Anaerobic co-digestion of pre-treated press mud and Molasses-based distillery wastewater enhanced biogas production

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Abstract. With goals in determining the effect of diluting the distillery wastewater (DWW) and of varying the amount of DWW and press mud (PM), anaerobic co-digestion study was carried out at mesophilic condition in a 2-L Erlenmeyer flask, with a working volume of 800 mL for Batch 1 and 1500 mL for Batch 2 experiments. For Batch 1, two different ratios of DWW and tap water, with 2:3 and 3:2, were used to assess the effect of dilution on the methane yield, where same volumetric amount of PM was added. For Batch 2, following ratio of PM and DWW were used: a) 1:0, b) 1:1, c) 1:1, d) 2:1, and e) 1:2. All samples had the same amount of inoculum, except that Batch 1 samples had bagasse. The parameters that were assessed after 42 days of digestion were: pH, COD, BOD, TSS, VS, Cu, Ca, Mg, Mn, TOC, TN, and methane yield. For the effect of dilution, a significant difference in the methane yield between samples with higher and lower dilution ratio was seen, and in the first batch, the optimal dilution ratio of DWW and H₂O, with 3:2 gave higher methane yield of 78.23% (v/v). Meanwhile, optimal volumetric ratio of DWW and PM from the Batch 2 experiments, with value of 1:2, gave the highest methane yield of 79.43% (v/v).

1. Introduction
Recent changes brought by industrial revolution pave the way to utilize amassed amount of fossil fuels to meet the energy needs, and these poise serious environmental challenges such as climate change, pollution, depletion of resources. This leads to the search for renewable and eco-friendly alternative energy sources to lessen greenhouse gases emission [1]. One of these renewable energy sources that gained increasing attention among researchers is biogas which can be derived from anaerobic digestion (AD) of organic wastes such as food and kitchen waste, agricultural waste, landfill waste and sewage sludge, spawning as well a potential source of agricultural fertilizer called as digestate [2]. A continuous supply of homogenous feedstock is required for this process; however, not all regions can do it due to increased number of wastes and their composition.

However, because of the insufficient vital trace elements/nutrients and quality of microbial content of one substrate, researchers have explored simultaneous digestion (anaerobic co-digestion) of two or more substrates to surmount the limitations of using mono-substrate, to have better yields and to ensure an economically sustainable biogas production [3,4]. Due to the synergistic effects of two or more substrates with complementary characteristics, co-digestion can lessen toxicity, improve pH buffering capacity and provide better nutrient balance [5]. The substrate’s characteristic is one of the most important factors that govern the biogas yield in AD processes. Thus, different technologies to enhance the biodegradability of substrates and maximize the conversion of organic matter into methane gas in AD are being widely studied. Researchers have also investigated the impacts of nutrient supplementation [6–9] and various pretreatment techniques such as thermal [10–12], mechanical [13], chemical [14–16], biological [17,18] and combination [19–21] of these methods to ensure a highly efficient biogas production.
Sugarcane press mud is a semi-solid by-product/waste from the sugar industry that is obtained from sugarcane juice clarification. Its properties depend on several factors such as the adopted processing of juice clarification, varieties of sugarcane, nutrients used, condition of the soil in the field and other environmental factors. It typically contains ~80% volatile solids that are biodegradable in nature; and its general composition makes it a viable substrate for AD processes [22,23]. However, the hydrophobic and non-degradable lignin content (9-14% on a dry weight basis [24]) in press mud and other fibrous materials prevents the action of microorganisms, making hydrolysis the rate-limiting step during digestion [16]. Among the different pretreatment methods, biochemical and biological treatments were utilized to improve the hydrolysis stage in AD in an eco-friendly way and without using expensive equipment in the process [25].

