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Research article

Efficient anaerobic digestion of a mild wet air pretreated molasses ethanol distillery stillage: A comparative approach

Getachew Dagnew Gebreeyessus [a,b,*], Trichur Ramaswamy Sreekrishnan [c], Andualem Mekonnen [d], Yonas Chebude [e], Esayas Alemayehu [f]

[a] Africa Center of Excellence for Water Management, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
[b] Department of UEM, Kotebe Metropolitan University, P.O. Box 31248, Addis Ababa, Ethiopia
[c] Department of Biochemical Engineering and Biotechnology, IIT Delhi, India
[d] Center for Environmental Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
[e] Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
[f] Jimma Institute of Technology, Jimma University, P.O. Box 378, Jimma, Ethiopia

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ABSTRACT

The effect of a mild, wet air pretreatment and the subsequent anaerobic digestion (AD) was examined on the recovery of a complex and toxic molasses ethanol distillery stillage. The biogas yield and organics removal due to pretreatment were compared with the raw stillage AD. The application of a scoria support in this industrial residue AD process stability was also assessed. Consequently, a statistically significant cumulative specific methane recovery difference (p-value = 0.000) with an almost complete biological oxygen demand (BOD) removal and a significant chemical oxygen demand (COD) reduction, which were 100% and 92% respectively were achieved. Additionally, the biogas recovery rate was hastened due to pretreatment. The application of scoria, whose property has been instrumentally inspected, has helped stabilize the pH in the AD systems. In a comparative approach, this study suggests the energy benefit and an ecofriendly discharge of stillage by the ethanol industry towards sustainability.

1. Introduction

The global ethanol production is increasing with time, even during this novel corona virus pandemic. Alcohol is produced for various purposes, including the uses for fuel, drinking, cleaning, and industrial chemical productions. Even the current pandemic too triggered the mass use of alcohol for drinking and cleaning as well as sanitizing purpose. Further, the making of alcohol intensifies with urbanization, especially in the developing world. In spite of the usefulness of alcohol, its production comes along a huge environmental problem. Regarding this water consuming as well as water polluting ethanol industry, a number of research report indicated that the recalcitrant nature, complexity and the magnitude of the problem due to ethanol byproduct release, which is mainly the distillery stillage. Stillage contains high COD, BOD, color-causing and refractory organics including the melanoidins, furfurals, phenolics, caramels, and odor-causing indole and skatole. These stillage contents are challenging the existing treatment technique, their discharge subsequently affecting the physical environment [1, 2, 3, 4].

In an attempt to replace the energy-intensive other stillage removal processes mainly evaporation and incineration, which increased the ethanol production cost, alternative techniques have been tested. So far, studies on the treatment of distillery stillage targeted the COD, BOD, and color removal via several methods including AD, oxidation, adsorption as well as diverse phytoremediation techniques [5]. For instance, Beltran and coworkers tested the combined effect of stillage AD and ozonation [6], Malik and colleagues reported the effect of combining AD and aerobic degradation, that even preceded by wet air oxidation (WAO) [7]. However, after all these efforts the COD removal

* Corresponding author.
E-mail address: getachewdagn@yahoo.com (G.D. Gebreeyessus).

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efficiency endured less than 80%. Additionally, most of the experiments were performed on a particular synthetic wastewater and on a laboratory scale, which do not bring a similar effect when it comes to the real scenario of full scale stillage treatment operations [8].

Despite its perturbing pollution potential, distillery stillage can still be used as a feed to bioenergy systems and other valuable byproduct making [5, 9, 10]. Nevertheless, the nonbiodegradable nature of stillage remained a challenge on the performance of those recovery techniques, which include its AD. Thus, the stillage COD residue remains 21000 and 50000 mg/l after AD [11, 12]. Consequently, a significant number of studies are recommending the feasibility of an integrated or coupled approach that include stillage pretreatment to enhance the performance on recovery and discharge limits.

In fact, the success and the economic viability of anaerobic conversion depends on its pretreatment, the nature of the feed and the scale of operation [13]. Further, the application of cheap and locally available materials to pretreat and enhance material and energy recovery from biowaste is worthwhile. Thus, the objective of the current study was to evaluate the recoverable energy potential of a WAO pretreated (WAOp) stillage against its ultimate biomethane potential (BMP). The current study also investigated the effect of the use of a vesicular rock, scoria, support if that brings an impact on the stability of the AD process. Accordingly, the results obtained based on standard methodology were promising.

2. Methodology

2.1. Experimental setup

The entire experiments spanning from the initial stillage sample characterization to its AD were accomplished as indicated in figure one. The purpose of the study was to compare an iron oxide coated sand (IOCS) supported WAOp stillage and the raw stillage on the subsequent AD, which was also supported with or without scoria. Thus, the setup included the relevant unit operations or processes in block diagrams (Figure 1A) and images obtained during the BMP test (top) and the batch AD (bottom) of stillage (Figure 1B). The effect of each operation was determined in between and before going on to the next process. The details of each block of operation are presented in different sections regarding the tests conducted, the instruments involved as well as the analyses performed.

2.2. Sampling

A cane molasses distillery stillage sample was brought from a process plant called Wave Distilleries and Breweries Pvt. Ltd. The factory is located in the nearby capital town of Aligarh, Uttar Pradesh State, India.

The adequate stillage sample was obtained on a clean and tightly closed plastic jar and transported to the laboratory within four hours. Following its arrival in the lab, subsampling was performed. After grabbing a homogenized subsample using a beaker, the stock was labelled and kept in a cold room (4 °C). The subsample was further subsampled and analyzed while keeping the remainder in a refrigerator all the time. In a different experiment, a mild IOCS based WAO had been performed using the same stillage. Thus, another subsample of the stillage has been the WAOp, which was performed for 4 h at the 60 °C with 3.5% IOCS loading. The WAO was done at atmospheric pressure and on a 1.5 l/min aeration rate, in which the pretreated stillage was also kept in refrigerator till usage in AD.

