AFRICA CENTER OF EXCELLENCE FOR WATER MANAGEMENT
ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
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Distillery Stillage Water Pollution Control: An integrated approach for efficient treatment and recovery

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Getachew Dagnew Gebreeyessus

A Thesis Submitted to
Africa Center of Excellence for Water Management (ACEWM),
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(Water Science and Technology)

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Declaration

I, Getachew Dagnew Gebreeyessus (GSR/9557/10), hereby declare that this research Dissertation titled *"**Distillery Stillage Water Pollution Control: An integrated approach for efficient treatment and recovery**"* has been developed by me and has not been submitted to any other institution for award of any academic qualification. The content of the dissertation has not been plagiarized and where works of other researchers have been used, they have been appropriately cited.

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A PhD DISSERTATION SUBMITTED
TO
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APPROVED BY BOARD OF EXAMINERS

This is to certify that we have read this PhD research and that in our opinion; it is fully adequate, in scope and quality, as a PhD dissertation for The Degree of Doctor of Philosophy in Water Management (Specialization in Water Science and Technology)

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Biographical sketch

Getachew Dagnew Gebreeyessus graduated in International Master of Science in Environmental Technology and Engineering from Gent University, Belgium in 2015. Later worked as lecturer, researcher and Undergraduate Programs Director at Kotebe Metropolitan University until 2018. Joining the Africa Center of Excellence for Water Management, AAU in 2018 to do the PhD in Water Management (Water Science and Technology). The following articles are published by the candidate or are under review after joining the PhD whereby the first two are from coursework while others are from the research project titled "**Distillery Stillage Water Pollution Control: An integrated approach for efficient treatment and recovery".**

1. **Gebreeyessus**, **G.D**. and Feleke Zewge. A review in environmental selenium issues. SN Applied sciences 1, Article number: 55 (2019). https://doi.org/10.1007/s42452-018-0032-9
2. **Gebreeyessus**, **G.D**. (2019). "Status of hybrid membrane–ion-exchange systems for desalination: a comprehensive review." Applied Water Science 9(5): 135 https://doi.org/10.1007/s13201-019-1006-9
3. **Gebreeyessus, G.D**., Yonas Chebude, A. Mekonen, and E. Alemayehu. Enhanced Recovery from Anaerobic Digestion of Molasses Distillery Stillage with Wet Air Pretreatment and Scoria Support. October 2020, AICHE annual meeting https://ann20-aiche.iposter
4. **Gebreeyessus, G.D.**, A. Mekonen, and E. Alemayehu, A review on progresses and performances in distillery stillage management. The Journal of Cleaner Production, 2019. 232: p. 295-307.
5. **Gebreeyessus, G.D.**, Yonas Chebude, A. Mekonen, and E. Alemayehu, Efficient anaerobic digestion of a mild wet air pretreated molasses ethanol distillery stillage: A comparative approach. HELIYON, 2020. 6(11)
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8. **Gebreeyessus G. D.**, Andualem Mekonnen, Yonas Chebude, Esayas Alemayehu. Effect of a mild iron oxide coated sand based wet air oxidation on the detoxification of molasses ethanol distillery stillage, Cleaner Engineering and Technology, submitted the revised manuscript.

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List of Acronyms

AD	Anaerobic digestion
AU	Atomic unit
BI	Biodegradability index
BOD	Biological oxygen demand
BVF	Bulk volume fermenter
COD	Chemical oxygen demand
CODsol	Soluble COD
CODt	Total COD
CWAO	Catalytic wet air oxidation
DO	Dissolved oxygen
EC	Electrical conductivity
FTIR	Fourier transform infrared spectroscopy
GHG	Greenhouse gas
HRT	Hydraulic retention time
IOCS	Iron oxide coated sand
MWW	Molasses wastewater
NALF	National Alcohol and Liquor Factory
OLR	Organic loading rate
SEM	Scanning electron microscopy
SRT	Sludge retention time
SS	Suspended solid
TDS	Total dissolved solid
TKN	Total Kejeldahal nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TS	Total solid
TSS	Total suspended solid
TVS	Total volatile solid
UASB	Up flow anaerobic sludge blanket
UV	Ultraviolet
VSS	Volatile suspended solid
WAO	Wet air oxidation
XRD	x-ray diffraction
WAOp	Wet air oxidation pretreated
WAp	Wet air pretreatment

Abstract

In spite of the increasing demand for potable and fuel ethanol, the distillery industries continue to be a major source of surface water and soil pollution, especially to developing nations, due to the release of a highly organic and complex byproduct- the stillage. Stillage contains solids, recalcitrant organics, persistent color, sulfate, chlorides, phosphorus and nitrogen, whose recovery and treatment plays a major role in environmental sustainability. In most cases, the application of single stillage treatment technology did not succeed. The objective of this study was to quantify molasses and stillage byproducts in Ethiopia and examine the feasibility of an integrated treatment approach towards sustainability. Standard methods were applied, beginning from a systematic literature review through primary data collection, stillage sampling and conducting a series of experiments. The trials were set-up based on a sound design followed by standard analytics and a statistical analysis of the results. The experiments started with determining the biochemical methane potential (BMP) test and then undertaking a mild iron oxide coated sand (IOCS) based wet air pretreatment (WAp) through anaerobic digestion (AD), adsorption and a polishing aerobic degradation.

From a quantitative characterization study, Ethiopia currently produces close to 1.8 million tons of cane sugar, with the release of over 300,000 tons of molasses as a by-product. Using cane molasses as a raw material and with the release of 431,000 m^3 of stillage into the environment, the potable ethanol industry in the country produces over 33,000 m^3 of ethanol annually. According to the BMP test run for 45 days at mesophilic condition, molasses stillage has the potential of giving 139 $NmlCH_4$/g-COD with 68% purity. A mild IOCS based WAp of the stillage sample at 3.5% IOCS loading, a temperature of 60 ºC, which was held for four hours at atmospheric pressure brought the desirable effect in detoxification. The result observed following comparison of raw and treated stillage from *E. coli* incubation at 37 ºC and 86 revolutions per minute of shaking demonstrated a statistically significant difference (p-value = 0.02) in toxicity. Successively, the consequence of a mild, IOCS based WAp on the methane (biogas) yield as well as the biochemical oxygen demand (BOD) and COD removal was compared with the raw counterpart using batch mesophilic (35±2 ºC) AD of stillage. Further, the effect of applying scoria (a highly vesicular igneous rock) support on AD process stability and performance was assessed for the first time. Consequently, a statistically significant (p-value < 0.05) difference in the cumulative specific

methane yield was obtained, which was 23.6, 24.2 and 84.0 in NmlCH_4/g-COD for the raw without scoria support, raw with scoria support and WAOp scoria supported stillage AD subsequently. The soluble COD (CODsol) after AD of stillage with scoria, without scoria and with scoria and WAp in mg/l was 30041.7, 30666.7, and 13375.0 respectively. With the WAOp and scoria supported stillage AD a BOD and COD reduction of ≈100% and 92% were achieved. These achievements are unique in stillage recovery and pollution remediation findings so far. Further, the biogas yield with respect to the reaction days (within 18 days) with a relatively stable process (average pH=7.7, 6.8 and 6.7 for pretreated and untreated stillage with scoria and without scoria) was hastened due to pre-treatment.

Regarding the adsorption trial on stillage after the batch AD, all the significant COD removals were observed after three hours of contact time for all doses of the treated scoria adsorbent. Maximum removal rate was at 0.03 mg/ml of scoria dosing and in three hours contact time whereby the final COD of the stillage did go lower only to 7500 mg/l.

In a related experiment, the effect of organic loading rate (OLR) and stillage WAp was studied in a continuous AD in scoria-packed column reactors. The removal of stillage COD was significantly better (*p*-value = 0.036), with an average of 13% difference, due to pre-treatment of stillage with an increased OLR to 2000 mg/L-d. In a further polishing aerobic treatment trial of the effluent from the stillage batch AD, a complete removal of the BOD and significant removal of COD, with an average effluent COD of 2278 mg/L was achieved. In fact, 68% of the COD removal occurred within eight hours of digestion. Despite the persistence of color, the removal of organics with bioenergy recovery from integrating the WAp, the batch AD and the aerobic remediations (˜99% COD and 100% BOD removals) of stillage appear to be a promising technique in its sustainable management while adhering to the regulators’ discharge limits.

CHAPTER ONE

Introduction

1.1. Background

The earth's freshwater availability is already inadequate and its regional distribution even makes it scarcer. In regions like the sub-Saharan Africa, lack of safe water is a deep-rooted problem exacerbated by the rapid population growth and urbanization that lead to fast economic development, to the level that some failing to maintain a clean environment and healthy ecosystems. Ecosystem productivity, food and energy are interlinked whereby ecosystem changes may be caused by slight water pollution. Mankind is the key player in affecting water and the environment that in turn impacts own lives. These brought water issues top on the list of development, urbanization and related agenda (Cosgrove & Loucks, 2015; Hand & German, 2018).

Fast urbanization and population growth drove industrialization such as the expansion of food and beverage industries, especially in developing nations, whereby ethanol factories can be mentioned. Sugarcane molasses ethanol production is on the rise even globally, especially following the notion on sustainability advantage of biomass-based energy production. The national situation is not any different, using cane molasses as a raw material, the potable ethanol industry alone produces over 33,000 m^3 of ethanol annually. The vast majority (80-95 %) of this production is technically by fermentation coupled with distillation processes (Latiff, 2011). However, this upsurge has become a vital concern regarding waste management policy that again depends on setting of standards, enhancing regulations and the technological advances.

Ethanol production gives rise to the release of vast quantities of complex distillery wastewater. For the production of a liter of cane molasses-based ethanol, it requires 4-5 kg of molasses as feed, with the release of 13 liters of stillage (Wilkie, Riedesel, & Owens, 2000). If not properly managed, the environmental problems from this particular residue are wide by impact, which include aquatic ecosystem toxicity and depletion of surface water, soil pollution, public health and aesthetic blur, all posing threats to whole-of-life sustainability (S. K. Tiwari & Prakash, 2021).

Moreover, diverse contaminants often interact synergistically to magnify the water pollution, thereby threatening resilience and sustainability. Hence, the treatment of industrial wastewater is seriously becoming thought-provoking for various stakeholders, primarily in the developing world. Therefore, the issue demands for solutions based on research in all aspects of integrated water management, which include water reclamation and reuse as well as organic management for energy production, and nutrient recovery, creating a more sustainable and looked-for future (Koop & van Leeuwen, 2017). Thus, an efficient recovery of industrial or domestic wastewater does not only help with energy self-sufficiency and decrease water pollution, but the use of biomass energy source also plays a considerable role, even in climate change mitigation thereby favoring fresh water availability (C. Xu, Nasrollahzadeh, Selva, Issaabadi, & Luque, 2019).

Concerning minimization of the environmental pollution due to stillage and the related technological gap, several research were conducted globally, mainly to remove COD and color (Lucas Tadeu Fuess et al., 2017; Sarat Chandra et al., 2014b). Most also focused on the bioremediation approaches to treat stillage, including the AD and aerobic breakdowns. Color removal research had applied physicochemical treatments including precipitation. However, the toxic and recalcitrant nature of stillage considerable limited its biodegradation resulting in lower treatment efficiency; mostly falling less than 80% in terms of the COD removal. In a related case, the AD of this acidic and corrosive biomass resulted in an average of 74% COD removal (Lucas Tadeu Fuess & Marcelo Loureiro Garcia, 2014).

Results of previous studies also showed that the use of single techniques, either the biological or wet air oxidation (WAO) as independent stillage treatments were inefficient (Kuhad & Singh, 2013; Luan, Jing, Piao, Liu, & Jin, 2017; Mantzavinos & Psillakis, 2004). Thus, ultimately the application of an integrated approach for the treatment of complex wastes like stillage looks to be promising (Ganesh M. Bhoite & Prakash D. Vaidya, 2018a). Consequently, combined treatment methods are largely recommended to tap bioenergy potential of stillage and minimize pollution including some form of pre-treatments.

Advanced oxidation techniques, mainly WAO has become a promising technology, especially under subcritical operations, to pretreat high solid wastewaters such as stillage due to its effect on COD and toxicity reduction (Luck, 1999). In a subcritical WAO the COD conversion is always lower than 70% (Luan et al., 2017), making it an ideal feed for the later AD as an environmentally friendly and energy saving alternative. Either homogeneous or heterogeneous catalysts can be applied in WAO including in-situ generation of the catalysts (Ganji, Doyle, & Ibrahim, 2011) depending on the type of operation sought, catalyst recovery and reuse, cost, product separation and the subsequent unit process. However, the milder application of WAO demands cheap, effective, non-toxic as well as resilient catalyst (Al-Atta et al., 2018). Accordingly, the current study used an iron oxide coated sand (IOCS) as adsorptive-catalyst, which is cheap and locally available material with no residual effect to following biodegradations.

Following the IOCS based mild WAO pretreatment of the real stillage sample, this study compared its effect in a batch and continuous AD with and without scoria support that brought a significant difference in COD removal as well as biogas recovery. The effect of aerobic degradation and scoria adsorption of the effluent from the batch AD was also evaluated for the final COD level of the effluent. Therefore, the current study aimed to develop a coupled technology consisting of the mild IOCS based WAO pretreatment, AD, adsorption and aerobic degradation.

1.2. Statement of the problem

The increasingly urban world population growth and industrialization elevated the fresh water demands while increased wastewater discharge negatively impacting surface and ground water quantity and quality. Such pollution resulted in severe consequences to downstream users and the environment at large which again affect aquatic life and aquatic products as well as public health that kept worsening (World Bank, 2006). Regionally, Uganda and Ethiopia, for instance, are the least on the list of the 96 countries concerning the safe management of water (The World Health Organization & The United Nations Childrens' Fund, 2017). Consequently, sound water management is demanding in those countries where urban rivers and urban inhabitants are a priority due to the urban concentration of the industries.

The rivers in Ethiopia's capital city are serving as open channels for the diffused and point-source wastewaters discharged into it without treatment. In relation to this, it was stated that 80% of the industries in the country are located in the metropolis, whereby 90% of them, including the distilleries, directly discharge their waste to streams and rivers. Among the 12 medium and large-scale potable alcohol industries in the country, 10 (83%) are located in Addis Ababa (Ethiopian Central Statistics Agency & Ministry of Finanace & Economic Development, 2013; Yohannes & Elias, 2017). Thus, based on estimates, survey results and some numerical analysis the ethanol distillery industries alone contribute about 0.02% of the city's daily wastewater by volume with its complex and undesirable nature.

The nature of stillage, especially its recalcitrance, together with technological gaps are contributing in the inefficiency of its management. Characteristically, cane molasses based ethanol distillery discharges a COD of 83000mg/l of stillage on average (Wilkie et al., 2000). Though considered as environment and energy benefiting process, the AD of stillage removes 75% of the COD on average (Lucas Tadeu Fuess & Marcelo Loureiro Garcia, 2014). Thus, the COD of stillage after AD reaches 21000mg/l on average. For some the COD of stillage after AD even remains between 21 and 50g/l (L. T. Fuess & M. L. Garcia, 2014; Wilkie et al., 2000) which is far greater than discharge limits (250mg/l COD) for EPAE. In addition to that, the dark brown color intensifies with AD and the recalcitrant organics in stillage complicates its AD (Ganesh M. Bhoite & Prakash D. Vaidya, 2018b; Sarat Chandra et al., 2014a).

A further aerobic combination did not even achieve above 88% efficiency (Malik et al., 2014). Aerobic systems alone do not appear successful either as it is characteristically incurring aeration cost, resulting in excess sludge. Recently, thermal or membrane based zero liquid discharge is being installed for stillage treatment technology, which seems attractive from avoiding non-compliance penalty perspective than sustainability. Additionally, it is typical of high cost and energy intensiveness that intensify as more water is recovered. The technology is not terminal in itself as the solidified stillage still remains to be managed (Maiti, Kane, Pandit, Singha, & Maity, 2021; Tong & Elimelech, 2016).

In this regard, increasingly stringent environmental regulations are forcing distilleries to either improve existing treatment, if any, or explore alternative methods of effluent management (Satyawali & Balakrishnan, 2008). The fact that the continuing stillage environmental pollution and the failure to meet discharge limits informs that there is a technology gap that has to be fixed through an ongoing study.

1.3. Research questions

1. Can the molasses and molasses ethanol distillery stillage biomass potential of Ethiopia be quantified?
2. Can a mild, IOCS based WAO pretreatment significantly improve the biodegradability of a molasses ethanol distillery stillage by removing toxicity?
3. Is there a significant difference in biogas production as well as stillage COD and color removal due to wet air pre-treatment?
4. Will the integration of WAO, AD and adsorption or WAO, AD and aerobic degradation improve COD, color and toxic removal efficiency and which one would be more efficient?
5. Can treated scoria adsorbent significantly reduce stillage COD and color after AD?

1.4. Objective

1.4.1. General objective

The general objective of this study was to develop and evaluate the treatment efficiency of an integrated IOCS based mild, WAO followed by AD, adsorption or polishing aerobic treatment of a molasses ethanol distillery stillage.