Distillery effluent is another waste from the bioethanol/sugar industry that can cause environmental concerns when discharged in bodies of water especially with its characteristic unpleasant odor. It also has low pH values and high biochemical oxygen demand (13,414–87,700 mg/L) and chemical oxygen demand (32,000–109,700 mg/L) [26]. Press mud requires water or liquid waste for digestion; hence, distillery effluent can be used as co-substrate since it also has potential in balancing the macronutrients (i.e. C, N, P, K, S and Mg) needed to enhance biogas production, as well as dilute toxic or inhibitory compounds [27]. For instance, López González et al. observed synergistic effects of press mud and vinasse (distillery wastewater) co-digestion due to the better nutritional balance of nitrogen, potassium and sulfur [24]. Moreover, micronutrient supplementation can also be applied to further stimulate methane production since some microelements (e.g. nickel, cobalt, iron, etc.) have been reported vital to the methanogen's growth and activity in AD processes [6]. Researchers have also explored the addition of microorganisms and enzymes as an alternative to physicochemical pretreatments of substrates [28]; however, direct dosing of enzymes into AD systems has received less attention. Enzymatic pretreatments, especially when combined with other pretreatments, can have great potentials in reducing/breaking down the recalcitrant lignocellulosic structure of sugarcane wastes, yet studies on their effects and efficiency remain limited at present and need further clarification by amassing research on this topic [29]. Not all of the enzymes can improve digestibility since the success of enzymatic pretreatments depends on several factors such as type of enzymes, their functions, stability, dose, etc [30,31]. Thus, further testing is needed to optimize this pretreatment strategy for certain types of substrate/waste. To our knowledge, there are no literature studies investigating the effects of biochemical pretreatment, as well as nutrient supplementation, in the codigestion of sugarcane press mud (PM) and treated distillery effluent (DE).

Thus, this study seeks to analyze the effects of supplementing press mud with distillery wastewater and bagasse in the improvement of biogas supply. Further, it will shed light in determining the effects of added nutrients, immobilizing substrate capability of bagasse, and other factors that may influence the biogas production.

More specifically, the goals of this study are to: (1) compare the methane yield of distillery wastewater co-digested with press mud to that of recent studies and (2) to determine the physico-chemical characteristics of the final digestate: pH, initial and final BOD/COD, %TSS (Total Suspended Solids) reduction, % VS (Volatile Solids) reduction, and C/N (Carbon/Nitrogen) ratio.

In this work, the substrates used came from Central Azucarera de Tarlac (press mud, distillery wastewater, and bagasse). Only batch experiments, in a mesophilic condition, were performed and halted after 42 days. It focuses on (1) the effect of diluting the distillery wastewater (DWW) with same amount of press mud, and (2) the effect of varying the volume of both DWW and press mud.

2. Experimental methodology

2.1. Pretreatment

The press mud was pre-treated by two-step hydrolysis before mixing with the solution. About 1108.8 g of press mud was soaked in 1 L of 62.0 mEq/L of Ca(OH)$_2$ for 15 hours. The alkali hydrolysate was
diluted with water up to 1200 mL then heated until boiling for about 20 minutes. The mixture was then allowed to cool for 7 days.

2.2. Experimental Design

Figure 1. Batch Anaerobic Digestion: (top) Pre-digestion Procedure and (bottom) Mesophilic maintained condition using Incubator Shaker

Anaerobic digestion batch experiments were conducted in 10 2-L Erlenmeyer flask, presented in Figure 1. For the first batch, about 300 mL pretreated press mud was added to 200 mL DWW solution and 100 cm$^3$ of bagasse. Sample 1 was prepared using pure DWW. For the remaining samples of batch 1, DWW was diluted with tap water in 3:2 and 2:3 volume ratio, and with and without micronutrients. For each sample, 20 g/L of glucose and 5 g/L of yeast were added, which were considered as macronutrients. Also, about 200 cm$^3$ of inoculum was added to each media. Details of Batch 1 sample
matrix are shown in Table 1, while the amount of micronutrients used in Table 2 is twice the recommended amount based on the literature [32]. To ensure anaerobic condition, nitrogen gas was purged in each media for about 15 minutes. For the gas collection, a 2-L urine bag was connected to each media bottle. All experiments were carried out at room temperature for a digestion period of 42 days. Also, the feedstock mixtures have different volumetric ratios of DWW and press mud: (1) 1:0, (2) 1:1, (3) 1:1, (4) 2:1, and (5) 1:2 as depicted in Samples 6 to 10. Samples 7 and 8 were of the same ratio; however, the former did only undergo thermal-dilution but not alkali pretreatment. For all samples under Batch 2, same amount of inoculum and nutrients were added as used in Batch 1. The total volumetric amounts of DWW and press mud used were increased by a factor 2.4 for this batch to compensate the excess amount of micronutrients added in Batch 1.

