Performance Evaluation of Co-substrates and Inoculums in Biogas Production from Distilled Cassava with Solubles

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Abstract

High rate of generation with adverse environmental and pollution characteristics of distillery wastewater has continued to worry distillery operators worldwide. This work investigated the comparative performances of cow dung versus poultry droppings as co-substrates and sewage sludge versus ruminal fluids of cattle as inoculums, in the anaerobic treatment of distilled cassava with solubles obtained as wastewater from a cassava-based ethanol distillery. D-optimal mixture design was used to generate ten (10) randomly executed digestion experiments for each co-substrate and each inoculum. Interactive effects of the proportions of the distilled cassava with solubles (50-100%) and each co-substrate (0-50 %w/w) on methane yield were first investigated, and the experimental condition that gave maximum yield was used in the experiments for inoculum performance evaluations within a range of 0-30 %w/w. Results showed that the distilled cassava with solubles has a relatively high carbon-to-nitrogen ratio (43.5:1) and thus required co-substrates for its effective anaerobic digestion. While maximum methane yields were recorded at the same co-digestion ratios of 62.5:37.5 for both co-substrates, its value for poultry dropping was higher at 11.66 ml/gVS) than for cow dung at 10.54 ml/gVS. Furthermore, using pre-degassed ruminal fluids of cattle and sewage sludge as inoculum respectively increased methane yield to 12.78 and 11.14 ml/gVS. The study concluded that poultry dropping had a more positive contribution to biogas generation than cow dung while the use of inoculums enhanced biogas production, with the ruminal fluids of cattle performing better than the sewage sludge.

Keywords: anaerobic digestion, biogas, distilled cassava, co-substrate, inoculum, mixture design

Introduction

Biofuels have been widely touted as credible alternative renewable energy sources (Shuba and Kiffle, 2018; Solomon et al. (2018)), and bioethanol is arguably the most produced and established of all the currently available options. Its numerous advantages include maturity of its production technology and wide applications; favorable energetic potential, and possibility of energy storage over a long time, unlike solar and wind sources –. World production was 124 billion litres in 2017 , and citing a 2007 biofuel policy document of the Nigerian National Petroleum Corporation, Ben-Iwo et al. (2016) reported that about 5 billion litres of ethanol would be required annually, for industrial and transport fuel applications in Nigeria. As such, increased awareness and favorable government policies have led to a recent surge in the number of cassava-based bioethanol production plants in Nigeria—. However, increased ethanol production leads to the generation of a highly polluting, acidic waste stream known as vinasse or distilled cassava with solubles (DCS). It is generated at a rate of 8-18 v/v of ethanol produced (Mostafa et al., 2019), it has a foul smell and high chemical oxygen demand (COD) that ranges from 62 to 74 g/l –. These anti-environmental characteristics of vinasse coupled with its large volume generation threaten the sustainable production of bioethanol and pose a severe challenge to distillery operations .

Anaerobic digestion (AD) technology could be used to treat distillery wastewater and also generate biogas in the process (Kotarska et al., 2019). In the right proportions, the use of a suitable co-substrate to adjust the properties of the primary substrate and inoculum to provide the much-needed microorganisms to kick-start the digestion processes can both enhance the quantity and quality of biogas production. Oparaku et al. (2013) reported that when cassava peel was co-digested with cow dung (CD), the highest cumulative biogas volume of 78.5 l was achieved, while Asikong et al. (2014) investigated biogas production from poultry droppings (PD), CD and water hyacinth. The study concluded that the highest biogas volume of 423.8 ml was obtained from the combination of all the three substrates. Canepa et al. (2017) compared the respective performances of the ruminal fluids of cattle (RFC) and sewage sludge (SS) as inoculums in the AD of pig faeces. The results showed that while the use of RFC led to higher COD removal and biogas

quantity of 0.012 l/d with 71.89 ± 7.60% of methane content, SS produced less (0.009 l/d) but better-quality biogas with 77.89 ± 6.74% methane content. In the production of methane from swine wastewater, Cordoba *et al*. (2016) evaluated the performances of rumen fluids of cattle (RFC), stabilized swine wastewater (SSW) and sewage sludge (SS) as inoculums. The study concluded that highest organic matter removal of up to 50% was achieved when SSW and SS were separately used while 15% was recorded for the RFC. Even though many studies have looked at various methods of reducing organic loads and improving biogas generation from vinasse, including those based on sugarcane , molasses , corn and sweet sorghum , there are however, very little information on characterization, treatment and utilization options for cassava-based wastewater (Usubharatana and Phungrassami, 2015).