2.3. Scoria preparation and characterization

Raw scoria, which is a highly vesicular, dark or brown colored volcanic rock was firstly obtained from two locations; the one near the Sagure town of the Arsi Zone and the other called Aluto calderra which is near Zeway town in the rift valley area of the Oromia region, Ethiopia. The scoria used in the AD system as support was simply broken down to a relatively uniform-sized gravel and got packed in the digester, which was within 40% by volume.

Based on elemental composition analysis, the raw scoria exhibited a higher oxygen, silicon and iron while having titanium as a least component. The raw scoria scanning electron microscopy (SEM) image also showed a rough surface appearance that may help attach the biofilms, which otherwise suspends in the bio-slurry during AD (Figure 2). The XRD image for the raw scoria also showed a non-amorphous structure while showing with a peak in intensity count between 25 to 30 positions.

2.4. The biochemical methane potential testing of the raw stillage

The BMP test is important to evaluate the extent of the recovery of a bioenergy contained in a biomass. Glass reactors of serum bottles, butyl septum as stoppers and aluminum crimp caps were used to fix the rubber lid after being clamped. Syringe and needle were also used to suck the gasses produced in the reactors.

The test was performed based on the guidance from published standards. The current study used triplicates of 130 ml volume glass bottles for stillage/inoculum mix and for inoculum only that were initially washed and dried. Afterwards, the test bottles were filled with 50 ml inoculum and 10 ml sample, while the blank assays were filled only with inoculum in order to deduct the average biogas yield brought only from the inoculum [14].

Though the results were normalized to standard temperature and pressure (STP) conditions (0 °C and 1 atm), the tests were performed at

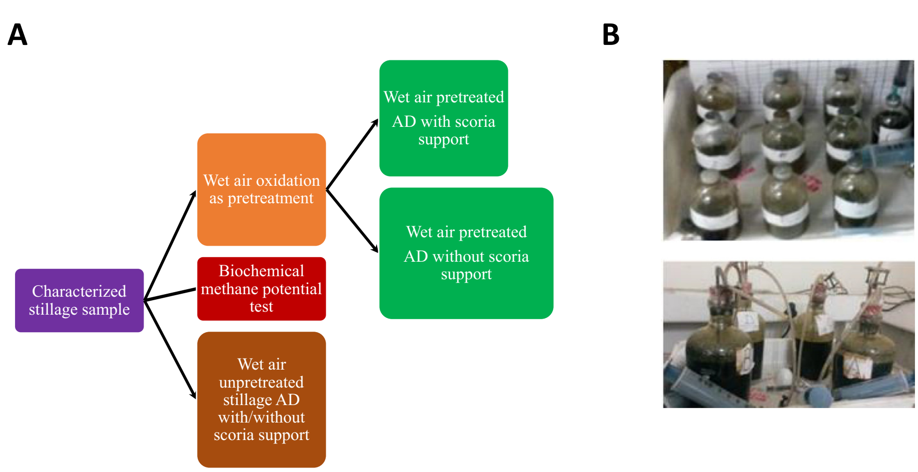

Figure 1. Diagram of the sequentially connected experimental unit operations (A) and the setups (B).

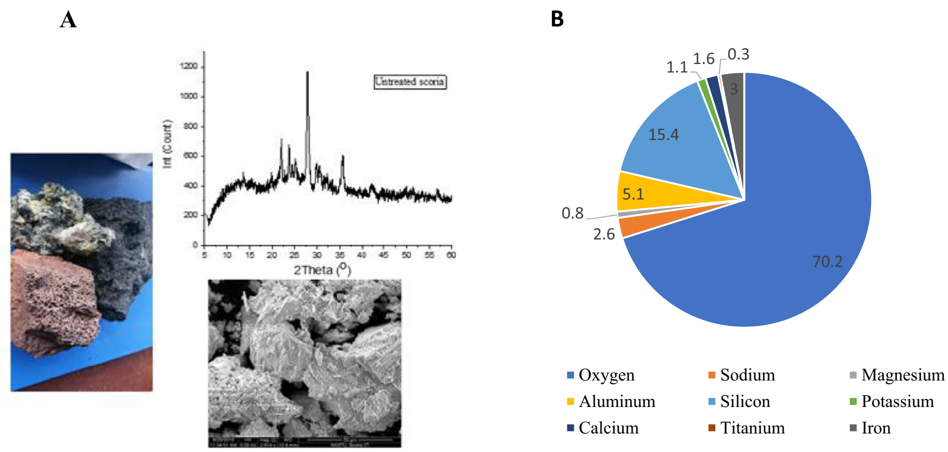

Figure 2. The x-ray diffraction, scanning electron microscopy image (A) and atomic percentage (B) of raw scoria.

mesophilic temperature. Further, the test bottles were manually mixed twice a day and the biogas and the methane contents were measured at the same time.

2.5. The batch anaerobic digestion of a cane molasses ethanol stillage

The batch AD tests were performed using two-liter volume glass bottles that were fitted with rubber cork into which holes were perforated for the insertion of the sampling and gas exit channels, using plastic tubes. The gas tube was connected to a graduated syringe. A known concentration of the COD was added as a feed parameter to those duplicates of test digesters. Like the BMP conditions, all the tests here too were performed at mesophilic temperature and the results presented were corrected to STP conditions.

2.6. Analytical methods and the data analysis perspectives

The analysis of the sample parameters was mostly performed according to the APHA standards guide book [15]. Additionally, methods published in trustworthy journals were also consulted. Routinely, pH was monitored using digital pH, conductivity and temperature probe (CyberScan pH 510, Thermo Scientific), which was periodically calibrated using standard buffer solutions of pH 4 and 7. Solids analysis was also performed according to the procedure outlined in the APHA's publication [16] in which measuring cylinder, crucibles, analytical balance, oven, desiccator and furnace were principally involved.