### Table 1. Controls Used in Batch 1 & 2

| Sample ID | DWW (mL) | H₂O (mL) | Nutrient | Sample ID | DWW (mL) | Press mud solution (mL) | Pretreated Press mud |
|-----------|----------|----------|----------|-----------|----------|------------------------|---------------------|
| 1         | 200.0    | 0.0      | ✓        | 6         | 0.0      | 1200.0                 | ✓                   |
| 2         | 120.0    | 80.0     | ✓        | 7         | 600.0    | 600.0                  | ✗                   |
| 3         | 80.0     | 120.0    | ✓        | 8         | 600.0    | 600.0                  | ✓                   |
| 4         | 120.0    | 80.0     | ✗        | 9         | 600.0    | 300.0                  | ✓                   |
| 5         | 80.0     | 120.0    | ✗        | 10        | 300.0    | 600.0                  | ✓                   |

### Table 2. Volumetric Amount of Micronutrients Used

| Nutrients | Amount (g/L) |
|-----------|--------------|
| NH₄Cl     | 4.00         |
| KH₂PO₄    | 1.00         |
| MgSO₄.7H₂O| 1.20         |
| MnSO₄.7H₂O| 0.04         |
| FeSO₄.7H₂O| 0.04         |
| NaCl      | 0.04         |
| CuSO₄.5H₂O| 0.04         |
| CoCl₂.6H₂O| 0.04         |
| ZnSO₄.7H₂O| 0.04         |
2.3. Analytical Methods

The initial and final values of physico-chemical characteristics such as pH, COD (Chemical Oxygen Demand), BOD (Biological Oxygen Demand), TSS (Total Suspended Solids), VS (Volatile Solids), Phosphorus amount, Total Carbon, and Total Nitrogen. The methane richness of biogas was determined according to the American Standard Test method ASTM D2504-88(1998) in the laboratory using a gas chromatograph thermal conductivity detector (GC-TCD).

3. Results and Discussion

Results from Figure 2 shows that the press mud mixed with diluted distillery wastewater with and without additional nutrients gave the highest methane yield of 61.3% and 78.23% (v/v), respectively.

![Figure 2. Summary of Batch 1 Data](image)

The pH of all samples decreases due to the produced acetate and fatty acids during digestion [32]. The methanogenic bacteria is mostly pH sensitive, since very low pH can cease chain of biological reactions in digestion. In anaerobic digestion, 4.0 to 7.2 was generally considered as the optimal pH of final digestate [33]. Sample 2, which already had an a very low pH initially, yielded lowest methane content. Sample 5 was able to maintain its pH around 5.0 before and after digestion, thereby giving the highest yield. The BOD/COD ratio in percentage depends upon the biodegradability of organic matter, which ranges from 0% (completely non-biodegradable) to 100% (completely biodegradable). Generally, it decreases before to after digestion (AD) due to presence of high proportion of nondegradable materials [34]. For sugar waste products, optimum % (BOD/COD) varies from 38.46% to 55.55%, which Sample 3 and Sample 5 followed [35].

An overabundance of nitrogen in the substrate (low C/N ratio) leads to excessive and toxic ammonia formation [36]. Sample 1 and 4, which did not have a significant methane yield, had both lowest and highest C/N ratio among them, respectively; meanwhile, the optimum C/N ratio found in this experimental study ranged from 78:1 and 72:1, seen from Samples 3 and 5, respectively. The effect of co-digestion along with the addition of micronutrients has been particularly a factor for the optimal C/N ratio values, as compared to the C/N ratio of DWW only, press mud (PM), PM(25%)+bagasse(75%), with values of 30.0, 25.0, and 24.7, respectively. No correlation has been found out for the %TSS reduction with respect to methane yield, since this study focuses only the mesophilic condition, with temperature gave a significant effect for the reduction of TSS from previous studies [31].