1.4.2. Specific objectives

1. To estimate quantity of molasses and characterize molasses distillery stillage nationally
2. To evaluate the effect of a mild, IOCS based WAO on the detoxification of molasses ethanol distillery stillage
3. To determine the COD, BOD and color removal efficiency from AD of stillage after WAO pre-treatment
4. To determine the coupling efficiency of a WAOp stillage AD and aerobic degradation
5. To evaluate effect of post AD stillage scoria adsorption on COD and color removal

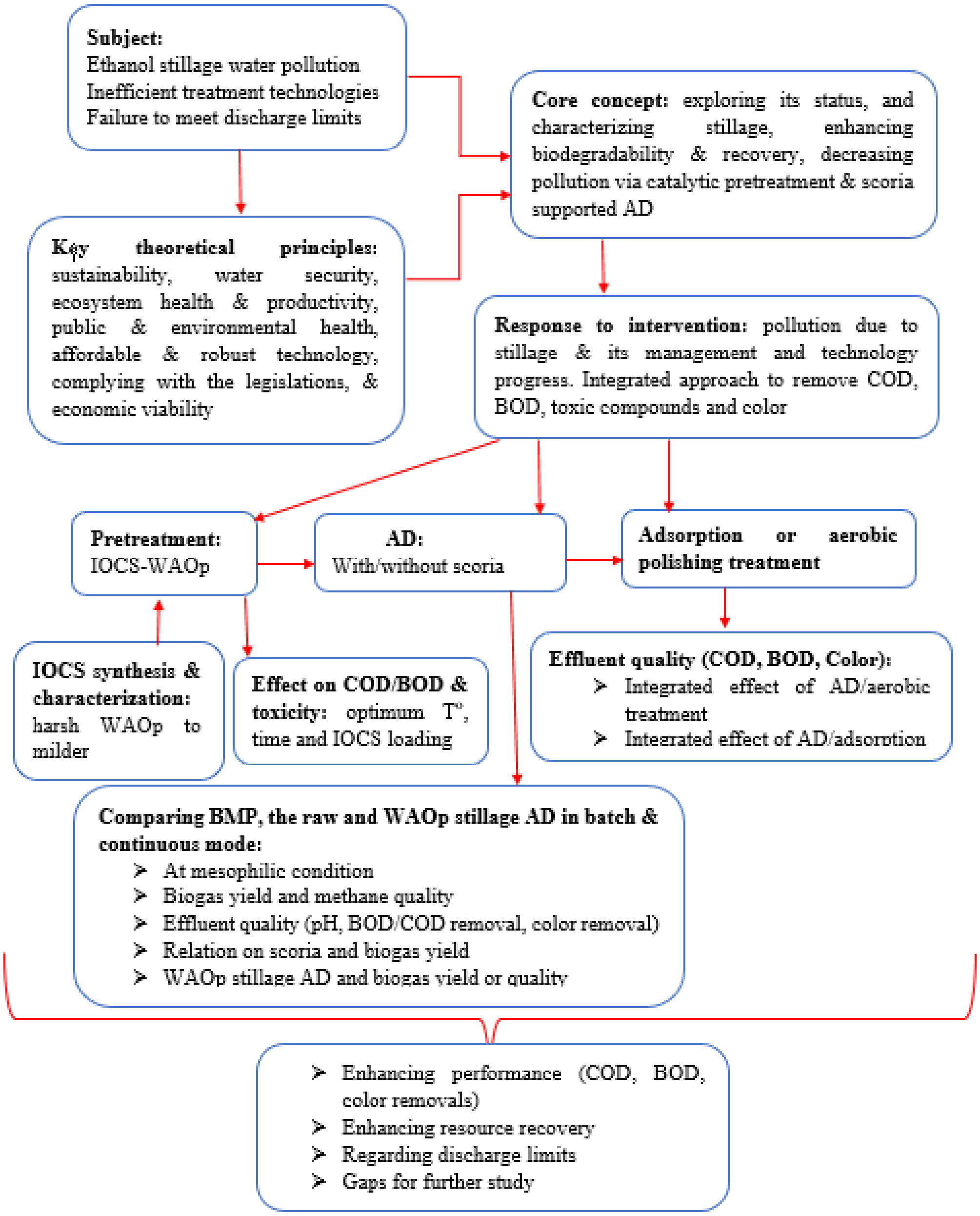
1.5. Significance and scope of the study

This study aims to generate technology as well as knowledge in the treatment of molasses-based distillery stillage for the ever-increasing distilleries in Ethiopia and elsewhere. It adds to the effectiveness of distillery stillage integrated treatment using catalyst and a natural support media. As a societal impact this study will contribute to the increasing alcohol industries towards meeting discharge limits set by the regulatory agencies. In doing so all that urban waters-mainly rivers and streams- are protected from being polluted from such point source pollution while fostering ecosystem productivity. In addition to that, gaining energy and soil nutrient after the AD will make a huge contribution to the sustainability of ethanol factories whereby the potential reduction of GHG emission and hence their ecological footprint will be significant. Further, this comprehensive work will contribute to reconcile potential conflicts between energy and environmental policies. Moreover, the research community and academia will benefit from such advances in knowledge and technology towards possible commercial scale application.

The scope of the current study is limited to laboratory scale, whereby the quantified and characterized cane molasses stillage pass through WAO to reduce COD, toxicity and hence enhance its biodegradability which is followed by an AD. The anaerobically digested stillage is then moved on to batch adsorption tests where adsorbent dosage and contact time were optimized. A polishing aerobic degradation experiment was also run to further remove stillage BOD and significantly minimize the COD. Lastly, the integral effect of treatments is evaluated against COD and BOD removals as well as toxicity and color reduction.

1.6. Project theoretical conceptual framework

There were four main packages of objectives in this study, i.e., testing the effect of IOCS on WAO, testing the effect of WAO in the subsequent treatments, evaluation of the later AD treatments and effect of polishing on effluent quality. This study was intended to be performed in phases as shown in figure 1A and 1B.



Subject:
Ethanol stillage water pollution
Inefficient treatment technologies
Failure to meet discharge limits

Core concept: exploring its status, and characterizing stillage, enhancing biodegradability & recovery, decreasing pollution via catalytic pretreatment & scoria supported AD

Key theoretical principles: sustainability, water security, ecosystem health & productivity, public & environmental health, affordable & robust technology, complying with the legislations, & economic viability

Response to intervention: pollution due to stillage & its management and technology progress. Integrated approach to remove COD, BOD, toxic compounds and color

Pretreatment:
IOCS-WAOp

AD:
With/without scoria

Adsorption or aerobic polishing treatment

IOCS synthesis & characterization: harsh WAOp to milder

Effect on COD/BOD & toxicity: optimum T°, time and IOCS loading

Effluent quality (COD, BOD, Color):
- Integrated effect of AD/aerobic treatment
- Integrated effect of AD/adsorption

Comparing BMP, the raw and WAOp stillage AD in batch & continuous mode:
- At mesophilic condition
- Biogas yield and methane quality
- Effluent quality (pH, BOD/COD removal, color removal)
- Relation on scoria and biogas yield
- WAOp stillage AD and biogas yield or quality

- Enhancing performance (COD, BOD, color removals)
- Enhancing resource recovery
- Regarding discharge limits
- Gaps for further study

A

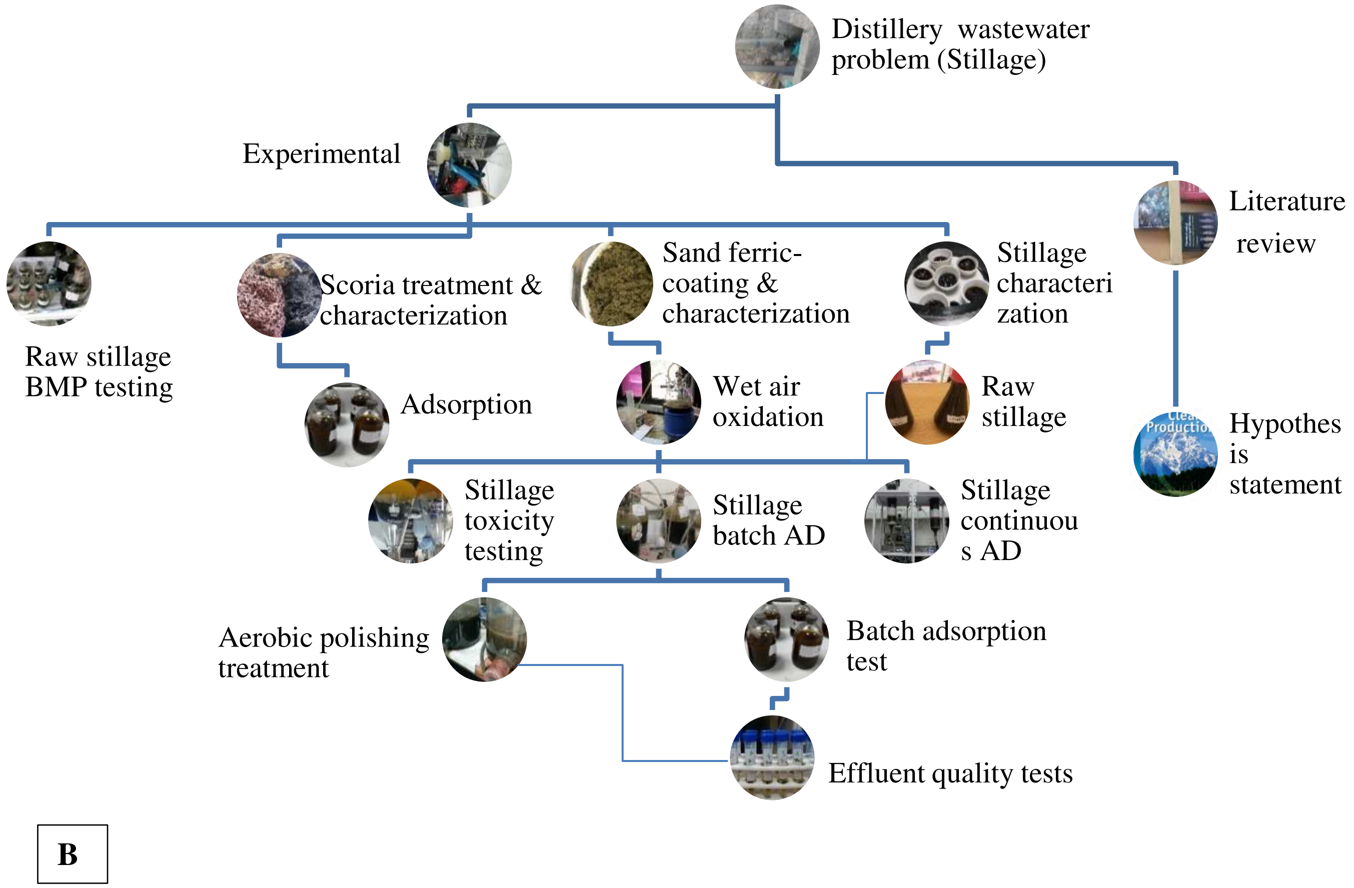


Figure 1 Conceptual framework (A) and structure (B) of the research project

1.7. Methods

The current study applied lab-scale experimental trials on a real stillage sample to see the effect of waste pretreatment, natural support materials and possible integration of remediation techniques for the organic's removal and recovery towards meeting discharge limits. The current study applied standard analytics and statistical tools to determine the variables and their effect as depicted on the project conceptual framework (Figure 1 A & B).

1.8. Findings

The current study determined the stillage production potential of Ethiopia from the potable and fuel ethanol sector. The drinkable ethanol production is around 33, 000 m3 annually from consuming 4-5 kg of molasses per produced liter of ethanol. Earlier review of the pollution problem and technological gaps in stillage management, treatment efficiencies of around 80 % were reported whereby most studies used lab-scale synthetic samples. A mild IOCS based wet air treatment of the stillage brought a minimum reduction in the COD while significantly reducing its toxicity. A batch AD of the wet air pretreated and the raw stillage clearly indicated a statistically significant difference in the biogas quantity and quality as well as reduction in COD. The pretreated stillage brought an almost complete BOD removal and a significant COD reduction after AD, which 100% and 92% subsequently. After a polishing aerobic treatment of the effluent from the batch AD, the results showed a complete removal of the BOD and significant removal of COD (around 98%), with final average COD of 2278 mg/L, whereby 68% of the COD removal was achieved during the first eight hours.

1.9. Outline of the dissertation

The entire document is organized in eight chapters. Following this introductory chapter, the state of the art of distillery stillage review is presented in Chapter Two. The review is published in The Journal of Cleaner Production Elsevier. Chapter Three addresses the first research question, which is about the determination of the quantity and character of local molasses and molasses ethanol stillage discharge. The manuscript is published in the Journal of Renewable and Sustainable Energy Reviews Elsevier. Chapter Four presents the IOCS based mild WAO experiment and the subsequent toxicity testing, which is under review in the Journal of Cleaner Engineering & Technology Elsevier. Chapter Five reports the effect of the mild WAp on the AD of stillage, which has been published in HELIYON Elsevier. Chapter Six discusses the effect of coupling the pretreated stillage AD with the polishing aerobic degradation, which has been published in Air Soil and Water Research SAGE journals. Chapter Seven discusses the adsorption experiment and the last short chapter, which is chapter eight talks about the overall conclusion of the current project.

CHAPTER TWO

Literature review

This chapter is based on a publication of the literature review on the projects research area as can be referred as: G.D. Gebreeyessus, A. Mekonnen, E. Alemayehu, A review on progresses and performances in distillery stillage management, J. Clean. Prod. 232 (2019) 295–307. https://doi.org/10.1016/j.jclepro.2019.05.383

A review on progresses and performances in distillery stillage management

Abstract

An increasing - and increasingly urban - world population has led to a boom in the distillery industry for the production of ethanol as potable alcohol, industrial chemical, and bio-fuel. Distillery industries, next to the paper industries, continue to be a major source for surface water pollution due to their highly organic and complex wastewater containing recalcitrant organics, persistent color, sulfate, chlorides, phosphorus and nitrogen. Despite that, advancements in distillery stillage treatment have not kept pace with the problem. This review examines the composition and the extent of stillage discharge into the physical environment with respect to advances in stillage management. Uniquely, this review addresses the local and global environmental burden of stillage discharge and the efficiencies achieved in stillage management. Typically, this review tries to bridge the gap in information between the traditional stillage treatment approach and the most current and advanced techniques. Moreover, the challenges of applying novel techniques in stillage management and the gaps for further research are discussed. Basically, the classical anaerobic-aerobic degradation is much explored technique to treat stillage so far. Though, the integrated stillage treatment approaches which include feed modification, physicochemical/biological hybrid systems, advanced oxidation systems and the catalysis degradation processes are recently being tested. Ultimately, it has become clear that a single technology cannot be sufficient to manage stillage. Thus, energy benefiting technology that leads to a sustainable stillage management through environmental conservation is suggested.

Keywords: environment; biodegradability; sustainability; wet air oxidation; anaerobic digestion

2.1. Introduction

The world population is growing faster while the accessible and fresh water resources of the planet remain limited and decreasing, partly due to industrial pollution. Fresh and unfrozen water of the earth constitutes only 1%, the bulk of which (99% of the fresh and unfrozen) is groundwater and only 1% is surface water found in lakes and rivers. Thus, protecting or proper use of the accessible fresh water resource is demanding. It is also the basis for driving the enforcement of stringent effluent discharge limits. As urbanization and industrialization progress, water demand and usage are followed by the increase in wastewater discharge affecting the natural water quantity and quality, especially on urban rivers. Additionally, discharged wastewater puts streams, aquatic life and public health at risk (El-Dessouky & Ettouney, 2002; ElMekawy, Hegab, & Pant, 2014).

Alcohol has been produced for millennia for drinking, industrial application, as well as fuel for transport (Kharayat, 2012). Distillery industries use sugarcane molasses or other locally available sugar sources to produce mild alcoholic beverages using a fermentation process followed by distillation. Thus, distillery stillage (also known as: spent grain, alcohol distillery waste, or vinasse) is produced as an unwanted bottom product. Distillery stillage is a complex, troublesome and highly oxygen demanding industrial organic waste which pollutes the environment.

The high organic content of stillage is attributed to the presence of lignin, sugar, hemicelluloses, dextrin, resin and organic acid as well as color. The color in stillage is mainly due to the presence of melanoidin, which result from the non-enzymatic reaction between reducing sugar and amino acids through the Maillard Reaction. In addition to melanoidins, the color in molasses distillery wastewater is attributed to polyphenols, alkaline degradation products of hexoses, and caramels in varying concentrations. The COD and the melanoidins are the critical pollutants from the alcohol industry (Arimi, Zhang, Götz, Kiriamiti, & Geißen, 2014). Furthermore, melanoidin cannot be decomposed by the conventional biological treatment method (Sirianuntapiboon & Prongtong, 2000).