Similarly, the analysis of nitrate and phosphate, COD (Closed Reflux, Colorimetric Method, 5220 D), and the color (Methods 2120C) were done using a spectrophotometric device. While the BOD was measured by titration (Method, 5210 B), the VFA and methane were determined using gas chromatography. Side by side, advanced instruments have been used for characterizing materials (scoria and the IOCS) including scanning electron microscopy energy dispersive X-ray analysis (SEMEDEX) and x-ray diffraction (XRD).

2.6.1. Standard preparation for the colorimetric chemical oxygen demand determination

The preparation of the standard for the spectrophotometric COD determination was made using the prepared known concentrations of potassium hydrogen phthalate (KPH) and a distilled water as blank. Concentrations of 50, 100, 200, 300, and 400 mg/l were synthesized after weighing of a known amount of KHP and preparing the stock solution. To prepare a 1000 mg/L COD standard stock solution, 850 mg of dried (120 °C, overnight) KHP was taken and dissolved in 1000 ml of distilled water. Afterwards, the optical density (OD) reading, which is also called absorbance, was taken at each concentration point and a standard curve of absorbance against the concentrations of KHP in mg/l was developed with an $R^2 = 0.998$. Then, the slope of the equation of the trend line was calculated for the later analysis of the unknown samples.

2.6.2. Standard preparation for the gas chromatographic determination of the volatile acids

At first, a stock solution of 1000 mg/l mixture of acetic acid, propionic acid and butyric acid was synthesized. Thereafter, the stock solution was diluted to 50, 100, 250, 500 mg/l solutions. A blank sample was prepared from distilled water. Accordingly, a method was developed within the range of the detection limits of the Gas Chromatography (GC) by diluting the samples. The lower detection being 10 mg/l which was almost detected whenever the blank could also be contaminated and the maximum detection was set to be far above the maximum possible presence of the acids in the sample. Whenever samples were run after the standards, chromatograms and computed concentrations were obtained. The goodness of fit, the R^2, was maintained around 0.988. Indeed, the GC system had an auto ranging Flame Ionization Detector (FID) which was set at a temperature of 300 °C to detect and quantitate from μg/l to g/l in a single injection. The carrier gas used was nitrogen, the burner was oxygen/zero air, and the fuel gas was hydrogen.

2.6.3. Gas chromatographic biogas analysis

A GC technique using 5700 NUCON was applied to measure biogas (nitrogen, methane and carbon dioxide). The GC carrier gas used was hydrogen. Though the GC had both FID and the Thermal Conductivity Detector (TCD), TCD was used in these testing at a detection temperature of around 90 °C. The oven temperature or the GC-injector temperature was always kept around 80 °C.

After attaining the aforementioned temperatures of the GC system in every testing, WinQCDS 8.0 crafted by Quazar Technologies Pvt. Ltd. was run from an attached computer. As a test run, a 1ml of atmospheric gas was injected every time to check the proper functioning of the chromatogram as well as to see the stability of the peaks. After a reasonable space of time, each of the biogas samples of 1ml volume was run every time. Reading the chromatogram, the three peaks of voltage (nυ) against time (seconds) were displayed as a report of the analysis where the peak around 20 s gives the methane value, which was taken as percentage methane.

2.6.4. Inocula preparation and activation

The inocula used for the AD were mixed from cow dung and sludge obtained from a working sewage anaerobic digester, whereas a sludge from waste activated sludge system was brought for the aerobic digestion of the stillage. The microbe activation and acclimatization were fostered by the addition of a lab synthesized glucose solution when necessary.

2.6.5. Biological oxygen demand analysis

The BOD of cane molasses stillage was analyzed at different times; when it is raw, wet air pretreated and after it is anaerobically and aerobically digested. In principle, the dissolved oxygen (DO) is used as a measure of the BOD. Thus, the DO buffer was prepared earlier using distilled water, aerator pump and air diffuser, which was connected with the pump by a narrow plastic tube. Before aeration begins, Mg SO_4, Fe Cl_3, Ca Cl_2, phosphate buffer which was comprised of KH_2PO_4, K_2HPO_4, $NaHPO_4.7H_2O$ and NH4Cl, 1 ml/l each, were added to the distilled water. Aeration was maintained for over 12 h.

Later, the stillage sample in two BOD bottles was analysed, one BOD bottle was incubated in BOD incubator at 25 °C for 3 days and the other was titrated to determine the DO (Method 5210 B) [17]. To do the titration, one mL of manganese sulphate solution and alkali-iodide-azide reagent was added to the BOD bottle. Afterwards, the bottle was closed and mixed by inverting many times and a brownish cloud appeared in the solution as an indicator of the presence of oxygen. After allowing the brown solution to precipitate out of the bottom, a 2 ml of concentrated H_2SO_4 was added. Then the bottles were closed and mixed well to dissolve the precipitated back. Finally, the 200 mL of each sample was titrated with a 0.025 N sodium thiosulphate to a pale-yellow colour. Then 2 ml of starch indicator was added that turned the sample blue in colour. Titration continued till the sample gets clear and the reading was noted for the calculation of the BOD.

2.6.6. The color analysis

The color of stillage was analyzed when it was raw and following biological treatments. The sample taken was diluted to the level that can be read within the transmittance reading range of the spectrophotometer used, JENWAY (xenon lamp light source) 7305. While using the standard, 10 ordinates and a spectral bandwidth of 5 nm were applied.

