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ABSTRACT: The objective of this study was to evaluate the treatment efficiency of a coupled stillage anaerobic digestion, which was performed in scoria-packed continuous reactors and following aerobic degradation. The optimum organic loading rate was determined for the continuous anaerobic digestion of a molasses ethanol distillery stillage with and without wet air feed pretreatment. The pretreatment of the molasses ethanol distillery stillage brought a significantly higher chemical oxygen demand removal in anaerobic digestion with an increased loading rate of 2000 mg/L d when compared with the raw stillage. The results also showed a complete removal of the biological oxygen demand following the coupling of anaerobic digestion with aerobic degradation. During the later stillage aerobic treatment, 68% of the chemical oxygen demand was removed within 8 hours of retention time. Despite the color, the removal of organics in stillage due to integrating wet air pretreatment, continuous anaerobic digestion, and aerobic degradation was successful. The pretreatment and hybrid technique also appears as a promising technique toward the sustainable management of stillage, thereby meeting discharge limit set for the ethanol industry by regulators.

KEYWORDS: Chemical oxygen demand, organic loading rate, pretreatment, scoria, stillage

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Introduction

The use of alcohol for drinking, cleaning, and fuel purposes is increasing with time and becoming a disintegrable phenomenon with population and economic growth as well as urbanization. The increase in use continued even under the COVID-19 pandemic time.[1-5] However, the mass production of ethanol has got pros and cons regarding the water, energy, and environment nexus worldwide.[6-8] The environmental concern with ethanol distilleries is mainly due to the massive release of stillage, which demands a sustainable stillage management. This has led to the need for the use of this third-generation biomass, distillery stillage, for energy generation, water reclamation, and soil nutrient recovery, thereby closing the gap in the economic cycle and ensuring the sustainability of such sectors, especially in the developing countries. For every liter of ethanol, an average of 13 L of stillage is produced as a bottom product.[9-11]

Despite the huge stillage production, the application of ethanol stillage for renewable energy and soil nutrition purpose is impeded by its recalcitrant nature caused by the use of agri-chemicals and due to the byproducts formed from some reaction between the sugars and the amino acids. Furthermore, stillage chemical oxygen demand (COD) and biological oxygen demand (BOD) fall far above the discharge limits set by the regulatory authorities for ethanol industries following conventional treatments to discharge their effluent to nearby environmental media.[12-14] In this regard, various stakeholders revealed their concern in minimizing the environmental footprint of ethanol industries while improving the energy self-sufficiency through recovery of byproducts, thereby ensuring sustainability. Due to the cost and techno-complexity of other management alternatives, stillage anaerobic digestion (AD) remains preferable as it is a robust, simple, and feasible technique.[15-17]

Despite the fact that detoxification and energy gain from stillage remained challenging, several research has applied synthetic wastewater to simulate stillage, whose products may not be realized in actual scenario.[18] Those AD tests on real ethanol wastewater remained at an efficiency of around 80% of COD removal,[19-21] whereby most of the studies were even performed under batch mode.[12,14] Few studies have been tested on the continuous mode, and the improved performance of AD was reported under fixed film digestion.[19] The fixed film AD used some media to pack the digesters with some synthetic medium, which would help attach the biofilm.[10,22,23]

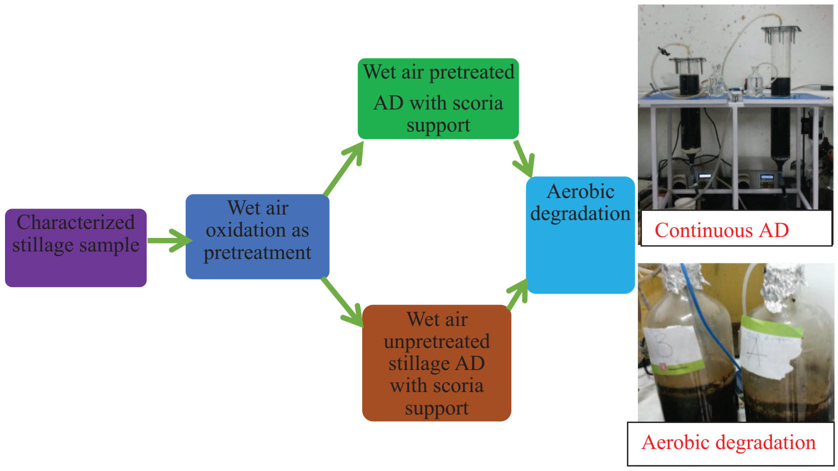

Figure 1. The diagrammatic and photographic setup of the experiments.