The distillery process technology produces about 13 (8-20 for fuel ethanol) liters of stillage on average for every liter of alcohol production. Thus, a typical large distillery which produces 150m^3/day of ethanol produces an additional 1950m^3 of stillage (McGee & Chan Hilton, 2011; Willington & Marten, 1982). By its nature, distillery wastewater is acidic, recalcitrant and contain highly organic substances that vary according to the raw material fermented (Latiff, 2011). Once released to the environment, highly colored compounds lead to reduced sunlight penetration in rivers and lakes, thereby reducing photosynthetic activity and dissolved oxygen concentrations and causing hazardous conditions for aquatic life, including fish death, suffocation, and ecological disturbances (España-Gamboa et al., 2011). In addition to oxygen depletion, stillage can also bring eutrophication of water bodies due to its plant nourishing nature (Kharayat, 2012).

Based on laboratory-scale studies on anaerobic treatment of stillage, the average final COD of the effluent remained high about 32967mg/l (Rameshwar & Chinnery, 1997).

Integrated approaches, especially those involving Advanced Oxidation Processes (AOPs) including WAO and the application of Fenton's reagent are gaining attention. For instance, the application of WAO in stillage pre-treatment has improved its biodegradability for the subsequent biological treatment while significantly removing recalcitrant organics. WAO is efficient when the process is catalyzed (Ganesh M Bhoite & Prakash D Vaidya, 2018; Sarat Chandra et al., 2014a).

More recently, the research in the treatment of distillery stillage is focusing on the integration of biological and physicochemical techniques. These studies are also integrating WAO, anaerobic degradation, aerobic decomposition and adsorption to effectively remove COD, toxic compounds and color from stillage (Aregu, Asfaw, & Khan, 2018; Ganesh M. Bhoite & Prakash D. Vaidya, 2018b; Sharafi et al., 2019).

Thus, the objective of this comprehensive review was to provide information to academia, industry, regulatory and professionals on the scale of ethanol production and stillage-related environmental problems as well as its management progress. Further, it identifies the gaps and challenges concerning discovery, scaling up as well as optimization of robust stillage treatment technologies.

2.2. Materials and methods

In this review content analysis was performed on reliable scientific data sources that include, peer reviewed journals, books or book chapters and expert-advice. Chronology was followed so that relevant past and most recent developments in stillage management are caught. The coherence of the review is in such a way that it informs the real progress of the stillage treatment, its magnitude and impact both on a local and global perspective.

Hence, 137 peer-reviewed and focused articles published during the past decades in the area of stillage management are studied for over a year, evaluated, and synthesized. Further inclusiveness of geographic extent is considered in order to keep balance on techno-economic and operational perspectives of stillage management. The center of evaluation of those stillage treatment reports includes COD removal efficiency, color and recalcitrant organics with respect to meeting stringent discharge limits as well as operating conditions.

2.3. Environmental and sustainability implications of stillage discharge

Worldwide, the management of water, wastewater and energy is challenging, particularly in food processing industries. In some developed nations effluents from such plants join the sewerage systems on their way to central treatment facilities and that rarely meets discharge limits set by regulators raising questions of water and energy efficiency (Klemes, Smith, & Kim, 2008). In this regard, countless industrial wastewater is ultimately returned to receiving water or land in developing countries without proper treatment (Thanapimmetha, Srinophakun, Amat, & Saisriyoot, 2017) and neither the regulatory nor the market system are able to control environmental pollution effectively.

From an ecological perspective, high levels of pollutants in river systems cause an increase in Biological Oxygen Demand (BOD), COD, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), color, toxic metals such as Cd, Cr, Ni and Pb eventually making such water unsuitable for drinking, irrigation and other ecosystem services (Kanu & Achi, 2011).

In the urban context, the situation is even complicated and is larger in magnitude due to the concentration of industries resulting in an unbalanced ecosystem (**Fig. 2**). Since the distillery industry is among the water intensive plants, it discharges a huge volume of wastewater back to the environment with its characteristic constituents putting urban rivers at risk.

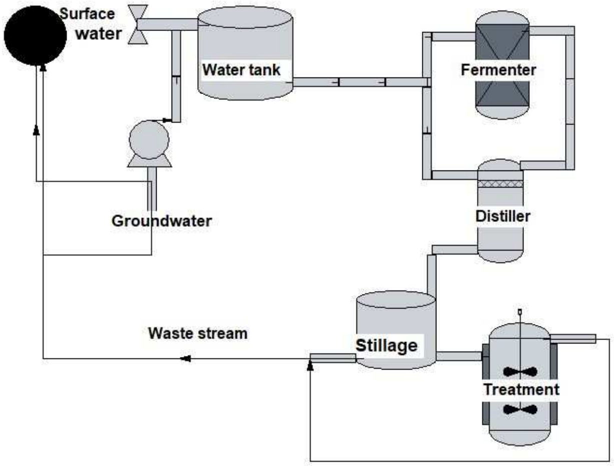


Figure 2 The connection between urban water resource and distilleries

Regardless of their economic importance, the distillery industries continue to pollute the environment and are next to paper industries by magnitude. That is partly due to their uniquely high oxygen demanding wastewater and persistent color as well as their heavy metal contents. Their impact is exacerbated due to their location which is close to surface water. The coloring agent in vinasse is an environmentally offensive and recalcitrant substance that does not only impairs light entry to water bodies but it is also an antioxidant, antimicrobial and cytotoxic (Thanapimmetha et al., 2017).

In relation to this, countries provide infrastructure and incentives based on evaluation of the wastewater treatment performances of food and agro industry, apply green branding, and certify green performances. Furthermore, the environmental concern and sustainability awareness of consumers and the general public, advocacy groups, international and local governmental and non-governmental organizations as well as eco-centric perspectives are taking their own share to improve performances (De Gisi, Petta, Farina, & De Feo, 2014).

Conventionally, list of ethanol industries typically operates their own complete on-site treatment systems in the developed countries much unlike the developing world in which their policies sometimes contradict themselves (Melamane, Strong, & Burgess, 2016). For instance, in Ethiopia the majority of the processing industries including a number potable ethanol producing factories do not comply with discharge limits. As shown in the annual report of the Central Statistics Agency of the country, the alcohol production was 54132hectoliters with an estimate of 13% annual growth rate in 2005. Thus, the country's potable ethanol production is currently estimated to be 265139.2hectoliters. For every liter of ethanol 13litters of stillage is released which results therefore in the release of 3,446,809 hectoliters of stillage every year, where all are discharged to the nearby environment without proper treatment.

Indeed, it is worth remembering that the former figure excludes the non-potable ethanol sector, which is even booming to produce a fuel mix that is influenced by the evolving policy and economic transformations in this country and elsewhere. In Ethiopia, an amount of 19 million and 804 thousand liters of which 18 million is blended with petrol, ethanol is produced annually from molasses alongside the 400 million kilograms of cane sugar production from the country's five sugar plants expanding to grow to 13 by 2020 (Ethiopian Sugar Corporation, 2016). This amount of ethanol production implies the cogeneration of over 260million litters of stillage per year from the non-potable ethanol sector alone, boldly challenging sustainability of the industry and the safety for the environment.

The driving principle to produce massive fuel ethanol is to replace fossil fuel, thereby reduce greenhouse gas emission (GHG) and cope the petroleum supply insecurity (da Silva et al., 2014; ElMekawy, Diels, De Wever, & Pant, 2013). Bio-ethanol can be produced using first, second or third generation biomass that determine its environmental advantage (Baeyens et al., 2015; Häggström, Rova, Brandberg, & Hodge, 2014; Lopes et al., 2016). Compared to the fossil fuel, the use of cane molasses ethanol can reduce GHG emission by about 40-60% with a relatively lower production cost (Manochio, Andrade, Rodriguez, & Moraes, 2017). However, experts argue that it can hardly reach the stated savings of 40-50% if full account of the problem is considered with respect to; land use changes, food-price, and the fundamental underlying causes including social equity (Ramos, Valdivia, García-Lorente, & Segura, 2016; Rosillo-Calle, 2012).

Therefore, from environmental pollution, sustainability and the aesthetic perspectives the distillery wastewater which is mainly composed of the stillage, fermenter and condenser cooling water has to be reconsidered not only in recovering the bio-resource but also the water contained in it (Pant & Adholeya, 2007).

2.4. Global ethanol production and stillage characteristics

2.4.1. Ethanol carbon source and stillage characteristics

Globally, the production of ethanol has increased with time. The USA and Brazil are the leaders in annual production, accounting for 94 billion liters of ethanol that amount is 85% of the world's total. The main reason behind is that these countries produce industrial ethanol and use it as fuel for transportation after the related policies are put in place (Krishnamoorthy, Premalatha, & Vijayasekaran, 2017; Lopes et al., 2016). Approximately 80-95% of this production were using the fermentation coupled with distillation processes (Latiff, 2011).

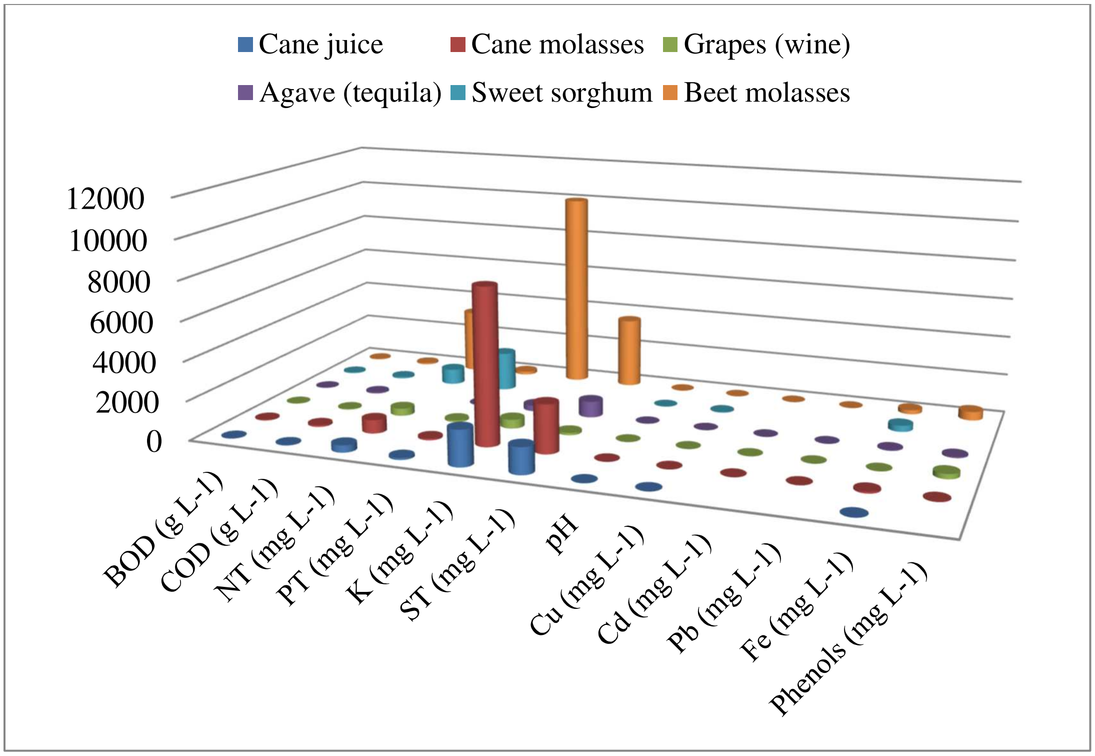


Figure 3 Composition of potable ethanol stillage (España-Gamboa et al., 2011)

Though distillery technology is the same everywhere, its stillage composition differs as a function of the raw material. The major variation in stillage composition includes the COD, N_T, P_T, K, and the S_T which affect its later management, mainly the AD. The stillage composition varies even with same sugar sources due to its processing. Figure 3 shows that cane molasses are the highest in COD content while potassium is far higher in beet molasses, though it is still high in cane molasses (L. T. Fuess & Garcia, 2017).

Even though beet molasses appears to be top in most of the stillage components among other raw materials, it is good to see from the nutrient proportion perspective to suggest AD as ultimate management. For instance, the BOD/TN ratio is the least for beet molasses while it is the highest for sweet sorghum and cane molasses, that are critical areas of nutrient or carbon supplement in biological treatment systems including AD or any re-fermentation (Choonut, Yunu, Pichid, & Sangkharak, 2015).

In fact, cane molasses are a cheap raw material for ethanol production. Cane molasses is the most applied raw material for potable alcohol production, particularly in Ethiopia. In cane molasses, the resulting stillage is high in the solids content. High solid content suggests dewatering through multiple evaporation using photo energy as stillage management alternative. In fact, dewatering through evaporation can be followed by condensation of water and densification of the cake. In that regard, determining the calorific value of the cake as biofuel and water reuse would be another researchable alternative to stillage treatment (**Table 1**), (Dirbeba, Brink, DeMartini, Zevenhoven, & Hupa, 2017).

Table 1 Typical composition of molasses based ethanol stillage (Willington & Marten, 1982)

Parameter	pH	Specific gravity	Temperature (^{O}C)	BOD	COD	Dissolved solids	Suspended solids	Ash	Organic matter
Quantity	4.8	1.05	90	45000mg/l	113000mg/l	10%	11%	3%	8%

From table one, it is clear that the organic matter and the solid content in molasses based stillage is so high and the challenge is that a proposed stillage management technology should accept that high, the solid as well as the acidic pH of the wastewater. In an earlier proximate analysis, the ash content of molasses based stillage was found to be 3.6% (Dowd, Johansen, Cantarella, & Reilly, 1994) which is even higher than the ash content indicated in table 1. In fact, such variation would be for some natural reasons, including changes in soil composition over time and anthropogenic manipulations that include the use of fertilizers. Further the components in cane molasses and stillage could even vary with time. Zhu and coworkers noted that the purity of sugarcane molasses decreased with time while the amount of colloid and ash increased (Zhu, Mao, Wang, Qin, & Wang, 2012).

Besides, the variability in the compositions of cane molasses and stillage can be ascribed to issues during cultivation and the technological process applied during sugar and ethanol production. The soil type, composition and moisture content can influence the cane produced. Afterwards, the molasses property will further be impacted by the unit process applied during cane juice clarification, evaporation, crystallization and bleaching. Also, the fermentation and distillation processes before stillage formation involves the addition of phosphate and sulfuric acid to enhance fermentation and adjust the pH of feed. As a result, the intensification of color and short chain fatty acids could occur due to fermentation. Additionally, the distillation process itself increase the solids content of the stillage (Arshad, Hussain, Iqbal, & Abbas, 2017).

2.4.2. Molasses based alcohol manufacturing process and stillage

Critical understanding of a certain product processing enables to figure out whether it is possible to redesign a sustainable process through modifying the raw material, redesigning the product or modifying the process itself as well as to minimize the environmental stress posed (Ke Wang, Zhang, Liu, Cao, & Mao, 2014). In relation to this, an alcohol manufacturing process involves mainly three steps, namely, the feed preparation, fermentation and distillation (**Fig. 4**). In an ideal distillation column, the overhead product will be mainly ethanol and the main bottom product that takes the greater share of waste in distilleries will be stillage water (Alkan-Ozkaynak & Karthikeyan, 2011).

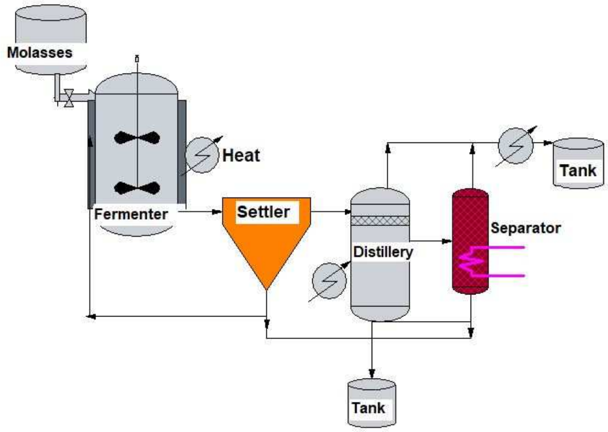


Figure 4 Process flow diagram for molasses ethanol distilleries

Figure 4 illustrates a summary of the process units to produce potable alcohol and industrial alcohol. Thus, environmental stressors exit points can be targeted for technical interventions along with the production line. Consequently, the issue with alcohol production is that distilleries discharge huge amount of organic waste that are rapidly degraded by microbes and simultaneously deplete dissolved oxygen in aquatic ecosystems (Beltran, Alvarez, Rodriguez, Garcia-Araya, & Rivas, 2001). Accordingly, that puts distillery industries, among the high carbonic waste emitters, 10,000-25, 000mg/decimeter cube of the BOD.

Besides, the focus of discharge of organic wastewater in alcohol processing plants are the fermenter and the distillery units. These units discharge a BOD of 50-60, COD of 110-190, total nitrogen of 5-7, sulfate of 7.5-9, phosphate of 2.5-2.7, chlorides of 8-8.5 and phenols of 8-10 all measured in unit of g/l (Kuhad & Singh, 2013). Bacteria and yeast are the major biotic components of this same wastewater (Bozell & Petersen, 2010).

2.5. Advances in distillery stillage treatment

2.5.1. Bio-based treatments of stillage

2.5.1.1. Anaerobic digestion process performances

Regarding enhancement of AD for COD removal and the minimization of stillage toxicity, several studies have recently evolved to include the application of some media/support. For instance, the use of natural zeolite as support media is reported by Montalvo and colleagues in a rigorous review published in 2012.