Therefore, this study aimed to find an appropriate co-substrate, inoculum and their respective relative proportions that can be used for maximum biogas production from the anaerobic codigestion of DCS. As far as we know, this is the first time that potential co-substrates and inoculums are being investigated with a specific purpose of maximizing biogas production from this bioresource (DCS).

MATERIALS AND METHODS

Materials

The DCS used in this study was collected in 2.5 l plastic sample bottles from the ethanol micro-distillery at the Bioresources Development Centre, Ogbomoso, Oyo State of Nigeria (8.06733, 4.36256), while PD and CD samples were collected from the Teaching and Research Farms of the Obafemi Awolowo University (OAU) in Ile-Ife, Osun State. The SS and RFC were sourced respectively from the wastewater treatment ponds located within the campus of OAU and the 'Odo-Eran' Abattoir, Ile-Ife. These samples were collected in waterproof sterile bags, and their pH values were recorded immediately before storage at 4 °C. Stones and other solid particles that are not biomass were removed before usage. All reagents used in this study were analytical grade and sourced from local vendors.

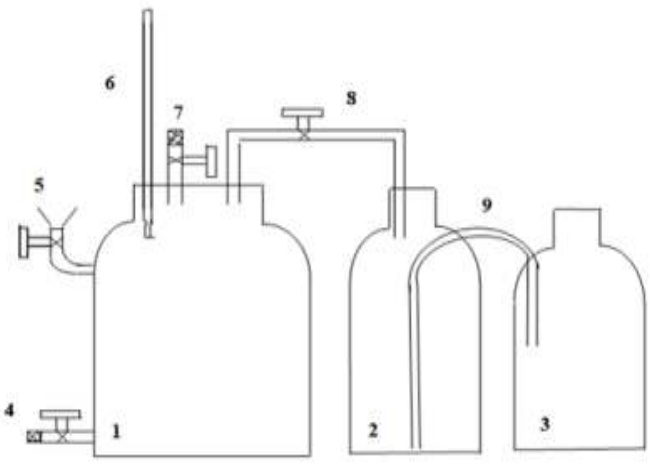

Fig. 1: Five-litre capacity anaerobic digester

Digesters were constructed from polyvinyl chloride (PVC) material. Each one consisted of a 5 l black-painted cylindrical bioreactor (Oloko-Oba *et al*., 2020) where digestion took place; a 2 l capacity tank partly filled with alkali solution over which the produced biogas was collected; and another 2 l tank that received the volume of alkali solution displaced due to biogas accumulation above the first tank (Figure 1). The reactor has a feeding inlet near the top and an outlet near the bottom for effluent discharge and sampling –. Gas sampling port is located on top of the reactor.

Analytical Methods

Standard Methods for the Examination of Water and Wastewater (APHA, 1999) was adopted to determine the physicochemical characteristics of all biomass (at room temperature) used in this study.

Legend:

1. 5 litre digester tank;
2. Gas holder filled with alkali solution;
3. Displaced solution tank;
4. Liquid sample outlet;
5. Feed inlet;
6. Thermometer;
7. Biogas sampling port (with cork);
8. Non-return valve on gas tube;
9. Solution tube

Design of Experiments

For each co-substrate and inoculum biomass investigated, D-optimal mixture design was used to generate ten (10) digestion experiments with weight percentage of volatile solids contents in DCS, PD, CD, SS and RFC taken as independent variables and the biogas methane yield (MY) as the response variable, with the aid of Design Expert software (Stat-Ease Inc., USA; version 11.0.5.0). Each digestion experiment was carried out in duplicates over an hydraulic retention time (HRT) of 30 days (Dahunsi *et al*., 2017) and the average MY calculated to establish the optimum mixing conditions for biogas generation . The optimum mixture condition from the co-substrate screening experiments was adopted as the primary substrate in the inoculums screening experiments. Tables S1 and S2 (Supplementary Materials) show the investigated variables and the range of values considered in the co-substrates and inoculums performance evaluations, respectively. All experiments were carried out at a mesophilic temperature of 30 ± 2 °C and a starting pH of 7 ± 0.2.