Although there are emerging AD techniques for an improved reduction in stillage COD,[24] still they are applied at a reduced COD feed concentration. Consequently, most investigations end up concluding on the importance of integrated stillage treatment, which include feed pretreatment, AD, and aerobic degradation.[25-27] Therefore, this study evaluates the effect of stillage pretreatment on organic loading rate (OLR) and the subsequent COD removal under continuous AD process as well as the removal of BOD and COD from coupling AD and aerobic degradation. It further examined the effect of scoria support, a natural vesicular rock, on the AD systems.

Materials and Methods

Experimental setting

The experiments were run in sequence as depicted in Figure 1. First, stillage brought from a molasses ethanol factory was characterized. Following that, subsample of the stillage was subjected to wet air pretreatment in a 4-necked glass vessel using air at a flow rate of 2 L/min and temperature of 60°C. The mild and iron oxide–coated sand (IOCS)-based (3.5% by weight) wet air pretreatment was conducted for a retention time of 4 hours using a batch system.

The optimal temperature-time-IOCS loading combination has been determined after conducting a separate experiment that considered them as factors for stillage COD and toxicity reductions, whereby the wet air experiment has been followed with toxicity testing. Temperature levels of 50°C, 60°C, and 70°C, and holding time levels of 2, 4, and 6 hours with no IOCS loading, 3.5% loading, and 7% loading have been tested while keeping the gas flow rate constant. The entire wet air oxidation (WAO) and toxicity testing study is under communication in a different journal.

After the WAO of stillage, the sample was moved onto a continuous AD alongside the raw subsample of stillage. Later, a predetermined soluble COD (COD_{sol}) containing reject water from the AD was moved onto the aerobic degradation bottle of 1-L volume glass reactor. The aerobic reactor was being sparged with air supplied from an external electric pump. Both the AD and aerobic degradation were performed at mesophilic temperatures (35 C ± 2 C) (Figure 1).

The experiments on biogas yield and COD removal have been conducted on a semi-continuous upflow anaerobic digester. Anaerobic digesters of 2 L working volumes were constructed in the lab using acrylic sheets, plastic funnels, silicon tubing, clamps, pumps, and a multipurpose sealant, among other materials. The digester construction included those accessories assembled: the feed tank, electric pumps fixed at 150 rpm, feed inlet, gas exit and effluent ports on which the silicon tubes were fitted, beakers to collect the exiting effluent, and the upright table to fix the digesters erected.

With an effective volume of 1.8 L, the anaerobic digesters were loaded with a varying OLR between 120 and 2000 mg-CODsol/L d whereby the hydraulic retention time was maintained at 15 days on average. The anaerobically treated stillage was coupled with polishing aerobic degradation, which was performed using 1 L volume batch glass reactors connected to electric pump and sampling ports (Figure 1).

Sampling

Scoria sampling. Scoria, which is a volcanic rock prevailing in the rift valley region of Ethiopia, has been brought from 2 locations whose coordinates are taken as indicated in Figure 2. The 2 sampling points were chosen to catch the possible effect of spatial variation on the property of the rock across the rift valley in the country. The scoria has been broken down to a relatively uniform coarse size and was washed with tap water and dried before packing to remove dirt.

The raw scoria has been examined for its morphology and elemental composition using X-ray diffraction (XRD), scanning electron microscopy (SEM) and scanning electron microscopy energy-dispersive X-ray (SEMEDEX) analysis. The SEMEDEX analysis of the scoria was performed in SwiftED3000 to quantify all elements whose analysis took