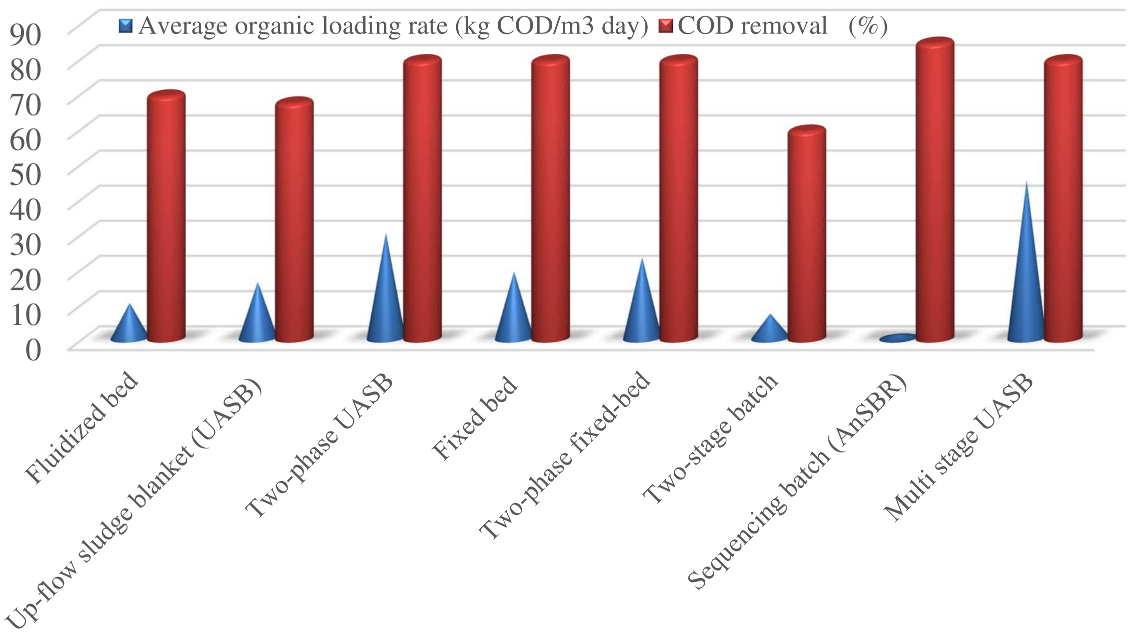


Figure 5 Performance of anaerobic digesters for stillage

According to Montalvo and colleagues (Montalvo et al., 2012), the use of zeolite in AD helped to immobilize the microbes, enhance the degradation of organics and served as ion exchange media. In addition, the application of zeolite as a microbial carrier in lactic acid fermentation of liquid stillage was found to be efficient (Djukić-Vuković, Mojović, Jokić, Nikolić, & Pejin, 2013). Contrarily, a recent study by Ayu and co-workers (Ayu, Halim, Mellyanawaty, Sudibyo, & Budhijanto, 2017) reported that use of zeolite showed no effect on palm oil mill effluent AD. This finding opposes the report by Fernández and colleagues (Fernández, Fdz-Polanco, Montalvo, & Toledano, 2001; Tada et al., 2005) who used activated carbon & natural zeolite as support to degrade vinasse.

Later, Pérez-Pérez and co-workers (Pérez-Pérez, Pereda-Reyes, Pozzi, Oliva-Merencio, & Zaiat, 2018) showed the importance of zeolite in stabilizing AD process during a synthetic swine wastewater digestion using an expanded granular sludge bed reactor. An earlier related study also noted that modifying the natural zeolite had an effect on the specific biogas yield (Milán et al., 2003). These studies highlighted the different effect of use of support may depend on the type AD feed.

Regarding the geography of anaerobic technology applied to stillage treatment, India is the most literature cited country. It has over 28 UASB, 20 bulk volume fermenters (BVF), and 13 down flow fixed film anaerobic reactors installed. By performance, the anaerobic contactors and fixed film anaerobic reactors sowed the best degradation efficiency. In addition, the mesophilic mode of stillage digestion is better in methane yield, methane productivity, and COD removal efficiency compared to the thermophilic mode but not in BOD removal (Wilkie et al., 2000).

Moreover, diverse AD technology configurations were applied to treat distillery stillage (**Table 2** and **Fig. 5**). In most studies the phased AD and fixed bed digesters showed better COD removal efficiencies. From operating conditions perspective, in Figure 4 and Table 2 it can be deduced that UASB digesters are tolerant to increased OLR, shorter digestion time; these digesters are at higher rate of performance with better COD removal. In addition, earlier studies showed the tolerance of UASB to system interruptions with uncompromised performance (Kaparaju, Serrano, & Angelidaki, 2010).

In fact, choosing between the thermophilic or the mesophilic temperature of AD will also have a huge impact on the kinetics, biogas yield and process stability even eventually affecting the sludge dewaterability of digestate (Getachew D Gebreeyessus & Jenicek, 2016; Sensai & Visvanathan, 2014; Tian, Mohan, Ingram, & Pullammanappallil, 2013). There is also interdependence among operation parameters. For example, choosing between the thermophilic and the mesophilic temperature of digestion affects the OLR that again disturbs dominance among the microbial community and hence the methane content (**Fig. 6**).

Table 2 Performance of anaerobic digestion for distillery stillage treatment and biogas production

AD technology	Organic loading rate (kg COD/m^3 day)	HRT (Days)	Biogas yield	Methane	Remova % COD	Support/Media involved	Authors
Fluidized bed	10	-	-	2L/day	70	Activated carbon & natural zeolite	(Fernández et al., 2001)
Up-flow sludge blanket (UASB)	15.34	2	380L/kg COD		68		(Saner, Mungray, & Mistry, 2016)
Two-phase UASB	16.5-44	-	-	16.5L/LCOD.day	80	None	(Shin, Bae, Lee, & Paik, 1992)
Fixed bed	3-35	1-20	-	-	>80	Polyethylene material	(Thanikal, Torrijos, Habouzit, & Moletta, 2007)
Sequencing batch	-	1-20	87L/day	-	77	None	(Tansengco, Herrera, & Tejano, 2016)
Two-phase fixed-bed	15–30	2.8		0.25–0.3L/gCOD	80	Polyurethane foam strip	(Lucas Tadeu Fuess et al., 2017)
Two-stage batch	6.5	5-21		1.9L/LCOD·day	60	None	(Ráduly, Gyenge, Szilveszter, Kedves, & Crognale, 2016)
Sequencing batch (AnSBR)	0.9± 0.2	6		0.29 L/gCOD	85	None	(Arreola-Vargas et al., 2016)
Multi stage UASB	30-60	4-8	-	20L /L. day	>80		(Yamada, Yamauchi, Suzuki, Ohashi, & Harada, 2006)

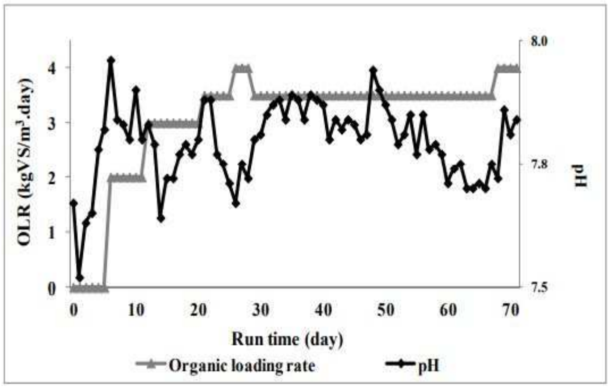


Figure 6 Interrelationship among HRT, OLR and pH in an anaerobic digester (Sensai & Visvanathan, 2014)

2.5.1.2. Further studies on anaerobic digestion and its coupling with wet air oxidation

Also, stillage treatment alternatives need to consider water recovery as water scarcity continues to be an issue. Cognizant of that, Yang et al., (Yang, Wang, Wang, Zhang, & Mao, 2017) integrated anaerobic-aerobic treatments followed by a chloride anion exchange for cassava stillage. In doing so, it was managed to completely recycle the water for the next ethanol fermentation. There was no observed difference between the use of the reclaimed and the tap water on the ethanol quality. This novel polishing treatment to stillage could even trigger ethanol production process flow diagram revision after optimization and scale up steps, contributing significantly to sustainability. During such experiment, ethanol fermentation was examined by measuring the mass of the flasks because the weight loss due to CO_2 release was proportional to the amount of ethanol produced (**Fig. 7**).

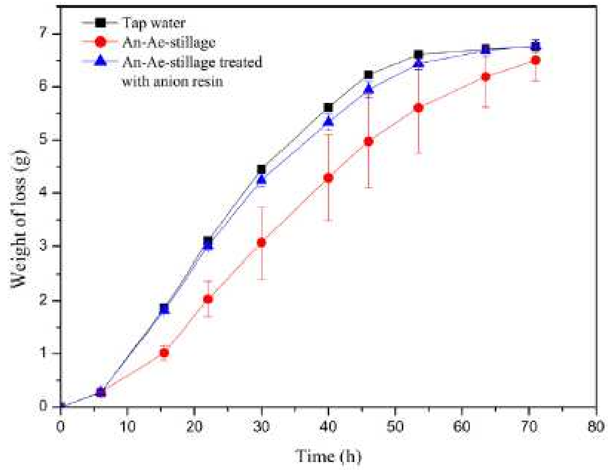


Figure 7 Comparison of the weight loss during fermentation in (anaerobic aerobic) An-Ae-stillage, An-Ae-stillage treated with resin and tap water (Yang et al., 2017)

Thus, research advances suggest that there is a need to reconsider the conventional stillage treatments, especially due to the coloring agents including melanoidin. Evidently, due to the complex nature of vinasse, the conventional anaerobic–aerobic treatment processes can accomplish the degradation of melanoidins only up to 6% or 7%. The color remains untreated, even though the COD content can be reduced by anaerobic fermentation as well as activated sludge treatments (Sirianuntapiboon & Prongtong, 2000). Therefore, advanced technologies that reduce the COD and simultaneously remove color from stillage are highly sought.

According to Kharayat , the removal of color and COD from the biological treatment of stillage did not enable to meet the discharge limits imposed by various agencies (Kharayat, 2012). Thus, coupling biological stillage treatment systems with other physicochemical methods is getting much explored. In this regard, WAO can be coupled with AD or other thermal treatments (Paradkar, Mudliar, Sharma, Pandit, & Pandey, 2016). WAO is in fact a preferred technique to treat organic pollutants at high solids concentration level, including stillage (Tarr, 2003).

Fortunately, WAO is suitable for aqueous wastes that are too liquid to incinerate but too solid to biodegrade (Belkadhi, Bouabdellah, Hammouda, & Ksibi, 2017). Furthermore, WAO is environmentally friendly compared to the conventional incineration since as it avoids emitting combustion gases into the atmosphere. More importantly, WAO degrades recalcitrant organics that would have been difficult to degrade biologically it rather improves biodegradability of the stillage for the later anaerobic treatment (Jing, Luan, & Chen, 2016). Typically, WAO process involves air or oxygen, aqueous waste, a bubble column reactor, pump, heat exchanger and separator after reaction is completed.

Regarding the mechanism and operation of WAO, initial preheating of the reactor is required to offer the activation energy (Joglekar, Samant, & Joshi, 1991). Next, the feed has to be pumped into the reactor at high pressure. Afterwards, air or oxygen is pump-injected into the reactor that already contained the fluidized feed at high pressure. After the oxidation begun, the reaction temperature could reach enough to proceed the reaction. Upon completion of the process, the pressure and temperature of the reaction need to be lowered before the gas and liquid content in the reactor are separated. The heat after cooling the reactor can be used to preheat the reactor for the next batch. The reaction mechanism in WAO involves typically three stages: initiation followed by propagation and the termination. The propagative stage releases the scavenging radicals that efficiently oxidize the organics in the system (Debellefontaine & Foussard, 2000) (**Fig. 8**).

The reaction mechanism in WAO involves typically three stages; initiation followed by propagation from an organic radical and the termination stages influenced by temperature.

Temperature mainly limits the initiation step in reaction-2. While the propagation stage, reaction-5, is rapid explained by their high-rate constant.

$O - O + R^{\cdot} \xrightarrow{yields} ROO^{\cdot}$ Reaction-1

$RH + O_2 \xrightarrow{yields} R^{\cdot} + HO_2{}^{\cdot}$ Reaction-2

$RH + HO_2{}^{\cdot} \xrightarrow{yields} R^{\cdot} + H_2O_2$ Reaction-3

$H_2O_2 + M \xrightarrow{yields} 2HO^{\cdot} + M$ Reaction-4

$ROO^{\cdot} + RH \xrightarrow{yields} R^{\cdot} + ROOH$ Reaction-5

To express the influence of WAO pre-treatment of stillage for the subsequent AD performance, the biodegradability index (BI) is used. The BI is the ratio of BOD_5 to COD and the higher the value so is the degradability. The untreated complex distillery effluent has a BI of 0.15-0.2 (Malik et al., 2014; Padoley et al., 2012; Sarat Chandra et al., 2014b). In this regard, Malik et al., reported that the performance of AD following WAO of complex distillery effluent showed better removal of COD (**Table 3**) compared to prior studies that applied ozonation or ultrasound pre-treatments. Such advances further claim the importance of WAO-anaerobic-aerobic degradation integration for further COD removal, 87.9% (Malik et al., 2014). COD removal of over 95% has been reported over a final batch digestion time of 33 days.

Further, WAO-pretreated distillery effluents' COD considered are different among studies and in most cases the pH of the stillage is unadjusted before WAO (Padoley et al., 2012). In addition to WAO, enzymatic and ultrasound pre-treatments are also investigated yielding best COD removal of over 62% from a concentration of feed ranging between 100,000–110,000mg/l that in fact followed aerobic oxidation (Sangave & Pandit, 2006).

Though COD removal and BI as a function of time and pressures sowed to have a positive relation, it can be deduced that the optimum removal of COD and the resulting increase in BI can be attained at an average temperature of WAO, 175°C. As Padoley et al., claimed in their work the desirable BI for the subsequent AD, which is 0.4, can be reached at a temperature of 175°C and 30minutes of reaction time and at 6bar of pressure (Padoley et al., 2012).

Nevertheless, better wet oxidation performance at a lower temperature and same reaction time at certain feed concentration study issues are in progress. For instance, from the use of catalysts improved WAO performance has been reported by Belkasemi and coworkers. The performance was highest using Mn/Ce oxides and Cu (II)-exchanged NaY zeolite catalysts as expressed in terms of the ratio of the residual Total Organic Carbon (TOC) to initial TOC (Khaled Belkacemi, 2000). The WAO-AD coupled treatment of stillage is also evaluated from the biogas yield and biogas quality perspectives. Based on the finding from Padoley and colleagues the methane composition as a percentage of total gas yield significantly improved for WAO pretreated sample with a peak in around 3days. Though, there was steady and slight difference in the total gas volume in favor of the biologically digested distillery wastewater.

Based on the information from table three it is evident that most coupled treatments are tested using batch reactors. In fact, most of the studies showed improved COD removal. Though, color removal is partly reported due to WAO pre-treatment of stillage followed by the AD and polishing aerobic degradations. So far, over 90% COD removal is reported from use of WAO pretreated AD of stillage that is frequently followed by the traditional oxidation. From an economic point of view a similar or even better COD removal is attained using catalysts, even at a closer pressure applied to WAO units (Ganesh M Bhoite & Prakash D Vaidya, 2018) (**Table 3**). Nevertheless, the coupling of AD, followed by WAO is not reported and it can be considered as a gap for further investigations.

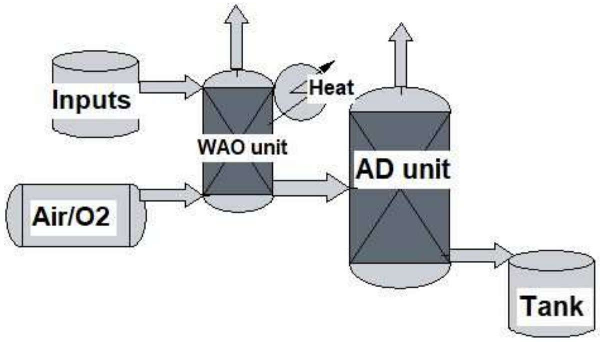


Figure 8 Wet air oxidation pre-treatment and AD of stillage

Table 3 Performance of wet air oxidation pre-treatment and anaerobic digestion for stillage treatment and biogas production

WAO pre-treatment conditions			AD	HRT (Days)	Biogas yield (l/l)	Methane (%)	Biodegradability index (BI)	Removal (%)		Post treatment	Reference
Temperature (^{O}C)	Pressure (Bar)	Time (Minutes)						COD	Color		
150-200	6-12	15-120	Batch	10	-	57	0.4-0.8	87.9	-	Aerobic degradation	(Malik et al., 2014)
150-200	6-12	15-120	Batch	-	1.06	49.6	0.88	34	-	None	(Padoley et al., 2012)
150-200	6-12	15-120	Batch	12	>1.1	64	0.55-0.88	54.75		None	(Sarat Chandra et al., 2014b)
150-225	6.9-20.7 (oxygen)	60	Batch	25	1.1	69	0.58	91		Aerobic degradation	(Ganesh M Bhoite & Prakash D Vaidya, 2018)

2.5.2. Physicochemical treatments

Although biological treatments are environmentally friendly or suitable for COD removal, physicochemical treatments such as adsorption, coagulation-flocculation, ozonation, electrochemical oxidation and electro-coagulation have been explored as complementary to stillage treatment. After biological treatments, the distillery effluents remain dark brown in color and contain some compounds which are toxic to microorganisms (Rodrigues, Fuess, Biondo, Santesso, & Garcia, 2014; Tchobanoglous & Burton, 1991). In that regard, different physicochemical techniques have been proposed to pre-treatment or post-treatment of stillage.