Overall, the sample with the highest dilution ratio yielded a high methane, while the use of micronutrients beyond the required amount from the literature must be taken into consideration since it
could inhibit the methanogenic activity of the bacteria. Meanwhile, the researchers of this study also attempted to measure the methane yield when no bagasse as an immobilizer was considered. The results showed that co-digestion of PM with 120:80 and 80:120 DWW-water ratio with added micronutrients gave the highest methane yield of 42.24% and 64.78% (v/v), respectively. Diluted and treated distillery wastewater in these two independent experiments show, therefore, better and reasonable results.

In Batch 2 experiments, the volumetric amount of DWW and PM solution was increased to compensate with the high concentration of trace elements (micronutrients). From Figure 3, Sample 10, with a ratio of 300:600 (DWW to PM) and added nutrients, yielded the highest methane content. No methane was found with Sample 6 particularly because of homogenous feed used (press mud) in a short span of digestion time.

A maintained acidic value of pH for the sludge indicates that anaerobic digestion was possible, seen on the lowest % pH change in Sample 10. The dramatic decrease of BOD/COD before and after digestion was seen in Samples 8 and 10, which gave a significant methane yield. There were no significant effects for %TSS, %VS, and %P reduction and their methane yield. Likewise, the trace elements (from micronutrients) such as Ca, Cu, and Mn, found in Samples 3, 4, and 5 shows that they contribute to the efficiency of AD, which has been similar to a previous study [31] ; however they do not directly affect the volumetric amount of the methane produced.

Table 3 shows the comparison effect of co-digestion along with dilution and ample nutrients, being more favorable to biogas production. The hot alkali pretreatment method in the experiment gave a better methane yield than that of using thermal water hydrolysis approach.

| Substrate                  | %CH₄  | Source |
|----------------------------|-------|--------|
| Bagasse (B)                | 26.00 | [37]   |
| Press mud (PM)             | 45.00 | [38]   |
| DWW                       | 50.00 | [39]   |
| PM + Vinasse               | 51.60 | [38]   |
| FW + Sludge + Glycerol    | 77.10 | [40]   |
| DWW + PM + B + nutrients  | 3.45  |        |
DWW/H₂O (120:80) + PM + B + nutrients  0.016
DWW/H₂O (80:120) + PM + B + nutrients  61.30
DWW/H₂O (120:80) + PM + B  1.36 This study
DWW/H₂O (80:120) + PM + B  78.20
DWW/PM (600:600)+nutrients (not pretreated)  5.88
DWW/PM (600:600)+nutrients  55.79
DWW/PM (600:300)+nutrients  34.40
DWW/PM (300:600)+nutrients  79.43

4. Conclusion
Overall, the main goal of this study, which was to determine the effects of co-digestion of pressmud and distillery waste water for enhanced biogas production, was achieved. Some important parameters had been assessed to analyze factors affecting the conversion rate. Pretreatment and nutrient supplementation have been successfully used to improve biogas production from the co-digestion of sugarcane press mud and treated distillery effluent.

Methane yield is affected by the sensitivity of microorganisms to pH variations. Meanwhile, its conversion rate has been analyzed as determined from the results of COD to BOD ratio, C/N ratio, and pretreated substrates. For the effect of dilution, a significant difference in the methane yield between samples with higher and lower dilution ratio was seen, and in the first batch, the optimal dilution ratio of DWW and H₂O, with 3:2 gave higher methane yield of 78.23% (v/v). Although micronutrients are necessary to microbial nutrition, this study shows that toxicity would occur if their concentrations do not go along with the amount of the substrates used. Also, optimal volumetric ratio of DWW and PM from the Batch 2 experiments, with value of 1:2, gave the highest methane yield of 79.43% (v/v). In addition, no significant correlation has been found between the %TSS, %VS, %TP reduction to the methane yield. Therefore, further studies have to be performed that could give more understanding on the biogas yield using the sugar refinery wastes.

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