Digestion Experiments and Gas Production Quantification

The required quantities of DCS and each co-substrate biomass stipulated in the experimental design matrix of Table 1 were calculated using Equation (1) and respectively loaded into each digester. An inoculum mix consisting of 25 gVS each of SS and pre-degassed RFC (Holliger *et al*., 2016) was added, and the volume brought up to the 3.75 l mark with distilled water. In order to

improve the anaerobic conditions and ensure stable pH inside the digester, two (2) drops of a 1 M disodium sulphide pentahydrate ($Na_2S.9H_2O$) solution and 10 ml of a 0.005 M potassium phosphate buffer (Silva and Dionisi, 2022) were immediately added before sealing off to commence digestion processes. Homogeneity of the digesting slurry was achieved by gently shaking the reactors for one minute, twice daily (Ghatak and Ghatak, 2018). The pH of the digesting substrate was periodically monitored and maintained within 7.0 ± 1 with the addition of either 1 M NaOH or 1 M H_2SO_4 as required. Biogas produced was allowed to bubble through and accumulate over a 3 M NaOH solution in the gas holder tank, displacing an equal volume of alkali into another tank. The scrubbed gas collected over the alkali solution was mainly methane, and its volume was first determined daily from Equation (2a) before it was converted to volume at standard temperature and pressure (STP) conditions using the ideal gas equation (Equation 2b). Both equations 1 and 2a were adapted from Alfa *et al*. (2014).

Where,

B = Load basis equivalent to 50 gVS of substrates loaded into the digester

VSi = Volatile solid of component 'i' loaded into the digester (i = DCS, PD, CD)

yi = Percentage of each component 'i' loaded into the digester

h = Height of displaced alkali solution in the tank

A = Cross-sectional area of the displacement tank

P1, P2 = Pressures at the digester operating and STP conditions, respectively.

V1, V2 = Measured methane volume at the digester operating and STP conditions, respectively.

T1, T2 = Temperatures at the digester operating and STP conditions, respectively.

Statistical analysis of experimental data

With the aid of the Design Expert software, experimental data from each digestion regime was subjected to analysis of variance (ANOVA) at 95% confidence level and then fitted to a quadratic model, which describes the significance and contributions of each mixture component on biogas methane yield.

RESULTS AND DISCUSSION

Physicochemical characteristics

Results from the physicochemical characterization of all the biomass are presented in Table S3 (Supplementary Materials). It shows that the DCS used in this study had a relatively high COD and VS contents of 73.65 g/l and 68.6 g/kg, respectively. These values indicate the presence of large organic matter with potential to cause serious environmental pollution if not properly handled but which may also be converted into biogas via anaerobic digestion . Although these values were both within the range reported in the literature for DCS, higher COD (109.7 g/l) was reported by Santos et al. (2019) for sugarcane vinasse, at room temperature. The BMP value of 247.1 Nml/gVS for the DCS compares favorably with those of substrate

$$Q = \sum_{1}^{N} (B/VS_i) y_i \qquad (1)$$

$$V_{biogas}|ml| = Ah \qquad (2a)$$

$$P_1V_1/T_1 = P_2V_2/T_2 \qquad (2b)$$

already established as feedstock for biogas production –, while its relatively high C:N ratio of 43.5:1 suggests the need for a high protein or nitrogen content co-substrates for its effective anaerobic digestion . The Table also shows that CD has higher COD (41.3 g/l) and total alkalinity, TA (52.7 mmole/l) than PD with values of 22.8 g/l and 31 mmole/l, respectively. PD on the other hand, had a desirably much lower C:N ratio of 4.4 as against 23.4 for the CD. The implication of this is that while higher alkalinity of CD could mean a more stable pH when CD is used as a co-substrate, a much lower C:N for PD could bring the C/N ratio of the digesting slurry to within the optimum value of 30:1 . The pH range of both inoculum (7-8) and the TA value of the RFC (>50 mmole/l) support good AD according to Holliger *et al*. (2016).

Performances of CD and PD as co-substrates

In most cases, the onset of biogas production generally started on the second day of digestion when PD was used as co-substrate while it started on the third day with CD as co-substrate. Performance evaluation was based on cumulative methane production per unit gVS of DCS loaded. At STP conditions, Table S4 (Supplementary Materials) shows that experimental conditions in which DCS and co-substrate were mixed in the percentage ratio of 62.5:37.5 gave maximum cumulative MY of 10.54 and 11.67 ml/gVS respectively for CD and PD, after 30 days of digestion. Figure S1 (a, b) in the Supplementary Materials compares the cumulative methane volume and yield profiles of the two co-substrates in their respective anaerobic codigestion with DCS at the optimum mixing ratio of 62.5:37.5. In addition to the early onset of biogas production, PD also performed better than CD as a co-substrate, in terms of methane volume and yield, throughout the digestion period despite the latter having higher VS and COD. This performance was probably because the PD contained easy-to-degrade organic matter and higher nitrogen contents to bring the overall C:N ratio to within the optimum range required for microbial growth . This result is similar to the outcome reported by Oparaku et al. (2013), which showed that PD exhibits a somewhat more positive synergy when co-digested with cassava peels for biogas production as compared to CD. Equations (3) and (4) describe the effect of each mixture component on methane production. They were obtained by fitting the experimental data from digestion experiments to quadratic models. When CD was the co-substrate, Equation (3) shows that for a unit