2.5.2.1. Chemical pre-treatments

At the heart of the stillage chemical treatment method is the chemical precipitation of soluble organic and inorganic substances, their sorption on an acceptable carrier and their flocculation with organic flocculants. The final products are sedimented flocculus and pretreated liquid. The flocculus can be separated by filtration and the filtrate can be finally treated biologically in lagoons with aeration. In this way, the limits for safe outflow into a recipient (river) can be achieved even though this alternative misses the recoverable energy benefit from stillage (Prajapati & Chaudhari, 2015).

After a lengthy process, flocculated stillage can be pumped into a filter presser where the flocculus is separated from the liquid phase. The concentration of the solid phase in the filter cake is about 20%. This mass can be composted or fed into a biogas plant. The filtrate with solid phase concentration of about 0.2% can be treated in aerated lagoons (Sajbrt, Rosol, & Ditl, 2010). Nevertheless, it results in a large configuration of treatment terrain and is mostly uneconomical. In recent times, the chemical-physical pre-treatment is being applied to fit the stillage for the subsequent membrane treatment so as to remove foulants and scalants rather than the minor chemical pre-treatments applied so far (Sankaran, Premalatha, Vijayasekaran, & Somasundaram, 2014).

2.5.2.2. Advanced oxidation treatment

In addition to the WAO pre-treatment, ozone destroys hazardous organic contaminants and has been applied for the treatment of dyes, phenolics, and pesticides (Kanimozhi & Vasudevan, 2009). Oxidation by ozone so far achieved 80% decolorization for biologically treated spent wash with simultaneous 15–25% COD reduction (Mines, Lackey, & Tribble, 2008). Ozonation also resulted in improved biodegradability of the effluent.

Ozone in combination with UV radiation enhanced spent wash's COD degradation; however, ozone with hydrogen peroxide showed only marginal reduction even on a very dilute effluent (Mines, Oglesby, & Lackey, 2009). Treatment of stillage using ozone appears to follow AD, which performed 95% COD removal (Kumar, Saroj, Tare, & Bose, 2006).

Recently, the use of WAO, especially the supercritical WAO, has emerged as an alternative in stillage treatment (**Table 4**). Moreover the application of hybrid AOPs including hydrodynamic cavitation and WAO brought synergistic effect in removing COD from distillery wastewater, including membrane technology to recover process water integrated with AD as secondary treatment (Kazemi, Tavakoli, Seif, & Nahangi, 2015).

Indeed, there are factors in using AOP in stillage treatment. In a recent study Yavuz investigated the effects of H_2O_2 concentration and pH in an electrochemical treatment experiment of a pretreated distillery wastewater. Interestingly the electro Fenton treatment showed COD removal efficiency of 92.6% with the additions of 0.3M$NaSO_4$ and 60 000 mg/L H_2O_2 at pH 4 where current density and H_2O_2 were the significant factors. Such result indicated Favorability of AOP as an alternative to stillage treatment over electro coagulation (Yavuz, 2007).

A more recent study by Tiwari and Sahu witnessed coupling of AOPs for further efficient removal of color and COD from the sugar industry wastewater that can meet discharge limits. The researchers attained removal of 98% COD and 99.5% color using chemical oxidation and electro-oxidation combined treatments. Though these methods appear efficient, the molasses based distillery stillage intensify color in the molasses through fermentation, which complicates the later management (A. Tiwari & Sahu, 2017).

Table 4 Performance of advanced oxidation processes in stillage treatment

AOPs	COD removal (%)	Color removal (%)	Comment	Reference
Electro-oxidation & chemical oxidation	98	99.5	The result is obtained before fermentation that intensifies color problem in stillage and is lab scale	(A. Tiwari & Sahu, 2017)
Electro Fenton	92.6	-	No report on color and the cost implication and it is lab scale	(Yavuz, 2007)

WAO	80.9	98	High strength initial COD, 60,000 mg/l, in the presence of catalyst and at a temperature reaching 400celicious	(Kazemi et al., 2015)
Pulse electro-Fenton	40.7	90	Lab scale	(Thanapimmetha et al., 2017)

Moreover, electrochemical methods like electron coagulation and electro Fenton have also been tested for stillage treatment though their feasibility remains uncertain. A study applied coupling of pulse electro-Fenton AOP to remove color in stillage. This novel technique offered to remove nearly 90% of color with an accompanying 40.71% COD removal in molasses wastewater at a pulse frequency of 2.5 kHz, 15.8% H_2O_2/wastewater ratio and >90minutes reaction time (Thanapimmetha et al., 2017).

2.5.3. Advantages and limitations of stillage treatment methods

The selection of a particular method to treat stillage globally depends on cost and efficiency. However, local contexts are also influencing its application. For instance, some European countries may have stringent discharge limits to be met at the expense of their trained personnel and advanced technology. In fact, earlier studies showed that a pilot-scale anaerobic membrane bioreactor can remove over 98% of the TSS from stillage with biogas recovery (Dereli et al., 2012). Thus, further optimization of membrane bioreactors is gaining attention in such context in current studies.

However, in such countries, especially in their urban areas land use could be a serious issue to be considered as limiting in selecting a particular technology. In relation to that, the scenario would be entirely different in developing countries. Nevertheless, the issue of sustainability appears to have unanimous consent that the biochemical operations may be favored so as to keep nutrient cycles in nature and to recover energy. Furthermore, the choice of a single technology may not be efficient, resulting in an integrated approach include the application of coupled WAO and AD (**Table 5**).

Table 5 Advantages and limitations of distillery stillage treatment methods

Method	Technology	Advantage	Limitation	%COD removal	Authors
Chemical	Electrochemical / Electrolysis	Efficient Feasible	Technically delicate No color removal is reported	89	(Vlyssides, Israilides, Loizidou, Karvouni, & Mourafeti, 1997)
	Ion exchange	Cost effective, Selective Good at removing color	Operational challenge Less efficient	23	(Krzywonos & Łapawa, 2012)
Biochemical	Aerobic biodegradation	Possibility of its use at high temperatures and no necessity for medium pH adjustment	No color removal is reported, Requires aeration Intensification of betaine	89	(Ryznar-Luty, Cibis, & Lutosławski, 2018)
	Integrated aerobic biological oxidation and ozonation	COD and polyphenol removal	Costly, Operation and maintenance issues	82	(Fernando J. Beltrán, Álvarez, Rodríguez, García-Araya, & Rivas, 2008)
	Microbial Fuel Cell	Electricity generation Reduced sulfate	Delicate technology	-	(Ha, Lee, Rittmann, Park, & Chang, 2012)
	Bioremediation	Sustainable and environmentally friendly, also removes color	Inefficient and delicate technology	Maximum of 60	(Krzywonos, Chałupniak, & Zabochnicka-Świątek, 2017)
	Up-flow Anaerobic Sludge Blanket	Energy gain, environmentally friendly,	Further treatment Inefficient	68	(Saner et al., 2016)
Physicochemical	Membrane separation	Efficient to meet discharge limits	Large investment cost Requires technical skill and membrane fouling,		(Prodanović & Vasić, 2013)
	Combustion	Low operating cost, Ease of operation, Energy gain, Low volume of final waste	High investment costs Emission (Sulphur and nitrogen)	Almost complete	(Sajbrt et al., 2010)

2.5. Conclusion, challenges and future research

Alcohol distillery industries continued to be an environmental challenge specially to surface water. In this regard, the scientific advances to treat stillage are slow and are less integrated with advances in agricultural waste valorization studies. Most advanced stillage treatment techniques tested so far are bound to laboratory scale, mainly due to their capital investment cost and operational complexity. Consequently, these industries continue to be environmental burden, especially in developing countries, if not worldwide, towards meeting discharge limits. Significantly, anaerobic-aerobic traditional treatments of stillage are most explored.

Recently, physicochemical approaches are being tested to treat stillage at least on a laboratory scale. Areas including the application of catalyst, cavitation chemistry by ultrasound and other advanced oxidation techniques needs further research in this regard. By coupling AOPs with AD stillage can optimally be treated so that the undesirable footprint of such industries can improve greatly. The application of physicochemical treatments, including adsorption, next to biological remediation is still valuable as color and COD remain in stillage after AD. Particularly the application of coagulation and flocculation is performing well despite its precipitate resulting in secondary waste. Therefore, the optimization as well as application of bio-based coagulants need further studies.

Ultimately, there is no single technology that can treat stillage so as to meet the stringent discharge limit as well as meeting the environmental sustainability requirements. Thus, there is a huge gap in research to be filled through an interdisciplinary approach which will develop an integrated treatment package and greatly improve the environmental performance and sustainability of distilleries in the current trend of urbanization.

CHAPTER THREE

This chapter is about objective one of the thesis based on a manuscript accepted in the journal of Renewable and Sustainable Energy Reviews as it can be referred as: Getachew D. Gebreeyessus, Andualem Mekonnen, Yonas Chebude, Esayas Alemayehu (2021). Quantitative characterization of cane molasses and molasses distillery stillage in Ethiopia. Ren. and Sus. Energy Reviews. Manuscript under revision.

Quantitative characterization of cane molasses and molasses distillery stillage in Ethiopia

Abstract

Characterization and quantification of the products and byproducts of the sugar and ethanol industries is an aspect of a sustainable natural resources management. The current study applied primary and secondary data collection as well as review of scenarios to quantify and characterize the products and byproducts of these industries. It also revealed the gaps in research and the relevant policies in discussing the alternative solutions available locally and globally. Currently, Ethiopia produces close to 1.8 million tons of cane sugar with over 300,000 tons of molasses as a byproduct. Using cane molasses as a raw material and with the release of 431,000 m^3 of stillage into the environment, the potable ethanol industry in the country produces over 33,000 m^3 of ethanol annually. Despite the expansion of sugar and ethanol industries both by the public and private owners, the environmental performance of the sector is apparently poor. These industries discharge the energy-rich “wastes streams” without proper treatment directly into the environment. Except for a few instances of the production of fuel ethanol from stillage and electricity from bagasse, the status of energy and valuables recovery from biomass in those sectors is quite lagging, which puts their sustainability at risk.

Keywords: Characterization; Environment; Molasses; Pollution; Quantification; Stillage

3.1. Introduction

In general, energy intensiveness, water consumption, biodiversity loss, and environmental pollution are big concerns in the sugar industry. Sugarcane is the feedstock for over half of global sugar production, predominantly in Africa, Australia and Oceania, and it was increasing by around 2.5 Mt or 1.5% yearly till 2004 (OECD, 2016). Despite associated debates on land use, land cover, pollution, biodiversity reduction and the ever-lower cost of sugar, sugarcane remains the only feedstock for sugar production in Ethiopia. and its cultivation continues to expand incrementally. In fact the production of cane sugar implies the release of solid, liquid, and gaseous wastes as well as byproducts (including bagasse, filter cake and vinasse), which are of input to alcohol production, and are discharged after transformation (Tena Gashaw, Mekbib, & Ayana, 2018).

Furthermore, cane molasses is the current, dependable raw material for the potable and fuel ethanol production in Ethiopia. After distillation of the fermented molasses, the primary waste stream is the spentwash, also called vinasse or distillery stillage (Stanbury, Whitaker, & Hall, 2017). Globally, the production of ethanol – whether as bio-fuel, for industrial applications, or alcoholic beverages – has increased with time. The United States of America (USA) and Brazil are the leaders in annual production, accounting for 94 billion liters of ethanol produced every year which is 85% of world production. This has come about due to these nations' production of industrial ethanol, as well as its use as fuel for transportation following the implementation of relevant policies (Krishnamoorthy et al., 2017; Lopes et al., 2016; Mikucka & Zielińska, 2020a). Global production has increased from 79 billion liters in 2008 to 94 billion liters in 2016/17 and continues to grow. The vast majority (80-95%) of this production was by fermentation coupled with distillation processes (Latiff, 2011).

The production of ethanol gives rise to the release of vast quantities of complex distillery wastewater (Mikucka & Zielińska, 2020a). For instance, the production of one liter of cane molasses-based ethanol requires 4-5 kg of molasses as feed, and results in the generation of 13 liters of stillage (Wilkie et al., 2000). It is estimated that over 30000 m^3 of potable alcohol is produced yearly in Ethiopia aside from the huge volume of fuel ethanol. The concern is therefore the environmental impact of the annual release of over 260 million liters of stillage, and which treatment strategies would be most effective in enlightening the maintainability of the sector (Getachew D. Gebreeyessus, Mekonen, & Alemayehu, 2019).

Stillage from cane molasses-based ethanol production typically has a chemical oxygen demand (COD) of 83 g/l (Wilkie et al., 2000). Even the anaerobic digestion (AD) of stillage achieves less than 75% COD reduction on average, thus failing to meet discharge standards (Lucas Tadeu Fuess & Marcelo Loureiro Garcia, 2014).

In fact, the COD of the stillage after AD remains between 21 000 and 50 000 mg/l (L. T. Fuess & M. L. Garcia, 2014; Wilkie et al., 2000) which is far greater than discharge limits; 250 mg/l COD as set by the Environmental Protection Authority of Ethiopia (EPAE). Moreover, the dark brown color intensifies with AD and the recalcitrant organics in stillage complicate treatment (Ganesh M. Bhoite & Prakash D. Vaidya, 2018b). These toxic, recalcitrant compounds are problematic in aquatic environments which receive the discharge (Sirianuntapiboon & Prongtong, 2000). For instance, heavy metal compounds present in stillage, including Cr, Cd, Hg, are known to be toxic to fauna and flora (Srivastava et al., 2017).

In this regard, unless the pollution potential of the sugar/ethanol industry is measured or quantified it will be impossible to control or manage the consequences. On one hand, the costs will emanate from the resulting air, water or soil pollution. On the other hand due to the lost value of byproducts, which could have helped for further productivity and thereby close the economic cycle (C. Xu et al., 2019).

Despite the studies reported so far, there is no clear view of the sugar and ethanol sector in the country; no full account of the situation has been quantified, characterized and discovered. The purpose of the current work is to bring the clear picture of the status of potable and fuel ethanol production in the country into image, to gauge the environmental performances of the sugar ethanol industry, as well as to review the energy potential of the biomass that is being lost in vain by the same sector. Moreover, this work is aimed at giving the basis for the ultimate proper management of the biomass resources based on a clear roadmap and in a sustainable way, which has to be backed by relevant policies and strategies as well as techno-legal grounds.

Thus, the current paper aspires the redirection of policies regarding resource recovery and waste management toward preventive, adaptive and reactive responses, bringing greater sustainability. Uniquely, the legal and technological dimension of the sector is explained based on a comprehensive methodology to bring a better impact.

3.2. Methodology

3.2.1. General

A mix of methods was applied to obtain a useful and meticulous data on the quantity and character of sugar and ethanol products and byproducts, which include a literature survey, production site inspection, official letter and email correspondences as well as experimental and numerical analysis. Consultations with experts and authorities has been done, which include focused group discussions (**Fig. 9**).

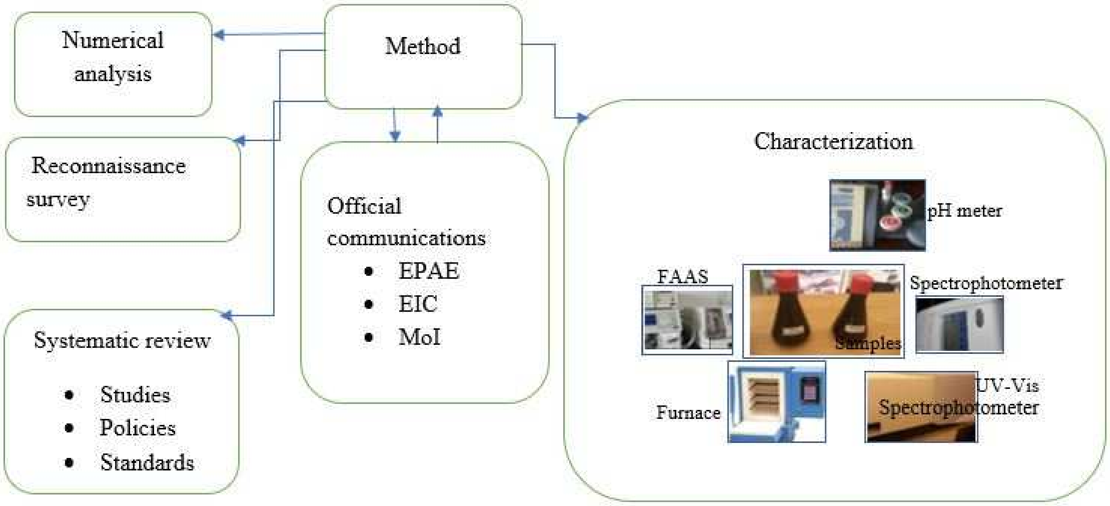


Figure 9 Simplified diagram of the methods and materials applied in the current study

3.2.2. Literature review and production site survey

Though the scope of this work appears to be geographically bound to Ethiopia, the context of sugar and alcohol production as well as environmental pollution policies and the effluent regulation systems are global by scale. Consequently, the local sugar ethanol production situations are elucidated from universal perspectives and scenarios. Hence, an organized analysis of the most recent and reliable publications was performed. Review of literature regarding cane sugar and ethanol production, quantification and characterization, the related global and national environmental performances and policies, as well as sector sustainability scenarios were performed. The review was done using the online tools that include Science direct, Scopus database, ResearchGate, and the Google scholar. Filtered by relevance and timeliness, the diversity of the journals was duly considered and the Harzing's Publish or Perish (©1900-2019 Tarma Software Research Limited) application was used for sorting purposes.