2.6.7. Nitrate and phosphate analysis

In this study, the NO_3 content of stillage was determined. To do so, two reagents (reagents 'A and B') including a standard were synthesized for the spectrophotometric determination of NO_3 by taking OD reading at 410 nm. Reagent A was prepared by mixing a 5 gm of salicylic acid ($C_7H_6O_3$) and 100 ml of concentrated H_2SO_4 whereas reagent B was a 2 N NaOH solution. Before conducting the procedure, a reference was prepared like for other tests which was the determination of a straight line showing the absorbance versus concentrations of a solution of 1.37 g of $NaNO_3$ in a liter of distilled water.

By procedure, a 100 μl of the sample was taken and mixed with 400 ml of reagent A and the mixture was incubated at 25 °C for 20 min. Following incubation, 9.5 ml of reagent B was added; then the mixture was vortexed and cooled before taking the OD reading.

Regarding phosphate analysis, two solutions (solutions A and B) were synthesized. Solution A was obtained by dissolving 25 g of ammonium molybdate ($(NH_4)_6$ Mo_7O_{24}) in 300 ml of DW. Solution B was made by dissolving 1.25 g of ammonium metavanadate (NH_4VO_3) in a 300 ml of boiling water which was cooled and later mixed with a 330 ml of concentrated HCl. The solution was cooled to a room temperature and mixed with solution A making up to one liter. A standard was prepared by mixing a 219.5 mg of hydrous KH_2PO_4 in distilled water, which is equivalent to 50 μg of PO_4^{3-}P. The test proceeds by taking 1 ml sample and 0.25 ml reagent and vortex mixing and resting for 10 min at a room temperature before taking the OD reading at 470 nm using the spectrophotometer.

2.6.8. Separation weighing and storage

Three centrifuges were used according to the size of the sample; the one for up to 2 ml (MiniSpin ML079, EPPENDORF®), the other for up to 50 ml (Centrifuge 5804 R, EPPENDORF®) and the rest for up to 2 L (SORVALL, LYNX6000 Centrifuge Thermo Scientific®) volume capacities per cell. Similarly, two different scales were used to make samples according to the size required; the first one ranges up to grams unit (Sartorius, Thermo Scientific®) and the other, which was bigger, has been used in kilogram scale. Regarding sample or subsample storage, small, medium and large (cold room) sized refrigerators were used according to the size of the sample. Otherwise, incubators have been used to store at thermophilic and other temperature condition if room temperature only suffices.

2.7. Data analysis

The data collected during the various experiments were first registered in a logbook. Later, the data recorded were cleaned and transferred to excel sheet in which average values and transformations were computed. Additionally, graphs and tables were also obtained in excel aside from using OriginPro 2016. The data in excel were finally analyzed in R version 3.6.3 (2020-02-29) for statistical significance and more.

3. Result and discussion

3.1. The cane molasses ethanol distillery stillage characterization

Based on the dominant wavelength identified (580 nm), the spectrophotometric color category, also called the hue, of the stillage sample has been determined as yellowish orange with 60% purity [16]. The stillage also showed a far higher total COD (CODt) concentration which was over 172000 mg/l [12]. It too contains over 230000 mg/l of solids that might be due to the applied vacuum techniques during distillation. Vacuum techniques assisted distillation often concentrates the bottom product higher than the normal distillation. Disproportionately, the amount of phosphate in this sample of stillage is far higher compared to nitrate, which could be due to the application of the diammonium phosphate to foster yeast growth before seeding the fermentation vat [18] (Table 1).

3.2. The biochemical methane potential experiment performed on the raw stillage

The BMP test was conducted during a batch reaction times of 45 days following the characterization of the stillage. The BMP test was aimed at quantifying the ultimate practical methane yield that can be recovered through AD, while at the same time evaluating the degree of conversion of the COD into biogas [13, 19, 20].

In this regard, the current study evaluated the BMP of the raw cane molasses stillage and presented the cumulative specific methane yield as well as the biogas produced. Based on the result, a specific cumulative methane of 139.3 $NmlCH_4$/g-COD with up to 68% methane in the biogas (49% on average) was obtained at STP (Figure 3). The methane yield in this BMP test was significantly lower when compared to other organic substrate sources and the obvious theoretical yield [14, 19, 21]. As many other studies argue, including Janke and colleagues, the lower yield could be related to the complex nature of the substrate as well as the additional effect contributed by the ethanol process technology [22].

However, the energetic potential of stillage has been suggested to be high enough that the AD of stillage is economically and environmentally recommended when compared to the other management alternatives, such as application to soil fertility [11]. Therefore, this low BMP result in combination with the high post-AD COD (see Table) suggests that stillage pretreatment is a necessity. In fact pretreatment of stillage could enhance its degradability and helps to tap the ultimate methane potential [13, 23].

The post BMP analysis of stillage showed a significant reduction in volatile solid (VS) and an almost similar pH when compared to the inoculum; however, the soluble COD (CODsol) remained was still higher (19.5 g/l) when compared with the inoculum (5.2 g/l). This high residual CODsol can be attributed to the poor degradability and/or biotoxic nature of the stillage, which needs attention in order to optimize the energy recovery. Except the propionic acid, the short chain acids were removed

Table 1. Physicochemical characterization of stillage sample brought from Aligarh, India in mean ± (SD).

Sample	pH	CODt (mg/l)	BOD (mg/l)	PO_4^{2-}(mg/l)	NO_3^{2-}(mg/l)	Color characteristics at pH = 6		NH_4–N (mg/l)	TS (g)	VS (g)	TSS (g)	TDS (g)	VSS (g)	VSS/TSS
Molasses stillage	4	172917 ± 9417	132500	5283.019 ± 801	1306 ± 96	Dominant wave length	580	417.8	232.72 ± 0.04	164.01 ± 0	18.16	214.56 ± 3.2	34.13	1.9
						Hue	Yellowish orange							
						Luminance	65%							
						Purity	60%							

CODt = total chemical oxygen demand, TS = total solid, VS = volatile solid, TDS = total dissolved solid, TSS = total suspended solid, VSS = volatile suspended solid.

significantly contrary to the COD, this latter case may further strengthen the issue of the existence of some recalcitrant organics in the system (Table 2).