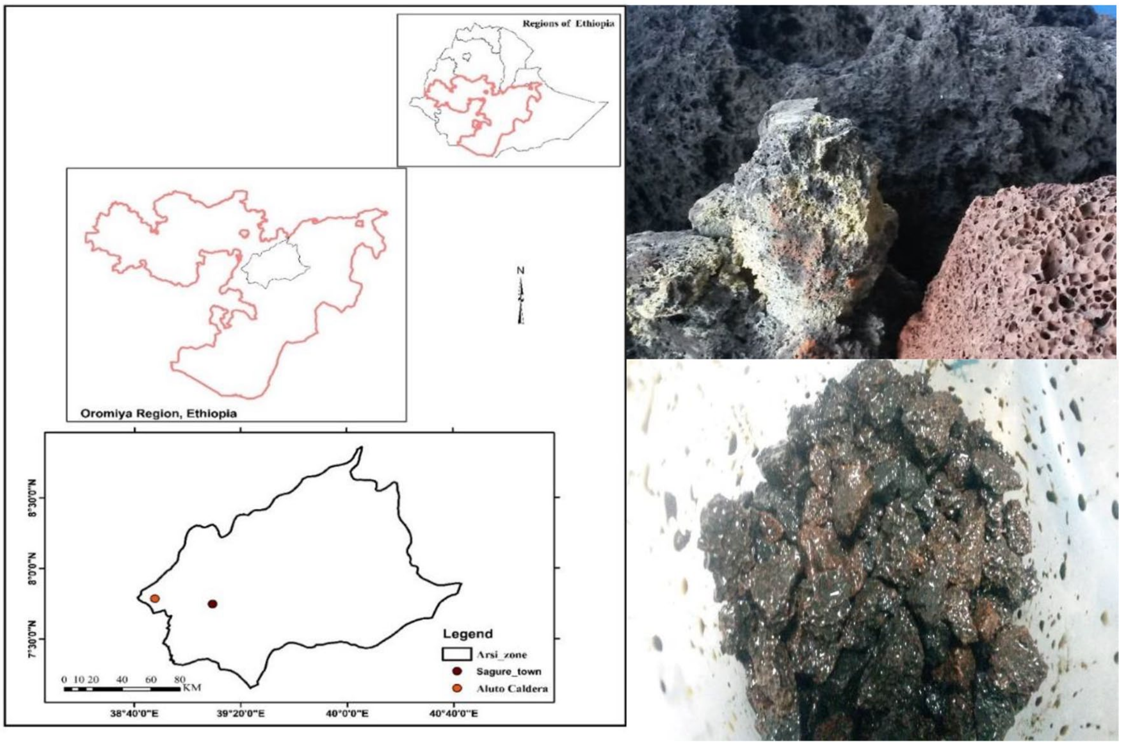

Figure 2. The location points (left) and raw (top-right) and used images (bottom-right) of scoria sample used for anaerobic digester packing.

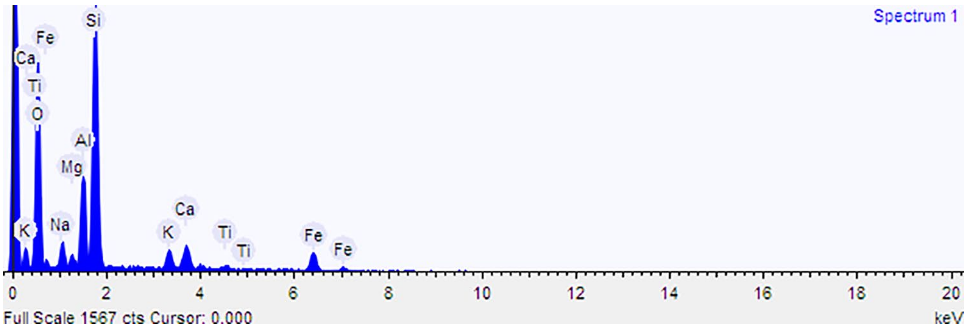

Figure 3. Spectrum of elemental composition of the raw scoria.

an acquisition time of 60 seconds, a process time of 4 minutes, and an accelerating voltage of 15 kV. No coating was applied. By composition, the top 4 elements are oxygen (70%), silicon (15%), aluminum (5%) and iron (3%) as shown in Figure 3.

The XRD image read at 2 thetas showed the non-amorphous nature of scoria. The SEM image of the raw scoria showed a rough surface nature, which may help biofilms attach over a wide surface area in the anaerobic digesters. A further analysis of the scoria using the Fourier transform infrared spectroscopy (FTIR) is also presented (Figure 4).