Production site observation and taking the relevant data using checklists were also performed. Hence the country's main potable ethanol plant, one which is publicly owned was visited to collect raw material, production system and environmental performance data. Additionally, the production capacity, the waste streams and overall management status was viewed.

Similarly, data on the status of the national sugar and ethanol production were obtained from the respective institutions. Moreover, three concerned authorities were approached regarding the details; the Ethiopian Investment Commission (EIC), the Ministry of Industry (MoI) of Ethiopia and Environmental Protection Authority of Ethiopia (EPAE) that included access to policy documents. Indeed, much of the data were obtained freely from the world wide web. The ethanol and sugar investment data were obtained from the EIC. The list of existing and operational potable alcohol industries was accessed from the MoI of Ethiopia.

3.2.3. Characterization of stillage

3.2.3.1. Sampling, test procedures and materials

The characterization of the stillage was performed in the laboratory using standard methods of testing and under different arrangements. Upon obtaining permission, composite samples of stillage have been obtained first from one of the production sites of the National Alcohol and Liquor Factory (NALF). Within two hours from sampling, the stillage sample was stored in a refrigerator set at 4 ^{o}C till subsequent subsampling and analysis. The characterization work has been performed using standard methods, which was mainly based on the American Public Health Association (APHA), Water Works Association (WWA) and Water Environment Federation (WEF) (APHA; WWA & WEF, 1999).

Microwave Plasma Atomic Emission Spectroscopy (Agilent 4200 MP-AES) was used to determine the metals, oven and furnace to evaporate water and organics, LAMBDA, 950 UV-Vis Spectrophotometer from PerkinElmer to determine phenol, and JENWAY 7305 spectrophotometer to measure the COD and color (**Fig. 9**). The laboratory tests on the selected parameters were performed in replicates and the calibration of equipment was done using standards.

Regarding metals analysis, pretreating a 10 ml subsample of the stillage obtained has been performed by diluting the sample ten times with distilled water (DW) using flask. Following dilution, the organic components in the sample were removed by digesting the sample using HNO_3

on a heating oven (set around 150 ^{o}C), which was performed five times in a safety cabinet. The pretreated sample has been put in a refrigerator for five days until instrumental analysis.

The phenol analysis has been performed in the lab under the Nutrition Center of the Addis Ababa University. To do the phenol testing, the subsample of stillage has been diluted using falcon tubes of 10 ml volume. The sample was pretreated before analysis. Plotting the reference curve using the known concentration of a reagent was done earlier. The stillage phenol content was quantified using the famous Folin-Ciocalteu test and the gallic acid, that are normally used as standards (Keskin-Šašić et al., 2012). The instrument LAMBDA 950 UV/Vis spectrophotometer with a Peltier-controlled PbS detector was used. To do that, a 0.02 ml of the liquified subsample was transferred into tubing containing 1.0 ml of a 10th watering down of the Folin-Ciocalteu's reagent. After ten minutes of waiting time, a 0.8 ml of Na_2CO_3 in DW (7.5% w/v) was mixed with the subsample. Then the tubes were stood still at room temperature for half an hour before taking the optical density measurements at 743 nanometers (nm). The total phenol was stated as gallic acid equivalents (GAE) as mg/g of the stillage. The quantity of polyphenols in the samples was derived from a standard curve of gallic acid in the range of 0.3 to 1.5 mg/L.

Biological oxygen demand (BOD) and COD analysis were performed at the Center for Environmental Sciences Laboratory, College of Natural land Computational Sciences, Addis Ababa University. Sub samples of molasses ethanol stillage were centrifuged at 9000 rpm for the soluble COD analysis, which was diluted before the spectrophotometer absorbance reading at 600 nm. Indeed, calibration of the instrument with known concentration of the BOD & COD samples has been performed before the testing. Brookfield DV-E Viscometer was used to measure the absolute viscosity of the stillage.

3.2.3.2. Standard preparation for the colorimetric chemical oxygen demand tests

Preparation of the standard for the spectrophotometric COD determination was made using a known concentrations of potassium hydrogen phthalate (KHP). DW was used as blank. Concentrations of 50, 100, 200, 300, and 400 mg/l were prepared after weighing a known amount of KHP and after preparing the stock solution. To prepare a 1000 mg/L COD standard stock solution, 850 mg of the dried (dried at 120 °C overnight) KHP was taken and dissolved in 1000 ml of DW. Afterwards, optical density (OD) reading was taken at each point of the concentration and a standard curve of the absorbance read against the concentrations of the KHP in mg/l was developed. The goodness of fit was $R^2 = 0.998$. Then the slope of the equation of the trend line was determined for the later analysis of the unknown samples (Getachew Dagnew Gebreeyessus, Sreekrishnan, Mekonnen, Chebude, & Alemayehu, 2020).

3.2.3.3. Biological oxygen demand analysis

The BOD of the stillage was analysed based on the difference in the dissolved oxygen (DO) concentration of the samples within three days. The DO buffer preparation was performed earlier using DW, aerator pump and air diffuser. Before aeration begun, Mg SO_4, Fe Cl_3, Ca Cl_2, a phosphate buffer which is comprised of KH_2PO_4, K_2HPO_4, $NaHPO_4.7H_2O$ and NH_4Cl, one ml/l each, were added to the DW. Afterwards, the solution was aerated for over 12 hours. Diluted samples of stillage were titrated using 0.025 normal $Na_2S_2O_3$ and a starch indicator in a BOD bottle (Getachew Dagnew Gebreeyessus et al., 2020).

3.2.3.4. The stillage solid analysis

A homogenous stillage sample was sufficiently mixed and a subsample of 100 ml was taken using a beaker. The sub-sample was continuously kept stirred using a magnetic stirrer till further subsampling. In the meantime, an aluminum foil was torn into rectangular pieces using a scissor. The foil cut into further pieces was molded into cups. The aluminum cups were then labeled. Part of the sludge sample was centrifuged at 1000 revolution per minute for 15 minutes to take the expressed water sample and subsequently analyze the dissolved solid (DS).

In each case, a 10 ml samples were taken using pipettes and were poured into the aluminum cups after weighting the cups themselves first. The sample within the cup was then dried at a temperature of 105±1 ^{0}C for 3 hours in an oven for total solid (TS) analysis. Later, following the cooling of samples brought from the oven using a desiccator, they were weighed using analytical balance. After weighting the dried sample and firmly folding each cup to avoid sample loss, it was then moved to a combustion furnace where it burns for an hour at a temperature of 550 ± 2 ^{0}C to determine the volatile and fixed solid (VS/FS). Then the samples after the ignition were cooled in a desiccator again before weighting, all performed according to method 2540 (A-F) of APHA standards (American Public Health Association, 2000).

3.2.3.5. The color analysis

Among the standard methods to determine color as outlined by APHA (Methods 2120B-D), 2120C was selected in this study since it is recommended for highly colored industrial wastewaters. Accordingly, the transmittance values in percent were obtained using a spectrophotometer (xenon lamp light source) and the 10 ordinates were selected for a fair accuracy whereas the spectral bandwidth was set at 5 nm (Getachew Dagnew Gebreeyessus et al., 2020).

3.2.3.6. Nitrate and phosphate analysis

In this study, NO_3 content of stillage was also quantified. To do so, two reagents ('A and B') and a standard solution have been synthesized for the spectrophotometric determination of nitrate for taking OD readings at 410 nm. Reagent A has been prepared from the mixture of 5 g of salicylic acid ($C_7H_6O_3$) and 100 ml of concentrated H_2SO_4 whereas reagent B was just a 2N NaOH. Before conducting the procedure, a standard was prepared like for other tests which is, in nitrate case, the determination of a straight line of absorbances versus concentrations of a solution of 1.37 g of $NaNO_3$ in a liter of the DW.

By procedure, a 100 µl of the sample was taken and mixed with the 400 ml of the reagent A and the mixture was incubated at 25 ^oC for 20 minutes. Following incubation, 9.5 ml of reagent B has been added, the mixture was vortexed and cooled before taking OD reading.

Regarding phosphate analysis, two solutions (A and B) were synthesized. Solution A was obtained through dissolving 25 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}$) in 300 ml of DW. Solution B has been made by dissolving a 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 a concentrated HCl solution. Later, the solution was cooled to room temperature and mixed with solution A making it up to one liter. A standard was also prepared by mixing 219.5 mg of a hydrous KH_2PO_4 in DW which is equivalent to 50 µg of PO_4^{3-}P. The test was followed by taking one ml of the sample and 0.25 ml reagent and vortex mixing before resting it for 10 minutes at room temperature. The OD reading was then at 470 nm using the same spectrophotometer (Getachew Dagnew Gebreeyessus et al., 2020).

3.2.3.7. *Data analysis and graphing*

The numerical analysis was performed to quantify products and by-products, including molasses and stillage/vinasse. To do so excel has been used. ArcGIS was used to locate and map the sugar and ethanol process factories in Ethiopia.

3.3. Results

3.3.1. Cane sugar and molasses production and challenges in Ethiopia

Based on official industry data and subsequent numerical analysis, Ethiopia produces a total amount of cane molasses close to 3.8 E5 tons/year, which is contributed by eight sugar factories distributed in different parts of the country. Disproportionately, the sugar factories are located in the center, west and the far north of Ethiopia (**Fig. 10 & Table 6**).

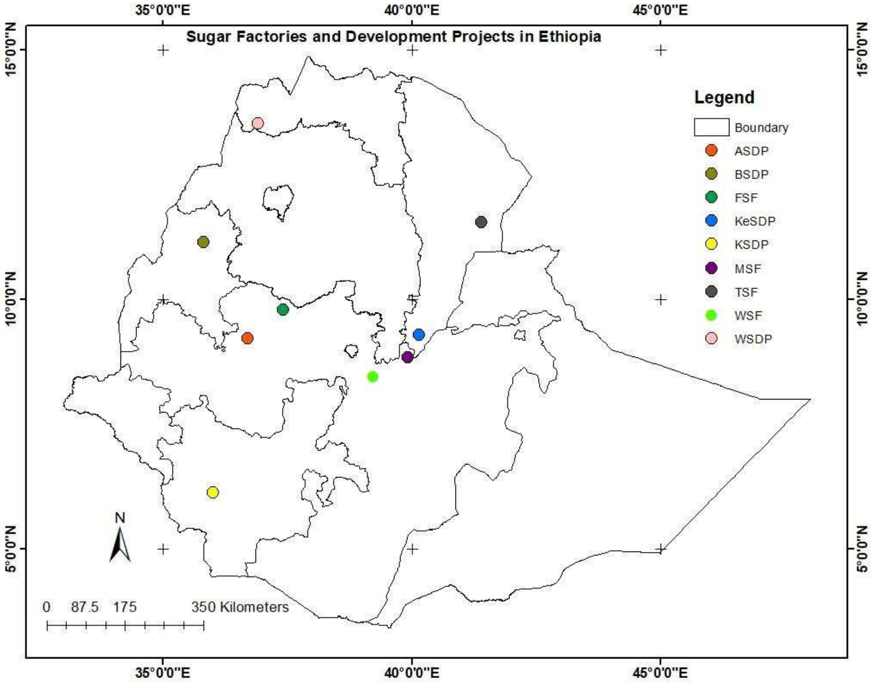


Figure 10 Map of sugar production factories in Ethiopia

ASDP= Arjo Sugar Development Project; BSDP= Beles Sugar Development Project; FSF= Fincha Sugar Factory; KeSDP= Kessem Sugar Development Project; KSDP= Kuraz Sugar Development Project; MSF=Methara Sugar Factory; TSF= Tendaho Sugar Factory; WSF= Wonji Sugar Factory; WSDP= Wolkyiet Sugar Development Project

The production of cane sugar in Ethiopia goes back to the 1950s. However, aggressive expansions have been made since 2014 with a rapid growth until recently. So far Fincha and Tendaho sugar factories are the biggest sugar producers with their respective magnitude of the molasses release. Wonji Shewa sugar factory is still expanding. Only three of the eight are ethanol producing, which are Metehara, Fincha and Arjo Diddiesa sugar factories (**Table 6**).

Since, 45 % of the molasses products are fermentable sugars (Lavarack, 2003), almost 1.35* 10^5 tons/year can be fermented in to alcohol. Accordingly, 50 % mass of alcohol can be produced from the sugar in every unit mass of cane-molasses on average, based on figures from earlier experiments and theoretic calculations. Thus, approximately 1.07* 10^5 m^3 of alcohol, with density correction, can be produced every year. However, the current sugar production capacity considered in this calculation does not include the expansion planned by most of the existing factories as well as the future commencement of five more plants which further elevates the alcohol and hence the stillage production of the nation (**Table 1**).

Table 6 Annual sugar and molasses production in Ethiopia

Name of sugar factory	Location /regional state	Year commenced	Expansion considered annual sugar capacity	Current annual sugar production in tons	Annual molasses production in tons	Status/remark
Wonji Shewa	Oromia	1954	222,700	17,400	2,958	Expanding
Metehara	Oromia	1970	136,692	130,000	22,100	Ethanol active
Fincha	Oromia	1999	270,000	270,000	45,900	Ethanol active
Tendaho	Afar	2014	300,000	240,000	40,800	Capacity: 124,068 tons of molasses annually
Kessem	Afar	2015	260,000	156,000	26,520	
Arjo Diddiesa	Oromia	2015	348,253	348,253	59,203	Ethanol active
Omo-Kuraz II	Southern Nation, Nationalities & Peoples	2017	302,000	302,000	51,340	
Omo-Kuraz III	Southern Nation, Nationalities & Peoples	2019	301,500	301,500	51,255	
	Total		1,839,645	1,765,153	300,076	

3.3.2. Quantification of the potable ethanol and stillage production in Ethiopia

Depending on cane variety and the growing environmental factors, roughly 2.5 % (1:39) of the cane harvested or 17 % of the cane sugar produced (1:5) ends up as molasses that contains 45 % fermentable sugar (Lavarack, 2003; Pérez & Fujita, 1997). By composition, the sugar, water and ash are major constituents of cane molasses that made molasses to be a good raw material for further food, drink or chemical production which has to be given attention (Clarke, 2003).

The source of alcoholic liquor supply in Ethiopia is both domestic and import due to the unsatisfied demand in spite of the sharp rise in local production, especially after 2005. Domestic production of alcoholic liquors during 1997-2000 was almost constant with slight fluctuations. During this period production ranged from 2,913,000 liters to 3,056,200 liters. But after the year 2000 the supply has increased consistently up to the year 2005. The production level, which was 2,955,200 liters, has reached to about 3,400,000 liters in the year 2002. By the years 2003, 2004 and 2005, the production has increased to 4153800 liters, 4,800,200 liters and 5,413,200 liters respectively. The annual average increase in the past five years was about 13 %. In spite of the annual average increase, currently it reached over 33,133,569 liters (**Fig. 11**).

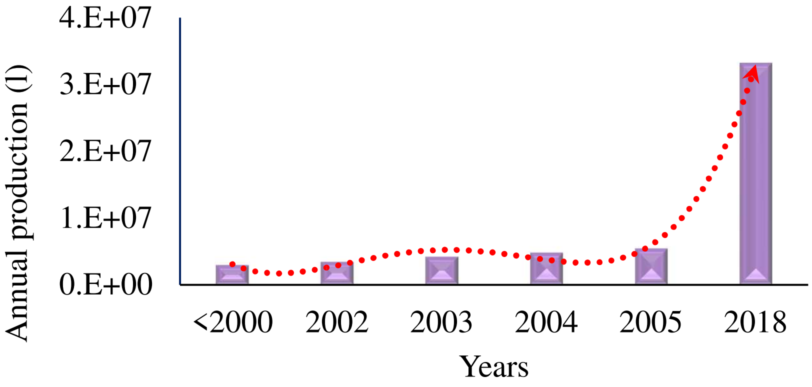


Figure 11 Potable alcohol production trends in Ethiopia

Due to the supply and demand mismatch, the quantity of alcohol manufacture in Ethiopia is rising sharply. The potable ethanol producing factories listed in table two are mostly either medium or large-scale by capacity. Thus, this list does not include those other small-scale productions. Further, there are informal or unregistered productions while for some accessing data are challenging, even though they are registered (**Table 7**).