3.3. The stillage batch anaerobic digestion experiment

The poor biodegradability of stillage used to be a hurdle in its AD. In turn, the inefficient AD is challenging the energy self-sufficiency and beyond efforts by the sugar and ethanol industries. Worst of all, the stillage released without proper remediation causes aquatic and terrestrial pollution whereby the DO depletion, aquatic ecotoxicity and soil infertility are mostly reported. In this regard, several technologies have been tested to treat stillage while some resulted in poor efficiency; others suffered poor economic viability. Aiming at sustainability, maximizing the recovery and the production of clean energy, stillage AD remained a necessity. The advantage of organics AD, especially of second and third generation biomasses is proving beneficial from an environmental and economic perspective with respect to renewable energy and valuable chemicals productions and including soil fertilizer. However, factors such as pretreatment, type of biomass, and digester operation parameters used to affect the performance and hence the profitability of an AD [13]. Amid such AD challenges, pretreatment of feed help improves biochemical process towards optimal production.

In the current study, stillage had been pretreated using an IOCS based mild WAO, which was performed at atmospheric pressure and insignificant temperature. The pretreatment was targeting the reduction of stillage toxic ingredients that include the phenolic compounds. Therefore, the energy recovery potential of the pretreated stillage in AD was compared with the one without pretreatment, while both were also contrasted with the ultimate BMP of stillage whose test was conducted for a period of 44 days. The AD of stillage was performed in batch mode. Along the biogas and methane yield, the operating parameters were also monitored, which was principally aimed at regulating pH.

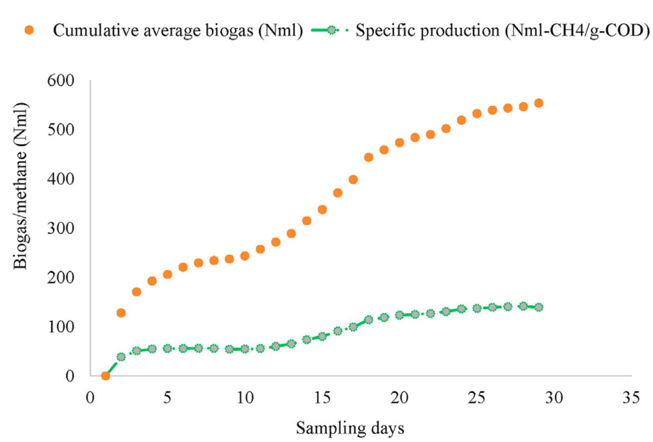

Figure 3. Averaged cumulative biogas and specific methane produced during the biochemical methane potential test on the raw molasses ethanol stillage.

3.3.1. Raw versus wet air pretreated stillage anaerobic digestion trials

The current study also explored the significance of the effects of the application of scoria support in addition to the WAO pretreatment on the specific biogas and methane yield as well as the AD process stability. Overall, the WAOp stillage followed by the scoria supported AD showed a significantly better yield in biogas and methane as well as an improved process stability has been observed with regard to the use of scoria support over the raw stillage AD. The specific normalized cumulative methane yield showed 23.6, 24.2 and 84.0 in ml/g-COD for the raw without scoria support, raw with scoria support and WAOp scoria supported stillage AD subsequently.

The improved process performance exhibited even by the non-pretreated stillage might have resulted because of the application of the raw coarse scoria as support. The scoria used proposedly helped in substrate shock minimization and thereby the attainment of a relatively optimal pH in the digesters (Figures 4 and 5). Further, nutrient leaching may occur, which would have benefited the microbiota. The analysis of the data in R, version 3.6.3. 2020-02-29, showed a significant difference in mean biogas quality between the digesters with scoria and the digester without, as measured in methane content (p-value = 0.000).

The two systems showed a closer variability and a significant mean difference in methane, 45 ± 22% for the former and 28 ± 20 for the latter. This difference in the performance of batch digesters due to scoria has proved the importance of using such natural and cheaply available materials in digesters' support instead of using a less or non-responsive support materials.

Previous studies support the proposed effect of such kind of material use. For instance, the use of scoria-compost mix as a biofilter has revealed a significant reduction in toxin removal from volatile organic carbon streams, xylene [24]. A related study also revealed the detoxification effect of scoria used as an adsorbent in the treatment of tannery wastewater [25]. Similarly, the current result of improved biogas and methane yield from the use of scoria support in AD might have resulted in reduced toxicity effect of stillage. Opposingly, scoria pack used in the AD systems can also have a substrate hiding effect as it can result in stillage inaccessible by the microbes, given a proper mixing may not be there.

However, use of scoria can still have positive implications in minimizing substrate shocking problems, while at some point the adsorbed feed served as spare provisions during suspended feed depletion. Thus, the former effect can either be compensated in some way that include the

Table 2. Post stillage biochemical methane potential test characterization result in means (±SD).

Parameter	Molasses stillage	Inoculum
pH	7.2 (0.04)	7.7 (0.02)
CODsol (mg/l)	19500 (34)	5222 (2)
TS (g/l)	46.1 (0.8)	25.3 (0.1)
VS (g/l)	21.8 (1.1)	13.3 (1.9)
Acetic acid (mg/l)	876 (91)	-
Propionic acid (mg/l)	1357 (55)	-
Butyric acid (mg/l)	594 (52)	-

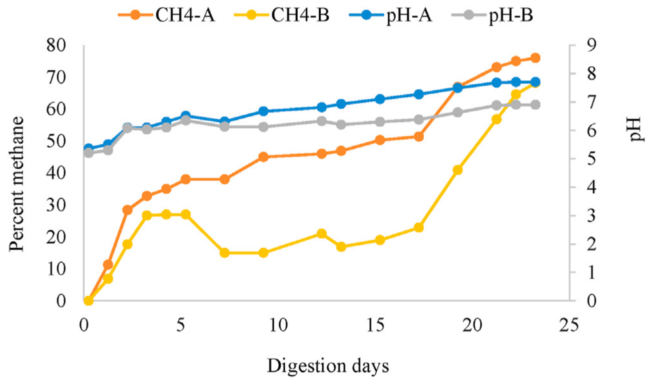

Figure 4. Difference in methane content between stillage batch anerobic digestion using scoria (CH_4-A) and without using scoria (CH_4–B) and pH.

application of mixing. Yet, it can be an advantage as a way of preserving the viability of microbes in the system enduring a span of the solids retention time.