Stillage sampling. A real molasses ethanol stillage sample was brought from Aligarh, Uttar Pradesh State, India. The sample was car transported in plastic container, which was placed in a box containing ice packs, that reached within 4 hours in the working laboratory where it has been further subsampled, analyzed, and cold-stored.

Inocula

Composites of inocula were grabbed from different places. One was from a lab stock of the AD crew maintained viable by feeding glucose solution periodically (Table 1), and the other was brought from a sewage AD process operating at a place in Delhi, India. The purpose of using mixed inocula was to establish the AD systems by quickly adapting consortia of anaerobic organisms, which include the use of sludge AD biomass. As a strategy to acclimatize the reactors, glucose solution was fed, especially during the establishment of the pseudo-steady-state conditions.

Analysis and equipment

Standard methods were followed during the determination of stillage parameters, which include BOD and COD, before and after treatment, mainly performed based on the American

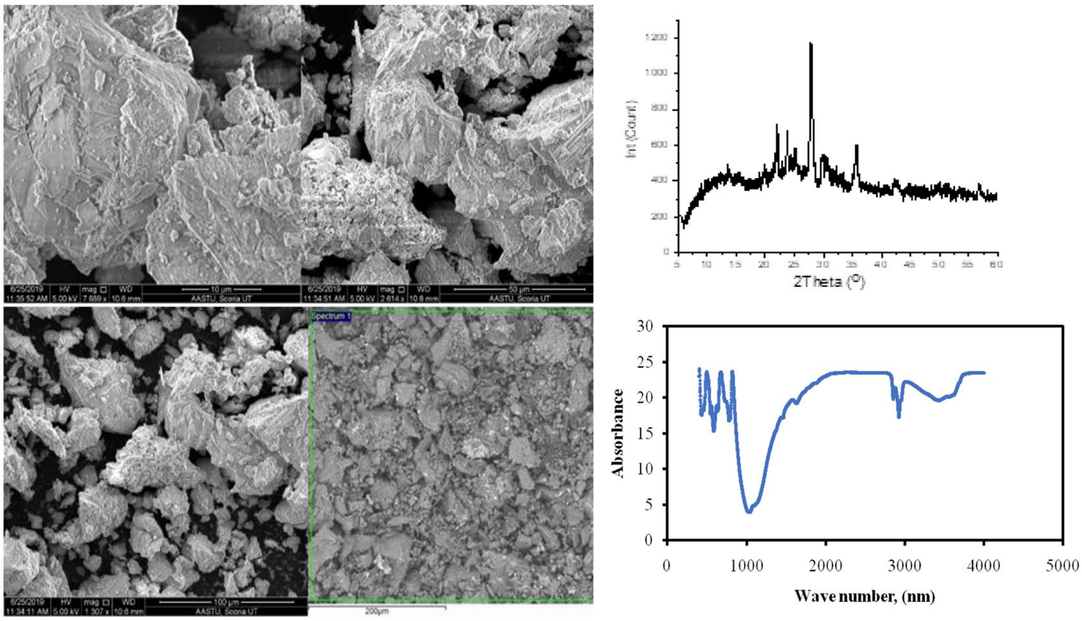

Figure 4. X-ray diffraction (right top) and scanning electron microscopic images taken at 10, 50, 100 and 474.1 μm resolutions (left) as well as the Fourier transform spectroscopy (right bottom) for the raw scoria.

Table 1. Formulation ingredients of glucose solution for inocula activation.

CONSTITUENT	COMPOSITION, G/L
Glucose (dextrose)	10
Yeast extract	0.34
Ammonium chloride	0.84
Potassium dihydrogen phosphate	0.136
Dipotassium hydrogen phosphate	0.23
Magnesium chloride	0.084
Ferric chloride	0.05
Calcium chloride	0.09
Distilled water	Rest

Public Health Association's outline.[28] However, other standard procedures were also referred from related peer-reviewed journals.[29,30] During analysis, prior calibration of instruments, which include the pH meter and the spectrophotometer (JENWAY 7305), has also been performed.