Though all the alcohol produced my not completely be based on cane molasses as raw material, approximately the current 3.4 x10^4 m^3 of ethanol production by the 17 factories is only 31 % of the total potential of cane molasses ethanol production, excluding the four missing factories. On average, these ethanol production factories are currently utilizing 84 % of their production capacity. Currently, the two largest potable ethanol producers in the country are Balezaf Ethiopia Liquor Factory and the National Alcohol & Liquor Factory, which produce over nine million liters every year. Name of the companies are presented anonymously for privately owned once as listed using the letters A to T (**Table 7**).

Table 7 Annual alcohol and stillage production profile of the private and public potable ethanol plants (Source: MoI, Ethiopia)

Company Name	Location (City/regional state)	Installed annual ethanol production in liters	Actual annual ethanol production in liters (80%)	Annual stillage production (m^3) (Approx. 13 liters/liter alcohol)
Liquor factory A	Addis Ababa	324,974	259,980	3.38E3
Liquor factory B	Oromia	13,884,000	11,107,200	1.44E5
Liquor factory C	Addis Ababa	344,843	275,874	3.59 E3
Liquor factory D	Addis Ababa	-	-	-
Liquor factory E	Addis Ababa	360,000	288,000	3.74E3
Liquor factory F	Tigray	2,643,300	2,114,640	2.75E4
Liquor factory G	Tigray	-	-	-
Liquor factory H	Addis Ababa	2,862,000	2,289,600	2.98E4
Liquor factory I	Addis Ababa	-	-	-
NALF	Addis Ababa	12,174,395	9,739,516	1.27E5
Liquor factory J	Addis Ababa	-	-	-
Liquor factory K	Addis Ababa	627,094	501,675	6.52E3
Liquor factory L	Tigray	1,350,000	1,080,000	1.41E4
Liquor factory M	Oromia	1,000,000	800,000	1.04E7
Liquor factory N	Addis Ababa	500,000	400,000	5.2E2
Liquor factory O	Addis Ababa	1,000,000	800,000	1.04E4
Liquor factory P	Addis Ababa	1,400,000	1,120,000	1.46E4
Liquor factory Q	Addis Ababa	900,000	720,000	9.36E3
Liquor factory R	Oromia	120,000	96,000	1.25E3
Liquor factory S	Addis Ababa	636,855	509,484	6.62E3
Liquor factory T	Amhara	1,289,500	1,031,600	1.34E4
Total		39,416,961	33,133,569	4.31E5

The major environmental concern with ethanol industries is the stillage discharge, which is a bottom product after distillation of the liquor. Considering an average 13 liters of stillage/liter alcohol produced, these potable ethanol industries generate 4.31*10^5 m^3 of stillage every year (**Table 7**). Unfortunately, these industries are built adjacent to rivers whereby it is easy for them to discharge the wastes into them and are concentrated in the capital city. (**Fig. 12**).

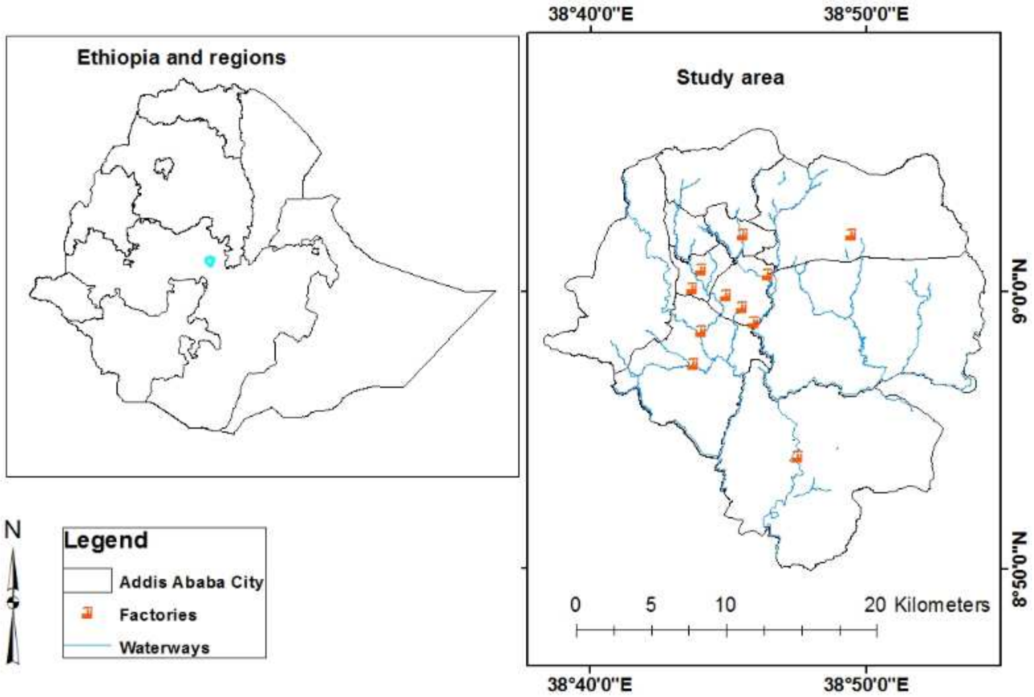


Figure 12 Map of the National Alcohol and Liquor Factory and other potable alcohol industries in Addis Ababa city, Ethiopia

3.3.3. Quantification of the non-potable ethanol and stillage production

The non-potable ethanol production is largely motivated to boost the country's green power supply and it can be produced from generations of sugar sources that can be grains, sugarcane molasses, or sugar beet molasses. Currently Ethiopia owns eight operating sugar production plants that produce over 1,787,645 tons of sugar and over 33,134 m^3 potable and 20,500 m^3 fuel ethanol yearly excluding the non-potable ethanol production by petroleum companies. Indeed, there are five more sugar production plants that are under construction with different fuel ethanol capacity, which include Omo Kuraz I and V (56,000 m^3) located in Southern Nation, Nationalities & Peoples, Tana Beles I and II (28,270 m^3) in Amhara as well as Wolkayt (41,654 m^3) in the currently Tigray region of Ethiopia planned to crush 84 tons of sugarcane per day (Ethiopian Sugar Corporation, 2016) (**Table 8**).

According to a news release by the country's public media in 2018, the Ethiopian Minerals, Petroleum and Bio Fuel Development Corporation and the Ethiopian Sugar Corporation — are going to jointly build an ethanol (power alcohol) refinery which will have a daily production capacity of 50 m^3 of ethanol from molasses raw material. The ethanol refinery will use molasses and bagasse produced at Omo Kuraz I and Omo Kuraz III sugar factories built by the Ethiopian Sugar Corporation (The Reporter, 2018). Thus, the expansion of sugar plants backed by the extensive sugar plantations will be inevitable.

Though there are far more plans to expand the ethanol production capacity from different generations of biomass, table three presented the current cane molasses-based fuel ethanol production in the country. The number of regions where these factories are located are three out of the ten in the entire country; Oromia, Afar and Southern Nation, Nationalities & Peoples' regions. However, there are expansion plans located in other regions, including the northern part of the country.

Table 8 Location, annual ethanol capacity and stillage discharge rate of fuel ethanol plants in Ethiopia

Name of sugar/ethanol factory	Location/regional state	Date of establishment	Installed annual ethanol capacity (m^3)	Annual ethanol production	Annual stillage production	Status/remarks
Wonji Shewa	Oromia	1954	12,800	-	-	To resume production
Metehara	Oromia	1970	12,500	12,500	162,500	Ethanol active since 2011
Fincha	Oromia	1999	8,000	8,000	104,000	Ethanol active & expanding to 20,000
Tendaho	Afar	2014	27,000	-	-	To resume production
Arjo Diddiessa	Oromia	2015	-	-	-	Has large ethanol potential envisaged

Kessem	Afar	2015	12,500	-	-	To resume production
Omo-Kuraz	Southern Nation, Nationalities & Peoples	2017	28,000	-	-	To resume production
South Omo Kuraz III	Southern Nation, Nationalities & Peoples		56,000	-	-	To resume production
AL-Habasha	Oromia	2007	-	-	-	Ethanol and ethanol products
Nile Petroleum Co. Ltd	Oromia	2009	-	-	-	Ethanol blending
National Oil Ethiopia PLC	Oromia	2011	-	-	-	Ethanol blending
Raj Agro Industries PLC	Oromia	2015	-	-	-	Ethanol active

3.3.4. Physicochemical characterization of stillage

3.3.4.1. The case study factory and potable ethanol production process

The case study plant, NALF, is among the biggest producer of liquor (alcoholic drinks), pure alcohol and denatured alcohol in Ethiopia. It supplies its products to the domestic and the international market, which include the exporting of its goods to countries such as USA, Australia, Israel, and South Sudan. NALF is a state-owned business organization and pioneer factory in manufacturing and distributing Extra Neutral Alcohol (ENA), denatured alcohol and different brands of liquor in Ethiopia. The factory is the first of its kind in the country when founded in 1920 by private owners. The current name was acquired after the merger of four companies in 1974.

NALF has four branches of installed alcohol production process plants whereby two of them are located in Addis Ababa city including the one called Mechanisa branch. A Mechanisa branch of

NALF is located in Mekanisa, Nefassilk sub city, Addis Ababa, Ethiopia (**Fig. 12**). Based on the site visit conducted, this particular plant principally involves mixing in a vat, batch fermentation, distillation and bottling process units to produce ethanol (**Fig. 13**) whereby it generates about 21,000 m^3 of stillage yearly along the production.

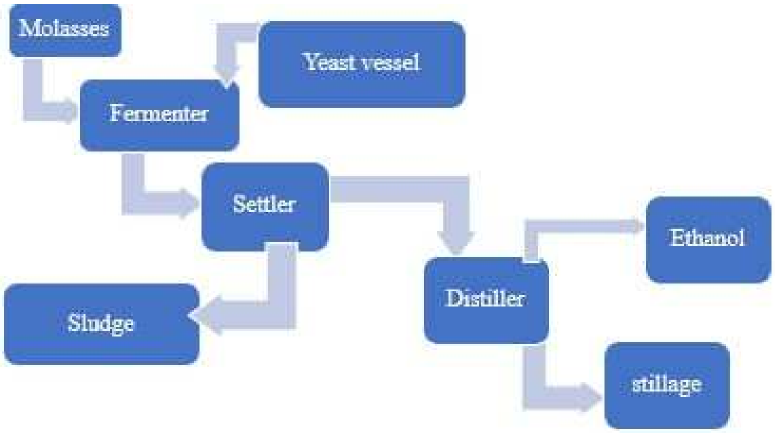


Figure 13 The conventional molasses-based ethanol production process

The molasses-based fermentation process takes place in a vat method (**Fig. 13**) with a holding time of 48 hours after being seeded with yeast mass that is cultivated in a separate incubator chamber. In the fermenter unit, sulfuric acid is added to precipitate limestone that is brought with the molasses and also introduced from the dug well process water source on site. In the yeast incubation chamber 0.3 kg/liter of nitrogen-phosphorus-sulfur composite is added on average to foster yeast growth. During molasses pre-treatment and fermentation, substantial volume of sludge is produced. By volume, 20 % of the molasses treated and 12 % of the molasses fermented are precipitated on average as bottom sludge. At the NALF, the Brix of the molasses is lowered from 75-80 to 28-34 by diluting with water during the molasses treatment.

Following fermentation, the liquor, which is also customarily called beer is moved to distillation columns to rectify the alcohol to the desired product quality. In fact, the huge stillage is coproduced at the mash column that is packed with 18 to 20 trays commercially where alcohol is fractioned from fermented mash so that alcohol vapor with a concentration of 35 -50 % by weight is taken at the top. Consequently, the alcohol is moved to the predistillation column where aldehydes, esters and other undesired fermentation by-products are removed and ethanol/water mixture is drawn from the bottom of the predistillation column while the impurities are taken from overhead.

Next to predistillation, the diluted alcohol stream from the nethermost of the predistillation column moves on to the rectification column, which is commercially packed with 65 – 72 trays to be concentrated and the concentrated alcohol product is drawn off of the top of the lower section, fuel alcohols are taken from the middle and spentlee (distillery wastewater) from the bottom. Lastly, the alcohol is purified in the methanol column from removing contents like aldehydes and methanol that escaped with the product obtained from the rectification column. The methanol column is commercially packed with 50 - 70 trays and consumes 1.5 – 2.5 MJ/kg of ethanol in the feed.

Currently, NALF is producing around 30,000 liters of Extra Neutral Alcohol (ENA), which is a food grade 95 % alcohol, per day as per its design capacity from its operating two sites; 18,000 liters from Mechanisa and 12, 000 liters from Sebeta divisions. This cane molasses-based ethanol production consumes 4-5 kg of molasses per liter of alcohol produced. Based on the daily ethanol production, a huge volume of sludge and stillage is generated from this single company alone. These include the generation of 20 % solid from the 4.5 kg/liter alcohol molasses use. Accordingly, from producing 30,000-liter alcohol/day, an amount of 27,000 kg/day sludge is generated.

The fermentation unit generates another volume of sludge that accounts for the 12 % of its daily volume. Hence it generates around 3,600 kg of sludge, assuming the density of water. Aside from the sludge generation, the ethanol plant produces 390,000 liters of stillage every day, which is normally contributed by the mash column. The stillage is released as a bottom product at a temperature reaching 90 ^{o}C with acidic pH and brownish color at the point of discharge.

Another environmental footprint this factory makes is the generation of one kg of CO_2 in every liter of alcohol fermented, which amounts to a daily release of 30,000 kg CO_2 from this single company alone. Further, the water footprint of this case factory accounts to the consumption of 250 liters/liter ethanol produced. So far, the environmental impact of this sector is excluding solid and other liquid wastes which may or may not have direct connection with the industry.

3.3.4.2. Amount of solid in stillage

The solids composition of the stillage obtained from potable ethanol plants in Ethiopia is within range of related findings from cane molasses-based ethanol stillage studies performed in the past though the degree of variability may differ (Mohana, Acharya, & Madamwar, 2013; Willington & Marten, 1982). Table four presents the amount and composition of the solids as well as their wet percentage in a liter of stillage sample. Relative to the total dissolved solids (TDS), the total suspended solid (TSS) appears low that it could be due to the low pH (Table 5) and very organic nature of the sample. The ratio of the volatile suspended solid (VSS) and TSS is found at 0.58 and this value together with the biodegradability index (BI) of the stillage which is found from the ratio of biological oxygen demand of five days (BOD_5) and the chemical oxygen demand (COD) as 0.18 is not favorable for any subsequent biological treatment. Compared to waste activated sludge with a VSS/TSS ratio of 0.7 (Getachew Dagnew Gebreeyessus, 2018), which is even considered as less biodegradable, the VSS/TSS ratio of stillage shows lower VSS value that is normally considered as an available organic material for biodegradation. Consequently, improving the BI of this stillage is a necessity if biological remediation or resource recovery should be considered in stillage management (Malik et al., 2014; Sarat Chandra et al., 2014a) (**Table 9**).

Table 9 Solid characteristics of stillage from the National Alcohol and Liquor Factory

Item	Mean value ± (SD) in g/l	Percent (w/v basis)
TS	177.3 (23)	18
TSS	52.99	5
VS	133.7 (23)	13
VDS	79.5 (3)	
VSS	30.9	
TDS	124.3 (2)	12
FS	43.6 (1)	4
FDS	44.8 (1)	

3.3.4.3. Physicochemical characteristics

Table four & five shows that water, solids and organics (BOD or COD) are major compositions in stillage driving its recovery. MP-AES have been used to determine the metals in stillage sample that is pretreated with nitric acid using an oven. According to the result obtained, the level of Pb, Cd and Zn in the stillage is below the target value to be present in soil as proposed by the World Health Organization (Kacholi & Sahu, 2018) (**Table 10**).

Table 10 The physicochemical characteristics of raw cane molasses-based ethanol stillage from the National Alcohol and Liquor Factory, Ethiopia

pH (at16°C)	TA (mg/l)	HCO_3^- (mg/l)	Cl^- (mg/l)	EC (µs)	Salinity (%)	Viscosity (Centipoise)	COD (g/l)	BOD (g/l)	SO4 $^{2-}$ (g/l)	Phenol (mg GAE/g)
3.99	4800	585.6	8236	3630	22	4.04	106±0.144	47.23±0.028	7.6	57.4±2.4
					Trace Metals (mg/l)					
Ca	Zn	K	Pb	Cd	Ni	Cr	Fe			
11580	0.69	1465.5	0.03	0.2	BDL*	BDL	147.3			

* BDL= Below Detection Limit; TA = Total alkalinity; EC = Electric conductivity

Heavy metals such as cadmium and chromium are present to an amount of 0.004 and 0.95 µg/l subsequently in digested spent wash or distillery stillage and to an amount of 0.025 and 0.17 µg/l in crude stillage (Jain & Srivastava, 2012). However, recent reports showed an even higher content of heavy metals including cadmium that is 2.3 mg/l in post-methanated distillery wastewater which is over 200 times the limit suggested by the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO). Furthermore, the heavy metals also form complexes with sulfides, and these complexes result in toxicity of the effluent especially with post methanated distillery effluent. Following the bio-methanation of the distillery seepage the heavy metal content is even become concentrated (Yadav & Chandra, 2019). In this regard, the supernatant was used in acute toxicity tests with the microcrustacean *D. similis* indicated that stillage at a concentration of 9.10 % is possibly noxious to river organisms (Messetti et al., 2017). In another characterization study, grain-based ethanol is a major production system in the USA (97 %) and even Brazil. Production cost and availability of the raw material as well as ease of wastewater treatment are among the reasons to shift from cane molasses-based distilling to grain-based distilling though it may differ among countries. Recently grain distilleries have been rising against cane molasses distilleries in Asian countries like India, especially fuel ethanol is being obtained from diverse biomass that includes lignocellulosic materials (Agriculture., 2006; Gnansounou & Dauriat, 2005; Lynd, Cushman, Nichols, & Wyman, 1991). Even though technoeconomic perspectives are equally important, spent wash from lignocellulosic and grain distillery were assessed for their physicochemical properties. Though it takes a relatively longer fermentation time, the lignocellulosic feed showed lower COD and solids content in the spent wash. Notably, the pH was favorable for the subsequent biobased stillage treatment. The grain spent wash is also less colored while containing less COD and solids compared to cane molasses (**Table 11**).

