of Biogas Production

Combinations C5, C6 and C7 were presented in **Table 2** and demonstrated no significant difference about biogas production. The difference in production was found significant ($p < 0.05$) between two combination groups as C5, C6, C7 and

C1, C4, C3, C2. Biogas production was depleted with the increasing of cow dung proportion in the mixture. In fact, cow dung contains macromolecules, the disintegration of which could produce acids components implicated in instability of process [8] [55] [57] [61]. They argument that some toxic particles or molecules could limit anaerobic digestion and consequently block biogas synthesis. This report was supported by Mekonnen [57] who worked on anaerobic co-treatment of tannery wastewater and bovine dung. It revealed that tanneries rich in chromium and sulphide, were highly toxic to the anaerobic digestion process causing fall of biogas production [57] [62].

3.4. Comparison of Combinations Activities

The influence of matrices characteristics on the selection of combination was based on principal components analysis (PCA). This analysis was used for description and visualization of combinations with three parameters studied: Biogas, Methane (CH_4) and Carbon dioxide (CO_2). The main components are shown in **Figure 2**. The matrix of linear correlations is shown in **Table 3**. The Kaiser criterion indicated that two (02) principal components (PC) should be considered. Indeed, the three (02) first main components have an Eigen value greater than 1 (**Figure 2**). However, PC3 accounts for only 0.75% of data variability (**Figure 2**). Therefore, only the first two (02) components describing 99%

Table 2. Biogas production after 25 days of anaerobic digestion (mean of 3 replicates).

Inoculum	Volume of biogas (mL)
C5	880.0[a]
C6	862.0[a,b]
C7	772.0[a,b]
C1	668[b,c]
C4	533.0[c,d]
C3	414.0[d]
C2	343.0[d]

In a column, values that have a different letter are not significantly different according to the Fisher test (LSD) at 5% threshold.

Table 3. Coordinates of variables and their contribution to identification.

Variables	Principal components		
	F1	F2	F3
Biogas (mL)	0.570	0.587	−0.575
CH_4 (%)	0.755	−0.097	0.649
CO_2 (%)	−0.325	0.803	0.499
Eigen values	1.719	1.259	0.023
Variance (%)	57.292	41.952	0.756
Cumulative (%)	57.292	99.244	100.000

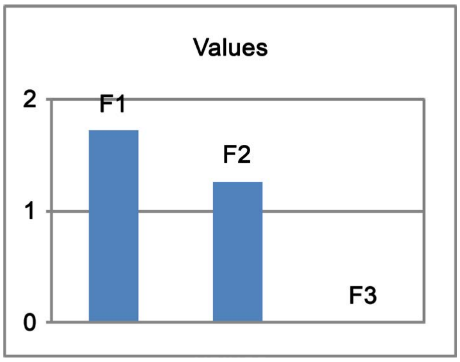

Figure 2. Results of principal component analysis.

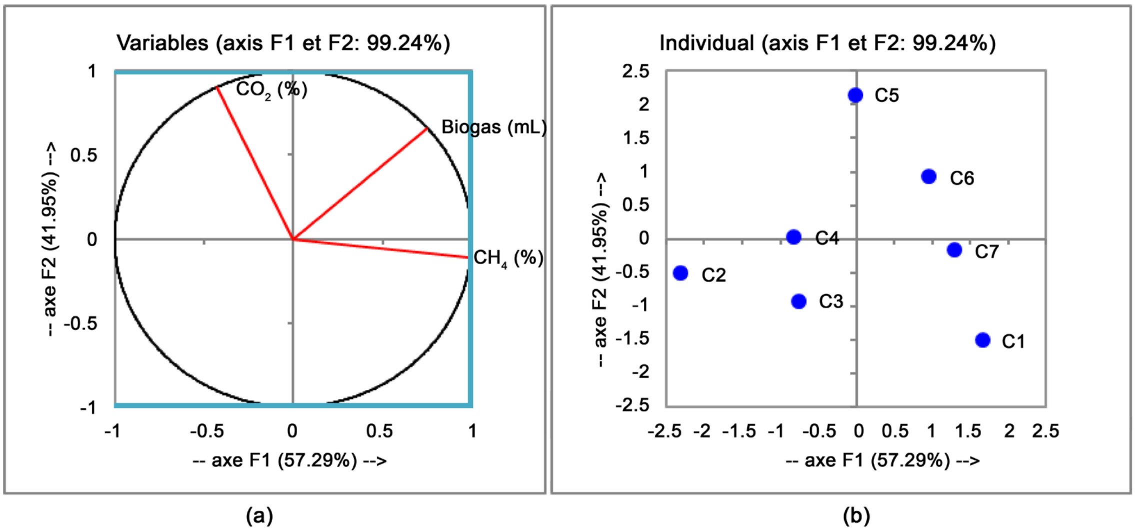

Figure 3. Principal component analysis (a) plot of variables biogas production, CO_2 and CH_4 proportions and (a) distribution of combinations on 1 × 2 axis of principal components.

of the data variability were retained for data description.