A thermal conductivity detector (TCD)-based gas chromatography (GC) was used for the analysis of biogas and methane (5700 NUCON). The GC carrier gas used was hydrogen performed at a detection temperature of around 90°C. The oven temperature or the GC injector temperature was always kept around 80°C. In addition, weighing (Sartorius; Thermo Scientific), pH measuring (CyberScan pH 510; Thermo Scientific), and centrifuging equipment (MiniSpin ML079, EPPENDORF/Centrifuge 5804 R, EPPENDORF/SORVALL, LYNX6000 Centrifuge Thermo Scientific) and advanced analytic instrument were used (INSPECTFS and SwiftED3000). The FTIR was performed using the instrument FTIR Nicolet iS05.

Data analysis

The meticulous data collected were first entered in Excel, and later statistical analyses were performed in R, version 3.6.3 (2020-02-29). The results obtained were also discussed by contrasting them with existing evidence.

Results and Discussion

The continuous anaerobic digestion experiment

Initially, the molasses stillage sample was characterized for its composition. The COD of the sample was so high, which was measured around 173 000 mg/L with a BOD of 132 500 mg/L. The solids in the stillage were over 232 000 mg/L with a considerably high phosphate that might have resulted from the excess use of phosphate compounds during fermentation to foster growth of yeasts (Table 2).

The startup periods. Following the completion of digester construction and setup, old stillage batch AD inoculum of 900 mL was shared between reactors A and B each packed with 400 mL scoria by volume on day 1 (October 30, 2019). The scoria, a vesicular volcanic rock, which was applied in the AD systems was selected to serve the purpose of biomass support and lower substrate shock. In addition, as a natural material it could not have a negative effect on the microbes over other synthetic support materials. Moreover, its abundance as a local rock and the possibility of nutrient leaching make it a promising substance to use.

Table 2. Physicochemical characterization of stillage sample brought from Aligarh, India, in mean ± SD.[31]

SAMPLE	PH	CODT, MG/L	BOD, MG/L	PO_4^{2-}, MG/L	NO_3^{2-}, MG/L	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	17.8	232.72 ± 0.04	164.01 ± 0	18.16	214.56 ± 3.2	34.13	1.9

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

Next day following feed characterization, 120 mL of centrifuged original stillage was pumped to each reactor in 850 mL of tap water to fill up to 2000 mL total volume of each reactor. However, the digesters were stuck for a few days with elevated pH and without considerable COD reduction, perhaps due to the unfavorable proportion of inoculum to substrate ratio. Consequently, modification of the contents of the reactors was performed after 2 weeks by emptying the old content with an entirely new one. Thus, on November 16, 2019, the inocula were renewed by adding a sludge from a working sewage sludge anaerobic digester and activating the biomass with glucose solution which was synthesized in the lab. The COD of reactors A and B was 1176 and 1155 mg/L, respectively. However, the AD was started at a lower OLR of 120 mg/L COD_{sol} by diluting 0.5 mL of the homogenized original sample with 999.5 mL of distilled water. From the day November 16, 2019, onward, the pH of the system was stable between 6.4 and 7.9 on average for both reactors as desired for the healthy operation of the AD.

Among other issues, AD process stability behaves as a function of substrate type; system susceptibility to xenobiotics; the mode of operation and temperature—continuous or batch or thermophilic or mesophilic; and the pH.[32] Thus, toward reaching stable operation, glucose solution and glucose stillage mix have been fed to digesters. However, pH drops of scale over 1.5 have been recorded in just 4 days during the second week, which is also followed with poor methane generation and lower percentages (10% methane). Consequently, a mix of anaerobic sludge from a working waste–activated sludge AD was obtained and supplemented to the current experiment.

Following the establishment of the pseudo-steady and stable condition during the startup period, the stability in pH and COD removal was monitored for over 40 days together with OLR variations and the COD removal examination as shown in Figure 5A and B. Figure 5A demonstrates a stable and also a relatively higher and consistent pH by reactor A. Figure 5B shows the stability of pH during variations in OLR after the pseudo-steady-state conditions by both reactors. Every time variables get tested, simultaneous runs of reactors A and B were conducted as replicates, which were labeled as A and B for the sake of monitoring and identification.