Based on observations from previous result in methane difference between scoria use and non-use, more AD testing on the use of scoria was conducted to further check the difference in both specific biogas and the methane produced (Figure 5).

The specific cumulative average biogas and methane produced by the digesters with and without scoria support were recorded after converting that to STP conditions. Later, it was evaluated if there was a significant difference between the two systems. Accordingly, the methane (p-value = 0.000) as well as the biogas produced (p-value = 0.029) showed a significant difference on average where the difference was more prominent in the case of methane yield. While the degree of variability in both cases was closer, the average production of the percent methane and the biogas volume was different. Both this and the former test results on the use of the scoria support of AD reinforces its use, perhaps in relation to biofilm attachment, substrate shock removal, and pH stability. Indeed, it would be worth considering the potential microbial nutrients contained in the rock materials that might have possibly leached and enriched the systems. Consequently, the result may be subject to further study to examine the phenomena in detail as well as consideration of the effect of other related minerals.

In another look, the batch AD results showed that the WAOp stillage had better extracted the ultimate methane or biogas potential of the cane molasses ethanol stillage when compared to the one without pretreatment. More importantly, the WAOp stillage AD demonstrated a faster degradation rate, giving the results in a relatively short time and its methane content reached over 70% indicating the biogas quality's advantage. More importantly, the COD removal of the pretreated stillage AD was over 92% when compared to the untreated one, which was 82%. That better removal of the COD would be due to the IOCS based WAO pretreatment, which would have caused the reduction in toxicity of the feed and enhanced stillage biodegradability. The improved biodegradability (the BOD/COD ratio) of original versus treated substrate was 0.57 and 0.73 respectively.

Both WAOp and raw stillage AD results of the current study, however, showed an above average COD removal, even before the aerobic polishing treatment, when compared to studies reported so far. For instance, in an enquiry on the energy latent of stillage, Fuess and Garcia claimed that, as an effective alternative treatment, the AD of this acidic and corrosive biomass can result in an average 74% COD removal [11] (Figure 6, Table 3).

The specific biomethane yield attained from such a third-generation biomass in this study is significant. In fact, the theoretical biomethane yield from a first-generation carbon source substrate is around 343 ml/gCOD. Using a concentrated molasses from a second generation bioethanol plant, Sarker and Møller, obtained 185 Nml-CH4/gCOD [26, 27]. Given that such methane yield was achieved from molasses after 95 days of digestion and with over 20 days of lag phase, the current biomethane recovery (80 Nml-CH4/gCOD) from a third-generation biomass and within 20 days of digestion was significant. Indeed, yield mainly relies on different factors including type of substrate, reaction time, pretreatment applied and process stability whereby the latter was also monitored in the current study.

Regarding the reactors' pH stability monitoring, the batch digesters did not show a significant difference between the one with an IOCS based WAOp stillage and the other with the raw stillage, while both being supported with scoria.

The AD process follows four major steps: hydrolysis, acidogenesis, acetogenesis and the methanogenesis. For methanogens, optimal pH needs to be between 6.7 and 7.4. A relative pH drop caused by the acidogenic bacteria is expected during the acidogenesis stage. However, the system has to recover itself into a suitable condition for methanogens which otherwise would compromise the methane content if that is not even worse to the level of pickling the anaerobic digesters. Though the pH in the batch digesters of the current study dropped to 5.3 in the first two days, later the digesters recovered themselves to be over 6 within the first five days without even a chemical addition to the desired range and therefore process instability was not a significant problem.

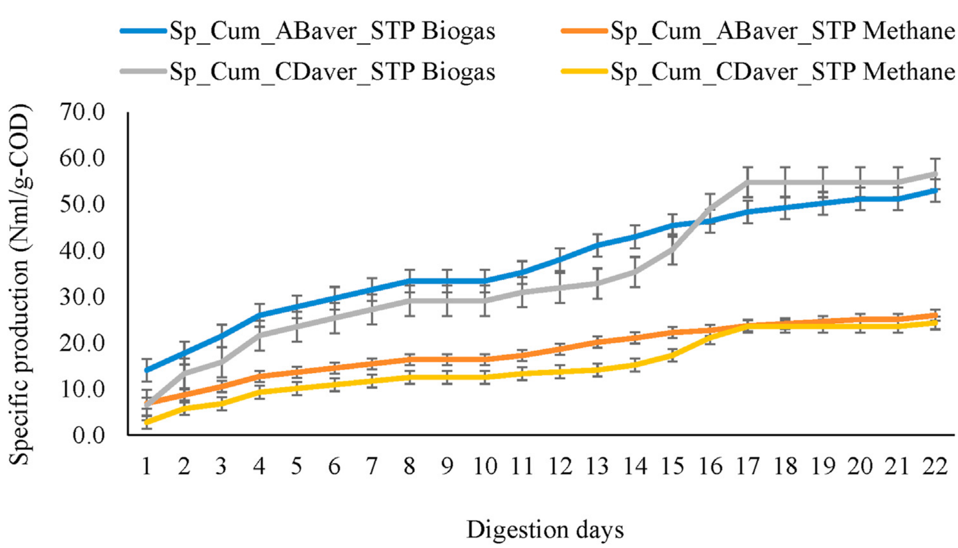

Figure 5. Difference in the normalized specific biogas and methane yield with respect to scoria support.