The typologies of variables (Biogas, CH_4 and CO_2) and samples on factorial planes constituted by axes 1 and 2 were presented in **Figure 3(a)** and **Figure 3(b)**, respectively. In these two figures, only variables close to correlation circle should be taken into account. In **Figure 3(a)** there are clearly three groups of variables close to the circle (Biogas, CH_4 and CO_2, so that the projections on the F1 and F2 axes are 99.24%). The first group consisting of the Biogas variable was positively correlated with the two axes F1 and F2. The second group (CH_4) was positively correlated with axis 1 and negatively with axis 2. The third group (CO_2) was negatively correlated with axis 1 and positively with axis 2. It is interesting to note that there is no particular affinity or opposition with the production of Biogas and the proportions of CH_4 and CO_2 (**Table 4**). Since the biogas consists of CH_4 and CO_2, the correlation exists between the productions of different gas. The representation of the combinations on the two factorial planes described by the axes F1 × F2 (**Figure 3(a)**) makes it possible to compare them according to the production of biogas and the proportions in CH_4 and CO_2. Combinations C5, C7 and C6 have a significant production of biogas. Combina-

Table 4. Pearson correlation matrix of variables distributions.

Principal components	Biogas (mL)	CH_4 (%)	CO_2 (%)
Biogas (mL)	1	0.659	0.269
CH_4 (%)		1	−0.513
CO_2 (%)			1

Table 5. Physicochemical characteristics of the best microbial complex.

Parameter	Means values	
	C7	C6
pH	7.21	7.68
TS (g/L)	10.52	17.48
TSV (%)	41.06	47.02
Ash (%)	58.93	52.97
COT (%)	23.81	27.54
Salinity (g/L)	3.5	7
TDS (g/L)	6.33	12.27
EC (mS/cm)	6.32	12.27
R (Ω*cm)	158.1	81.6
VFAs (mg acetic acid/L)	1320	3036
TAC (mg $CaCO_3$ /L)	520	1310
Methanogenic bacteria (CFU/mL)	3.5×10^5	3.5×10^5

CD = cow dung; WW = wastewater; C7 = 10CD + 90WW; C6 = 30CD + 70WW; TDS = Total dissolved solutes; EC = Electrical conductivity; R = Resistivity.

tions C1, C7 and C6 have high proportions of CH_4. And the combination C5 has a high proportion of CO_2.

3.5. Characteristics of the Sludge Produced

The physicochemical characteristics of the C7 and C6 sludge were presented in Table 5. Organic matter was respectively 41.06%, 47.02% for C7 and C6. VFAs and TAC concentration were 1320 mg acetic acid/L, 520 mg $CaCO_3$/L for C7 and 3036 mg acetic acid/L, 1310 mg $CaCO_3$ /L for C6. These characteristics are comparable to those of [50] [63] [64] [65] [66]. Ali Shah [67] showed that physicochemical parameters influence the densities of archaea and bacteria in the medium. In died, according to Aguilar [68] significant inhibitory effect was observed in the process at 10,000 mg/L of VFAs. Vedrenne [69] was found 2000 - 3000 mg/L with the same substrate. The alkalinity of the digesters should not exceed 1000 mg of $CaCO_3$/l of alkalinity according to Hawkes *et al.* [70]. The VFAs values in the case of C6 allow adaptation to microorganisms with high values of acid production in the digesters. Methanogenic bacteria were estimated to 3.5×10^5 CFU/mL for C7 and C6. Ueki *et al.* [71] obtained respectively 7.17 ×

10^4/mL or 1.6×10^6/mL with H_2 or acetate as the substrate, in anaerobic digestion of municipal sewage sludge.

4. Conclusion

The evolution of the pH showed that the formulation with the low proportion in cow dung had the best production profile. C7 and C6 gave best production of CH_4 and CO_2 then also exhibited the growth of methanogenic bacteria. Their physico-chemical characteristics showed an interesting media rich in organic matter, VFAs and TAC which could maintain microbial flora stability. The combination C6 was presented good conditions of growth and maintenance associated to anaerobic digestion of bacterial consortia. It buffering properties can use to prevent medium acidification during VFAs production. To conclude C6 could be considered as best inoculum for organic waste digesters.

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Conflict of Interest Statement

We declare that we have no conflict of interest.

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