The slightly lower pH on the second day of the pseudo-steady operation of reactor "A" was associated with the relatively higher initial COD and the subsequent hydrolysis and acidogenesis activities happening in the system. Although the slight variation in pH between the 2 systems persisted nearly all the time, the pH of both systems was maintained between 6 and 8 during the entire startup period. Maximums of 8.0 and 7.7 and minimums of 6.7 and 6.1 pH have been recorded for digesters "A" and "B" subsequently. However, the pH monitored showed a significant difference between reactors "A" and "B" ($P = .00$) based on a *t* test performed in R. Despite their major disparity in pH, the 2 digesters were within desirable ranges of pH except the slight increase recorded by

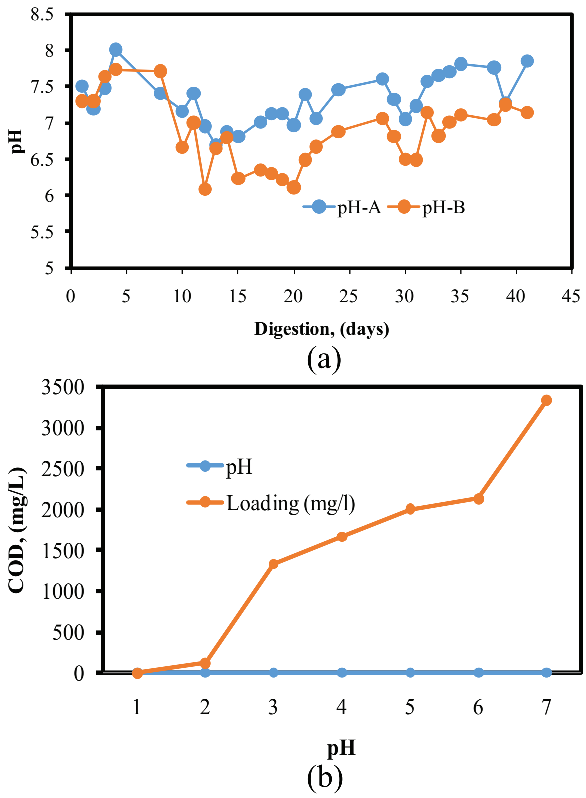

Figure 5. Results of pH monitoring (A) and pH versus loading rate (B) in the continuous anaerobic digesters over time.

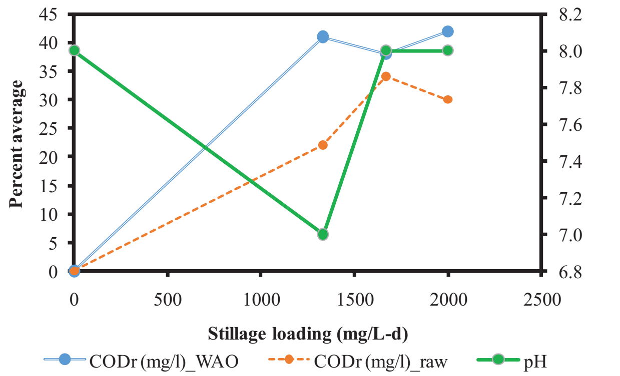

Figure 6. Stillage anaerobic digestion average pH and COD removal difference between wet air pretreated (CODr (mg/L)_WAO) versus the raw feed (CODr (mg/L)_raw).
COD indicates chemical oxygen demand; CODr, COD removal; WAO, entire wet air oxidation.

digester "A." After the establishment of a relative stability in pH, the test runs were started aiming at the COD removal efficiency of both digesters fed simultaneously, and the OLR and feed were changed over time.

COD removal efficiency variation with feed pretreatment. Average values from both digesters were taken for nearly 2 months after establishing pseudo-steady-state conditions whereby the OLR was varied between 2 and 3 weeks. The entire runs were repeated except changing the feed type from the raw molasses ethanol stillage to a WAO pretreated one. The COD after the stabilized pH was showing negative removal for the first 2 days, which could be due to leaching from the packing material, release of extracellular polymeric substances in the sludge inoculum, poor mixing in the system, and the use of undiluted samples for the COD analysis. Despite the accumulating COD, the pH of both reactors was on a slight increase, which would be due to the effect of the packing material.

In recent times, AD feed pretreatment is becoming increasingly important for efficient bioenergy, resource recovery, and environmental protection, which depends on the nature of the biomass. Molasses ethanol distillery stillage can be pretreated using various advanced oxidation techniques. However, its high solid nature and recalcitrant organic content fit to the application of WAO. The wet air oxidation pretreatment (WAOp) was important in lowering the solids while minimizing the toxic and recalcitrant nature, thereby enhancing the biodegradability of the stillage that would help in the later biological treatment stage.