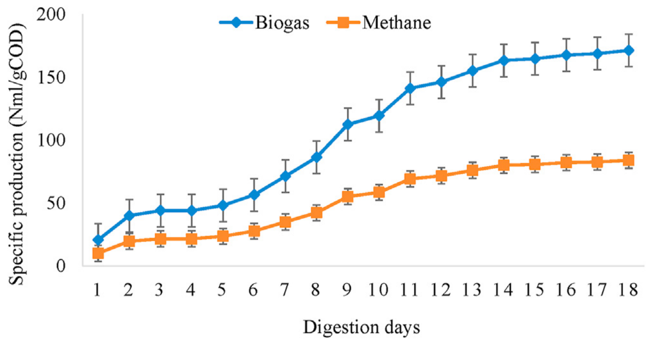

Figure 6. Normalized biogas/methane yield on anaerobic digestion of a wet air pretreated stillage.

Both reactors showed a close reading in pH (Figure 7). However, these average similarities in pH, that is, 6.8 for the one fed with WAOp stillage and 6.7 for the other which was fed with raw stillage, were maintained after adding a 2M NaOH solution for the former digesters during the first week. Otherwise, it was expected that the lowering in pH by the reactors fed with the WAOp stillage would have suffered souring. Since the degradability of the substrate improved significantly after wet air pretreatment, the rate of degradation was faster as expected, which has brought the drop in pH in the early days of digestion which would have resulted otherwise in process instability.

3.3.2. Comparison of the specific methane yield among treatments with reference to the ultimate methane potential of the stillage

The cumulative specific methane yield obtained from the BMP, the raw batch and WAOp stillage AD tests were compared at STP conditions. Relatively, the WAOp stillage AD better exploited the methane potential with even shorter digestion days. Despite the possible impact on the percentage methane of the biogas yield, especially in the early stages of the AD, the ‘rate’ of degradation and hence the speed of recovery during the first two weeks was best performed by the WAOp stillage. In fact, the slight peak over the scoria supported WAOp AD by the BMP test result in the first week was due to the difference in inoculum to substrate ratio whereby a 5:1 inoculum to substrate ratio was applied. Thus, given the small amount of substrate tested (10 ml) and the 50 ml inoculum applied, most of the substrate was quickly consumed during the first week in the BMP tests. This, in fact, came to a compromise in biogas quality expressed as percent methane.

Indeed, the cumulative methane yield by the WAOp stillage surpassed the one obtained by the BMP test in the second week. Apparently, the former was significantly higher compared to the other two tests conducted on the same scale; both were without WAO pretreatment, but one was without scoria and the other was with scoria support. Indeed, with a narrow margin, the scoria supported was over the non-supported, especially in the first two weeks (Figure 8).

3.4. The color analysis

First, the color of stillage has been checked visually and the true color was determined using the standard methods, before any treatment and after each treatment. Among the standard methods that are outlined by APHA (Methods 2120B-D) to determine color, method 2120C was selected in this study since it is recommended for highly colored industrial wastewaters by the same standard. Accordingly, the transmittance values in percent were obtained using a spectrophotometer (xenon lamp light source), JENWAY, 7305, and the 10 ordinates were selected for a fair accuracy whereas the spectral bandwidth was 5 nm.

With any treatment, the color change observed was from yellowish orange to greenish yellow, which would be due to the interference of the

Table 3. Post anaerobic digestion characterization of stillage with respect to pretreatment in mean ± (SD).

Parameter	With scoria	Without scoria	Scoria and wet air
Ph	6.8	6.7	7.7
TS (g/l)	73.8 (3.3)	73.2 (3.7)	31.6 (6.7)
VS (g/l)	30.3 (0.8)	28.5 (0.7)	14.1 (5.4)
Acetic acid (mg/l)	2301 (120)	4281 (5)	2987 (157)
Propionic acid (mg/l)	2558 (65)	2719 (46)	1939 (83)
Butyric acid (mg/l)	875 (19)	884 (11)	498 (16)
CODsol before AD (mg/l)	172917	172917	168889
CODsol After AD (mg/l)	30041.7	30666.7	13375.0
CODsol removed (mg/l)	142875	142250	155514
Removed actual (g/l)	28.6	28.5	19.4
CODsol removed (%)	82.6	82.3	92.1

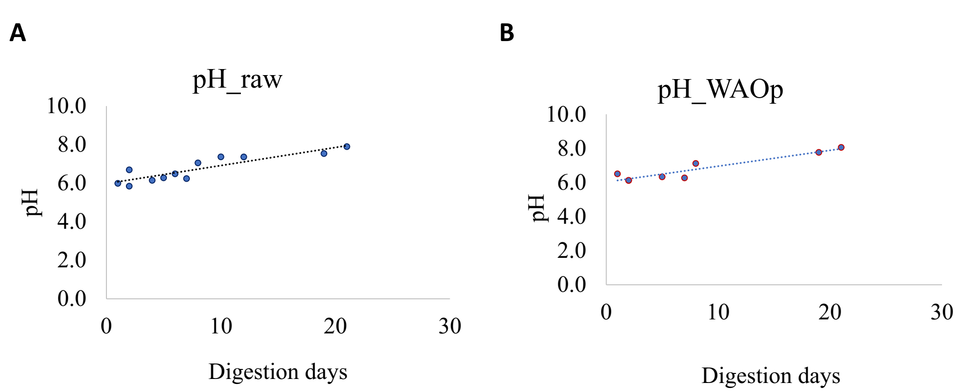

Figure 7. Average pH results of the digesters fed with raw stillage (A) and a pretreated stillage (B).