increase in the percentage of CD loaded, MY increases by a factor of 9.69 whereas, the increment is higher at a factor of 10.94 for a percentage increase in the quantity of PD loaded, according to Equation (4). ANOVA and fit statistics of these model equations are listed in Table S5 (Supplementary Materials). The p-values of the models are significant (< 0.005) with adequate precisions (above 4). Also, the coefficient of determinations (R2) of each model is close to unity with low standard deviations which indicate high model predictive accuracy..

Where,
Y_CD and Y_PD represent methane yield with CD and PD as co-substrates, respectively.
A, B and C denote percentage proportions of DCS, CD and PD respectively loaded into the digesters.

Performances of SS and RFC as inoculums

It was generally observed that methane production increased as the proportion of inoculum increased up to about 25% point, after which a further increase in inoculum proportions led to a drop in production. However, maximum cumulative methane volume and yield at STP conditions were achieved for both inoculums at the same optimum mixing condition of 75.1% main substrate and 24.9% inoculums, as shown in Table S6, after 30 days of anaerobic digestion. The Table also shows methane yield values of 11.14 and 12.78 ml/gVS respectively for SS and RFC as inoculums, where DCS* is the main substrate representing 62.5% DCS and 37.5% PD. Figure S2 (a, b) in the Supplementary Materials graphically compares the performances of SS and RFC when separately used as inoculum in the anaerobic codigestion of DCS and PD, at the optimum mixing condition. In terms of the total volume and yield, it can be seen that RFC performed better than SS from the beginning to the end of the digestion processes. The better performance exhibited by RFC could be attributed to its higher TA of 72 mmole/l which promotes stable digestion environment (Holliger *et al*., 2016).
Although, microbial counts were not carried out, open treatment pond as the source of the SS samples used in these experiments could have caused a reduction in the proportions of methanogens in them. Methanogenic bacteria are strict anaerobes that are very crucial to methane formation (Ghasimi et al., 2015). This phenomenon was also observed by Canepa et al. (2017) who reported higher biogas volume and COD removal

$$Y_{_CD} = 8.14A + 9.69B + 6.1AB \quad (3)$$

$$Y_{_PD} = 8.31A + 10.94C + 6.51AC \quad (4)$$

$$Y_{_SS} = 8.74\alpha + 11.12D + 1.77\alpha D \quad (5)$$

$$Y_{_RFC} = 8.42\alpha + 12.54E + 4.33\alpha E \quad (6)$$

when RFC was used as inoculum in the anaerobic digestion of pig faeces, compared to SS. Equations (5) and (6) obtained by fitting experimental data to quadratic models describe the contributions of each inoculum to methane production.

While Equation (5) shows that a unit percentage increase in the quantity of SS loaded into the digester increases MY by a factor of 11.12 ml/gVS, Equation (6) shows a corresponding incremental factor of 12.54 ml/gVS, which is a higher contribution from RFC. The ANOVA and fit statistics of these model equations as shown in Table S7 (Supplementary Materials) indicate good fit and high model predictive accuracy.

Where,
Y_SS and Y_RFC represent methane yield with SS and RFC as inoculums, respectively.
α,D and E denote percent proportions of DCS, CD and PD respectively loaded into the digesters.

CONCLUSION

In this study, the performances of CD and PD as co-substrates and, SS and RFC as inoculums, in the anaerobic digestion of DCS were investigated, in terms of methane yield. The results show that the combination of PD and RFC respectively as co-substrate and inoculum produced the highest cumulative methane volume and yield. The study provided useful information on optimal proportions of DCS/co-substrate and inoculum, for maximum methane yield. Therefore, it would be useful to cassava-based agro-processing industry, including the distillery operators, as it suggests alternative waste management/utilization option for DCS, which allow higher bioenergy recovery that could improve the sustainability of ethanol production.

RECOMMENDATION

Further studies may be carried out to evaluate the performances of other animal manures and potential inoculums in the anaerobic codigestion of DCS.

DECLARATIONS OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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