After giving some time to run both the systems, the 2 reactors were compared against feed type for COD removal efficiency with respect to the OLR applied. The OLR and the COD removal efficiency in percentage were compared between the WAO_p and the raw feed. The averaged organic/stillage loading rate against the COD_{sol} removed has been compared between the wet air pretreated (CODr (mg/L)_WAO) and the unpretreated (COD_r, mg/L) for which the graph shows a relatively better performance of the earlier. Furthermore, the 2 systems showed inverse relation in COD removal against loading from 1667 mg/L d upward. Based on a simple linear regression modeling of the COD removal, a visible difference between the WAOp feed and the raw feed has been observed, which is $CODr = 0.022 \times OLR + 2.7381$ and $CODr = 0.0167 \times OLR + 0.6264$, respectively. Indeed, the difference in the regression constants as well as the regression coefficients was tested for statistical significance. Based on Wilcoxon signed rank test with continuity correction, both variables have proved to be different ($P = .036$) with an average of 13% difference in COD removal.

Despite the decline in the average COD removal at 1667 mg/L OLR, the WAO_p feed showed increased removal. The improved removal continued even on the highest OLR of 2000 mg/L that signals the opportunity to higher loading possibility of such pretreated feeds in AD while maintaining better efficiency in the COD reduction. On the contrary, the unpretreated feed showed a steep decline in COD removal with such an increase in OLR (Figure 6). In fact, physicochemical pretreatments are reported to recover more methane, thereby removing more COD in AD at various loading rates.[33,34] Although the data obtained in this study were for comparison purpose, the issues with continuous operation of the systems, air interference, and hence poor mixing contributed to the overall low performance of both systems.

Although the focus of this study was to see the effect of the stillage pretreatment on the removal of the COD in AD with respect to varied OLR, the methane content of both systems was also followed periodically. As a result, a negligible variation in percentage methane content was obtained between the digesters and among the varied OLR, ranging between 37% and 40% on average. However, a relatively better percentage of methane (up to 42%) was frequently recorded by the digester, which was fed with a WAO_p stillage. In a related experiment, the reject water from the AD testing was moved to a polishing treatment—the aerobic degradation.

Aerobic batch digestion of the postanaerobic digested pretreated stillage

Although there is a relatively recent attraction in AD, aerobic degradation used to be the main systems in wastewater treatment since earlier times. The oxygen molecule being the terminal electron acceptor, the degradation of organic molecule results in the transformation of organic molecules to cell masses and mineral byproducts, including the endogenous respiration. In such schemes, the rate of oxygen consumption stoichiometrically links the organic utilization rate to cell mass buildup in the system.[35] Aerobic systems are preferred mainly for their shorter hydraulic retention time, although the process demands many variables with a higher biomass yield resulting in a huge mass of sludge.

The mechanical supply of air, nutrient system supplement, and diversification of the consortia of organisms as degrading crew among other environmental factors used to be the process performance determinants. However, aeration cost and the resulting biomass buildup that gives up secondary waste are among the influencing parameters to prefer anaerobic systems to it. On the contrary, the application of either of the 2 systems alone is less efficient, especially in the treatment of complex industrial waste including distillery stillage.[32] Therefore, the coupled application of both systems was reported to have a desirable effect on the degradation of stillage including the application of different pretreatments. The aerobic degradation potential of the stillage after the WAO_p AD was performed in this study to see whether the final removal would be desirable to meet discharge limits, which is becoming widely and increasingly stringent.

With the perspective that the coupling of aerobic and anaerobic digestion of organic matter increases the removal efficiency,[36] the anaerobically treated stillage was moved to aerobic digesters. Aerobic reactors with glass bottles of 1 L volume were constructed to run the experiment. Rubber corks, plastic tubes, and air blowers were used to mechanically supply air, insert the feed, and remove samples, as well as for monitoring the systems, which were running in duplicates for over 8 days. During those experimental days, the COD removal of the feed, including the glucose concentration supplement, was monitored along with pH and dissolved oxygen (Figure 7).