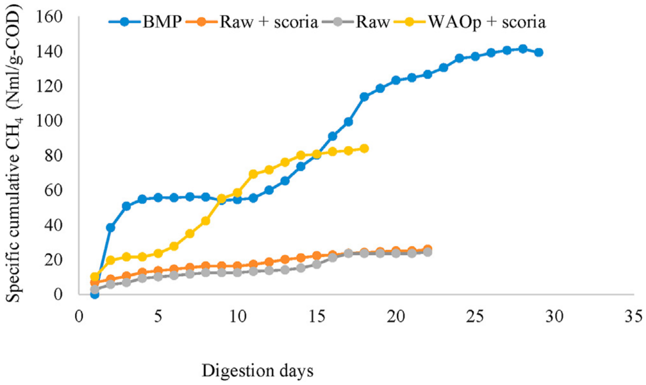

Figure 8. Comparative efficiency in methane recovery of treatments compared to the ultimate biochemical methane potential of stillage anaerobic digestion.

its toxicity and complexity. In a related fact, it may be unnecessary to carry on aerobic digestion on a WAOp stillage after AD that will have a huge cut in the cost of such industrial wastewater treatment. In other words, over 92% COD reduction in the stillage achieved may suffice, compared to the cost implication of connecting the aerobic degradation unit as final treatment.

Therefore, such renewable and clean energy recovery efficiency with a mild pretreatment makes stillage a valuable candidate for AD towards energy self-sufficiency. With a toddling move and a varying momentum among countries and even continents, the world is transferring towards dependency on sustainable energy sources against fossil fuel dependency, which is blamed for its atmospheric pollution effect due to greenhouse gas emission and its associated economic instability potential. One such activity of the change is aimed at enhancing the economic competitiveness of the bioethanol sector whereby stillage AD in those process plants is deemed to improve the overall energy balance of the sector. In-depth review by Cesaro and Belgiorno suggested the need to widely transform stillage AD to an industrial scale if issues of net energy gain and process stability are managed even at their conclusion of up to 80% COD to biogas conversion [28]. Thus, this study shows the enabling conditions that can significantly support the intended move. Furthermore, the result obtained in this study contributes to the effort towards the compliance of the stringent discharge standards, at least to fit the standards to join municipal sewerage systems.

Table 4. Summary of color characteristics and pH of raw molasses ethanol stillage and after subject to biochemical treatments.

Sample	pH	Color properties			
		Dominant wave length (nm)	Hue	Luminance (%)	Purity (%)
Raw stillage	6.04	580	Yellowish orange	65.0	60
WAOp	4.12	570	Greenish yellow	81.6	40
Original	3.96	570	Greenish yellow	93.1	40
After AD with scoria	6.80	570	Greenish yellow	91.3	60
After AD without scoria	6.70	570	Greenish yellow	94.3	60
After AD with WAO & scoria	7.70	570	Greenish yellow	94.6	40

Table 5. Summary of the biological oxygen demand analysis tests on the raw and treated molasses stillage.

Sample	DO average (mg/l)		BOD average (mg/l) ± (SD)	BOD/COD	Remark
	Initial (mg/l)	Final (mg/l)			
Raw stillage	6.9	4.25	132500 (3536)	0.57	
After WAO	7.2	1.55	169500 (6000)	0.73	
After AD of raw	9.4	7.60	12000 (2400)	0.40	CODsol
After AD of WAOp	9.3	8.30	0	0.00	CODsol
Blank (Seeding)	9.5	8.50	1 (0)	n/a	

microbial activities and any process issue, such as the addition of inocula, dilution effect as well as pH. The major range in color parameters was seen due to percent purity followed by percent luminance, while the dominant wavelength showed very close values, varying between 550 nm and 580 nm, among treatments (Table 4). Visually, there was an almost negligible difference in color before and after as well as among treatments. The lowest purity has been obtained from the sample collected after the WAOp and anaerobically digested stillage. The intensity of light emitted per unit area as expressed in percent luminance in this study has generally increased following treatments, which may signal the decreasing effect of molasses stillage towards light transmission when it is released to aquatic systems.

3.5. The biochemical oxygen demand test results

The BOD of the stillage was tested several times at different stages. Initially, it was found to be around 132500 mg/l. Afterwards, the testing was conducted following the WAO, the AD of the raw stillage and the AD of the WAOp stillage (Table 5).

The application of WAO pretreatment improved the efficiency of the AD of the stillage towards a complete degradation of the biodegradable fraction, perhaps in a short time compared to the sample without WAO pretreatment. On the other hand, WAO improved the biodegradability of the stillage as it has influenced its bioamenability due to the reduction of

4. Conclusion

The application of an IOCS based mild WAOp to stillage as an advanced oxidation process together with the application of a scoria supported AD resulted in higher efficiency through the elimination of the most toxic components of stillage, probably by adsorption. The batch AD of a mild WAOp cane molasses distillery stillage can completely remove the BOD with a significant removal of the COD. The energy gained from this third generation biowaste was considerably high, promising self-sufficiency for the ethanol sector. Further, the energy recovery rate was hastened by the AD of the WAOp stillage. Scoria support during the AD also brought stabilization of pH during the first few days. Thus, the current work highlights on the use of cheaply and locally available materials, including IOCS and scoria, in environmental technology applications that can help solve industrial pollution. The color in the stillage, however, remained almost as it was, which may trigger the search for the simultaneous integration of color and COD removing adsorbent material. The direct testing of phenols in stillage before and after treatment is recommended for future research.

Declarations

Author contribution statement

Getachew Dagnew Gebreeyessus: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Trichur Ramaswamy Sreekrishnan: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Andualem Mekonnen: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yonas Chebude: Performed the experiments; Analyzed and interpreted the data.

Esayas Alemayehu: Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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