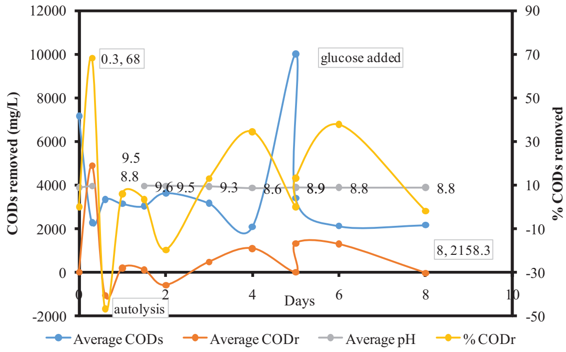

Figure 7. Monitoring the result of soluble chemical oxygen demand removal and pH in the aerobic degradation of the wet air pretreated and biomethanated molasses distillery stillage.

Although there were drops of up to 2 to 3 mg/L of dissolved oxygen in the systems during the initial stage and when glucose supplement was performed, mostly the dissolved oxygen was around 6 mg/L. Although the optimal pH was around 7 and a pH of 6 to 9 is tolerable,[35] there were times when the pH of the systems went over 9, especially during the first 3 days of the experiment. Consequently, regulating the rise in pH was done by adding more inoculum on the third day and by using a 2% HCl solution, which was added to a volume of 5 to 10 mL in each reactor.

Unfortunately, the nonbiodegradable COD remained resistant even after the aerobic degradation period was over. The best COD removal (68%) in the process was attained in just 8 hours. In fact, the final corrected COD after aerobic degradation was still around 2278 mg/L; however, it was far better when compared with the removal of the same, which was obtained from prior studies, and of course that was far better than the same COD measured after the AD was completed.[27] Such removal efficiency was superior compared with reports based on coupled treatment studies.[14,19] During the aerobic test, the least COD measured was attained within 4 days. Besides, it has to be noted from Figure 7 that the negative removals were representing the effect of the glucose solution added on the fifth day, and it has been understood that its addition had no effect on enhancing the COD removal despite the expectation that it could have done otherwise (Figure 7).

The aerobic degradation has improved the overall average COD removal efficiency of the integrated treatment in this study. Thus, the elimination efficiency attained was above 90%, which is very much closer to the distillery wastewater (DWW) discharge limit set by the authorities. The later argument is valid by considering the final stillage would be mixed with other wastewater streams in an ethanol industry. More importantly, the current integrated biological treatment has completely removed the BOD of the stillage feed. In most countries including Ethiopia and India, the DWW discharge limits are set at 250 mg-COD/L and 60 mg-BOD/L or less.

In this regard, the discharge limits can be set at 2 levels: one for connecting to the local municipal sewage system and the other for joining water bodies or just the natural environment including the land. Hence, DWW discharge limits set to linking to sewage can easily be met.

However, considering the huge water portion in the entire DWW, the degree of reduced COD reduction level together with the total BOD removal in the current hybrid treatment can meet the discharge limits on its own even without the need to join other municipal waste streams. However, not only pollution minimization reclaiming of biogas but also the recovery of water has to be given emphasis in forthcoming studies as the sector is among major water-consuming industries.[37]

Conclusions and Recommendations

The COD removal by the continuous AD of stillage alone was not sufficient either to meet discharge limits or to efficiently recover the renewable energy potential contained in molasses stillage. The percent average COD removal in the AD of a WAOp stillage was always better than the raw counterpart, which suggests that pretreatment of the cane molasses distillery stillage can improve the removal of the COD, thereby enhancing the energy recovery under optimum OLR. The application of scoria packing could help absorb substrate shock in the AD systems; thus, it stabilized the system pH. Shorter holding time, which is around 8 hours, would be enough to remove a significant portion of the COD in aerobic degradation of stillage. The stillage AD-aerobic degradation approach significantly improved organic removal, especially with wet air pretreatment. However, the regulation of the pH to an optimum level was a challenge in aerobic systems. Existing ethanol distillery industries have to adapt to locally available, robust, and sustainable techniques to recover their residue, which is produced massively, and thereby protect the natural ecosystem.

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Author Contributions

All authors contributed equally to the development of this work, from the collection and organization of data to the analysis and interpretation, including creating the analysis graphs.

Data Availability

Data on this paper can be accessed based on request.

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