Table 11 Physicochemical characteristics of stillage obtained from lignocellulosic and grain feeds fermented in the lab, mean (±SD)

Sample type	pH	COD (mg/l)		Phosphate (mg/l)	Nitrate (mg/l)	Color			TS (g)	TSS (g)	FS (g)	TDS (g)	VSS (g)	DFS (g)
		Total	Soluble			Characteristics	At pH =7.6	At pH =2.7						
Stillage from ligno-cellulose feed fermented for 72 hours	5.8	44330 (2667)	22,520	721.7 (25.1)	6.4 (1.0)	-	-	-	54.6 (2.3)	4.6	3.1 (0.5)	30.0	5.5 (2.8)	4.0 (1.0)
Stillage from ligno-cellulose feed fermented for 96 hours	6.1	35670 (0)	18,299	377.4 (0)	20.3 (4.1)	-	-	-	39.5 (5.8)	11.7	3.4 (0.4)	27.8	12.5 (5.5)	4.2 (2)
Grain spent wash	2.7	77670 (9900)	40,625	299.5 (22)	28.1(2.8)	Dominant wavelength	580	575	95.4 (17.5)	50.2	6 (0.1)	45.2	50 (3.7)	5.8 (2.1)
						Hue	Yellow-yellowish orange	Yellow						
						Luminance	67 %	51 %						
						Purity	80 %	60 %						

3.4. Discussion

3.4.1. Ethanol production from cane molasses and the stillage discharge issue

Ethanol and other types of bio-diesel making can be performed using a variety of biomass as raw materials which include molasses. The fuel ethanol production in Ethiopia dates back to late 20th century following the feasibility study conducted by the country's ruling government, which was financed by the United Nations Industrial Development Organization (UNIDO) and contracted with a Finish company in 1972. By that time the country had 46,000 vehicles which were considered as future users of ethanol blended gasoline and result in cut of crude oil use. Recently, however, the country has near a million vehicles on its streets. Indeed, this time fuel ethanol is not sought only to be blended with gasoline, but also to produce electricity and also be used in aviation fuel so that huge foreign currency saving can be achieved.

The fuel ethanol sector in Ethiopia is booming far beyond higher in magnitude compared to the potable ethanol sector. Recently the National Oil Ethiopia, a private fuel company, inaugurated distilleries with a capacity of 1,000 m^3 ethanol production per day in a small town of Dukem located near east of the capital city.

The major contributing factor to the increase of production was the existence of a wide market for alcoholic liquors in the urban and semi urban areas and the enabling environment created for private investment. Although there are a number of small to medium factories that produce alcoholic liquors the NALF, a state-owned enterprise, is the largest domestic producer.

The practical annual water consumption in these process plants ranges between 18 & 19 liters per liter of alcohol for all purposes, accounting for nearly six million liters, in the potable alcohol production, of which, the 14 liters per liter alcohol is the vinasse, on average. Though, over five million liters of vinasse is released from the potable alcohol factories, the current average production of vinasse in the country is estimated to be in the hundreds of millions of liters annually from both the potable and fuel ethanol processing industries. The untreated potable ethanol vinasse, along with a COD of 80,000-100, 000 mg/l, among other sensitive parameters, are discharged into a nearby watercourse. Such discharge is equivalent to the release of over 334075.32 kg COD/year or 915.3 kg COD/day and that daily release can completely take up the 7 mg/l dissolved oxygen (DO) in a 130753.6 m^3 of water at the expense of the aquatic lives (Rameshwar & Chinnery, 1997).

Despite the higher order increase of alcohol production in the country, neither the regulatory, nor the market system are efficient to control the environmental pollution problem, mainly due to technological lag. Nevertheless, progressively strict environmental rules are obliging distilleries to improve the prevailing treatment and also to explore other methods of effluent management. Along the transformation of the production technology that maximize alcohol production and minimize water and biomass loss, the adequate treatment of stillage through energy and materials recovery is therefore imperative before discharging the wastewater.

3.4.1.2. Nature and characteristics of the toxics released from distilleries

The nature or composition of stillage is mainly related to the nature of the raw material applied, chemicals added and the unit operations adapted by the distilleries. Distillery liquid waste is comprised of phytotoxic, antibacterial and recalcitrant, as well as, color complexes including phenols and polyphenols. Further, such wastewater contains heavy metals, which have negative effects on micro-organisms and plants at disposal sites (España-Gamboa et al., 2011). Vis-à-vis their antioxidant properties, melanoidins are toxic to many microorganisms involved in wastewater treatment that include genotoxicity (Taylor et al., 2004). In addition, these colored effluents pose a hazard to tourism because of their bad aesthetic imparts usually accompanied with their bad odor (Chauhan & Dikshit, 2007; Rameshwar & Chinnery, 1997).

Further, the highly colored compounds lead to reduced sunlight diffusion into streams and lakes, thereby, hindering photosynthetic action, depleting the dissolved oxygen concentrations and causing hazardous conditions for aquatic life like fish deaths, suffocation, and ecological disturbances (Kharayat, 2012). The color causing mixtures include polyphenols, caramels, melanoidins and alkaline degradation products of hexoses. These polyphenolic composites exhibit antioxidant, anti-microbial, anti-carcinogenic, free radical hunting and metal chelating possessions (Chowdhary, Raj, & Bharagava, 2018).

A recent study also demonstrated that sugarcane-molasses distillery waste contains androgenic and mutagenic compounds, both in distillery sludge and leachate, that cause serious consequences for water-based plants and animals. This deduction was made following a chromatographic detection of compounds dodecanoic acid, octadecanoic acid, n-pentadecanoic acid, hexadecanoic acid, b-sitosterol, stigmasterol, b-sitosterol trimethyl ether, heptacosane, dotriacontane, lanosta-8, 24-dien-3-one, 1-methylene- 3-methyl butanol, 1-phenyl-1-propanol, 5-methyl-2-(1-methylethyl) cyclohexanol, and 2-ethylthio-10-hydroxy-9-methoxy-1,4 anthraquinone as major organic pollutants along with heavy metals (Chandra & Kumar, 2017).

3.4.2. The local environmental and regulatory status of stillage discharge

The EPAE has made an evaluation of the pollution status of the NALF sometime in the past. Based on the result obtained at a different test date, the inspection team of the organ has compared it with the set limit values and already labeled it as priority polluter. NALF showed variable but almost six years of unacceptable limit of discharge pertaining BOD, COD, ammonia and phosphorous concentrations and obviously failed to meet discharge standards. As the biomass are discharged without treatment the recoverable are lost in vain in addition to the environmental impact it already brought. It has not been the only process plant that failed to meet discharge standard either.

The laboratory evaluation of sampled ethanol and other factories located in Addis Ababa city also showed variability and in disobedience with the discharge limit values.

From the legal perspective, Ethiopia has a rather sound framework that begins within the country's constitution through the declarations in proclamations to a formation of supervisory organizations. For example, under the environmental right of people in the law of the government of the Federal Democratic Republic of Ethiopia (FDRE) article 44, number 1 states that "all people enjoy the privilege to a clean and healthy environment". Deduced from that right is the enablement of the EPAE to regulate the discharge of effluents by formulating standards based on the Environmental Pollution Control Proclamation Number 300/2002. However, the implementation and the environmental performance of the nation is apparently poor.

In addition, the EPAE together with the Ministry of Economic Development and Cooperation published an environmental policy document that considers issues of wastewater recycling, and the sustainable use of renewables by placing the assurance of improved environmental sanitation towards achieving sustainable urban development. Regarding hazardous material and pollution control policy, the same document outlines the need to adhere to the known precautionary principle that aims to minimize or possibly prevent the discharge of such materials in to the environment (E. P. EPA & MEDC, 1997).

On the other hand, there are development related policies and strategies that are being implemented at a relatively faster rate, of which Ethiopia's Growth and Transformation Plan and Ethiopia's Climate-Resilient Green Economy- strategy can be mentioned. The latter strategy envisages 32 % of the gross domestic product to be contributed by the industry by 2025 with a co-increase of average per capita income. Anticipating the issues of sustainability, this same strategic document indicated possible mitigation alternatives, including afforestation (FDRE, 2011).

Regardless, almost all the industries including the distilleries continued to pollute the environment. Few studies evidenced the indiscriminate discharge of domestic and industrial wastewater, mainly in the capital city where over half of the industries are located, into the water, soil and the air. Such practices resulted in the change of the physical, chemical and biological nature of rivers and the groundwater therein. Having observed the situation and predicting the trend, ecological crisis appears unavoidable, especially in major cities including the capital (Worku & Giweta, 2018).

The control and regulation of the polluting industries is hardly practiced in Ethiopia mainly due to a lack of competent personnel and the technical facilities (Alemayehu, 2001; Bezuneh & Kebede, 2015; Cheever). On the contrary, countries like India are advancing their standards and are benefiting through the application of "zero liquid discharge" technology by the distillery industries (Muhammad & Lee, 2019; Saha, Balakrishnan, & Batra, 2005). Even though Ethiopia has formulated a law on environmental protection, the factories' negative impact on the environment is increasing continuously. The country, like other developing nations (Ramjeawon, 2000) in the world, has developed and approved regulatory document that controls the discharge from different industries consisting of distilleries. Thus, there was a five year grace period for the industries to adjust themselves to the level of the regulation, however, the industries nor the regulatory has achieved their target (Nyssen, Haile, Moeyersons, Poesen, & Deckers, 2004).

Reconciling economic activities and environmental problems is challenging the developing countries to the extent of impacting the choice of technology from a pollution potential perspective. As people in the field do argue that pollution itself is a market problem, market-based pollution control instruments can also be considered as alternative solution to environmental problems through the application of emission permit trading, price instruments, subsidy systems, and voluntary environmental agreements. Given the limitations within the market-based pollution control system, stringent regulations through legal controls and actions are unavoidable, especially to prevent pollution from the source (Stavropoulos, Wall, & Xu, 2018). In fact, it is relevant to consider an awareness raising program in the society as part of a cooperative effort among religious and other institutions, especially in developing nations like Ethiopia. Further, the issues of sectoral collaborations and institutional memory preservation, as well as, statistical approaches for evidential proof have to be strengthened in the state.

3.5. Conclusion

Sugarcane is the sole source of sugar production and cane molasses are the dominant raw material for the potable alcohol production in Ethiopia. The sugar industries in the country are not energy self-sufficient and a huge amount of biomass is wasted as bagasse, filter cake and molasses. After consumption of huge land and water, the expanding sugar industry releases 3.01E5 tons of molasses annually and its ethanol potential is underutilized. Similarly, the stillage from ethanol distilleries is being discharged without proper recovery and treatment into the environment.

The ethanol industries release 1kg sludge/liter of alcohol only by the fermentation unit which is also a bioresource lost in vain that otherwise could have been a means of recoverable energy as well as nutrient. Further, the release of 1kg CO_2/liter of alcohol is a concern, which can be captured and transformed into other biochemical products. Despite the formulation of good policies and standards, their implementation is quite poor in the country and it is not coping with the ever-expanding sector that calls for prompt and concerted action to improve the sectors' environmental and economic performance. In this regard, the quantification and characterization of the products and byproducts can help the various stakeholders to make an effective management strategy and develop achievable interventions.

Moreover, waste valorization and possible no liquid discharge have to be considered through the implementation of biorefineries for coproduction instead of using the traditional technologies by aiming sustainability. By doing so, such industries can close the gap in economic cycle and ensure sustainability through better environmental performance. In this regard, revision of the production process through smart and improved development and updated environment policies towards resource efficiency is important. Besides, the introduction of emerging technologies to production, resource recovery and waste treatment contributes to the betterment of these industries particularly in energy, nutrient and water sustainability. Further studies on the use of CO_2 as a carbon basis for fermentation or biomass production is suggested. Advancing the alcohol separation techniques, including operations in vacuum systems, the application of emerging biotechnologies as well as fermentation feed modifications are necessary.

CHAPTER FOUR

This chapter is based on the objective two of the research project and the manuscript is under revision in the journal of Cleaner Engineering and Technology. It can be referred as: Getachew Dagnew Gebreeyessus, Trichur Ramaswamy Sreekrishnan, Andualem Mekonnen, Yonas Chebude, Esayas Alemayehu (2021). Effect of a mild iron oxide coated sand based wet air oxidation on the detoxification of molasses stillage Journal of Cleaner Engineering and Technology. (Under revision)

Effect of a mild iron oxide coated sand based wet air oxidation on the detoxification of molasses stillage

Abstract

Ethanol distillery stillage, a bottom product of the liquor distillation, is produced massively everywhere, as a known environmental pollutant and yet it is a potential source of bio-products. However, its complex nature, containing recalcitrant organics and toxic compounds impedes its biodegradability thereby challenging the efficiency of its bioremediation approaches. Thus, enhancing its biodegradability by applying pretreatments aiming at detoxifying the stillage are deemed important. In the current work a mild iron oxide coated sand based wet air oxidation was examined to see if the toxicity and hence biodegradability of the stillage improves. The findings showed that the addition of the iron oxide coated sand (3.5%) during wet air oxidation was a real factor in bringing the difference in the chemical oxygen demand of the stillage before and after treatment which was applied at a temperature of 60 ^{o}C for 4 hours and at an atmospheric pressure. Consequently, a significant difference in the toxicity of the pretreated stillage was observed following a microbiological culture testing, when compared with the raw stillage based on *E. coli* growth evaluation as determined spectrophotometrically at 600 nanometers. This suggests that the biodegradability of stillage has significantly enhanced. Given sand and iron are abundant and iron oxide coated sand can even be integrated from scrapings of an aeration-filtration operations of groundwater treatment plants, this finding proposes a sustainable pretreatment option to toxic and recalcitrant organic industrial wastes including the distillery stillage towards an efficient bioresource recovery.

Keywords: biodegradability, *E. coli*, iron oxide coated sand, stillage, toxicity, wet air oxidation

4.1. Introduction

Distillery industries that use molasses as feed for ethanol production are increasing with time, especially following urbanization, which is particularly a phenomenon in the developing countries. Wilkie and colleagues (2000) reported that the release of molasses distillery stillage from the fuel and potable ethanol process industry is characteristically complex and huge in magnitude. Thus, stillage needs proper handling aiming at maximizing resource recovery and minimizing the ecosystem damages including surface and groundwater pollution. High chemical oxygen demand (COD), biological oxygen demand (BOD), and color causing as well as the recalcitrant organics including the melanoidins, furfurals, phenolics, caramels, in addition to odor causing indole and skatole contents are among the hurdle in stillage bioremediation (Wilkie et al., 2000).

On the other hand, Mikucka and Zielinska (2020) recently reported that the indiscriminate discharge of raw or partly treated distillery stillage is hazardous to ecosystems (Mikucka & Zielińska, 2020b). At different levels of biological organization, stillage affects the ecological integrity, biodiversity and hence ecosystem sustainability due to the presence of organic acids and recalcitrant compounds that include polyphenols, tannins and metals. Sousa and coworkers (2019) studied the toxicity of stillage on fish and plant species deep into its effect on germination as well as the embryo level and have found that stillage causes moderate to high toxicity. It has brought varying toxicity among the tested organisms, including bacteria, invertebrates, plants and animals. Such discovery alarms the critical ecological effect of stillage thereby demanding its proper treatment before discharging it into the environment (Sousa et al., 2019).

As bioremediation challenge, almost half of the organics in stillage are biologically nondegradable. Consequently, alternative removal technologies, including the multi-effect moisture elimination are applied towards meeting discharge standards set by various nations. Despite that, an earlier technoeconomic evaluation of evaporative technologies by Barta and coworkers (2010) showed the poor energy efficiency affecting the overall economy of the ethanol production sector (Barta, Reczey, & Zacchi, 2010). According to Santos and workmates (2014), in spite of its nonbiodegradable nature, stillage remains to be a potential source of biomaterials, biogas and other valuables (Santos, Ferreira Rosa, Sakamoto, Amâncio Varesche, & Silva, 2014). For instance, the anaerobic digestion (AD) of stillage is inefficient mostly due to its recalcitrant nature. Yet, as studied by Asato and coworkers (2014), the AD of stillage can have energy, nutrient and water recovery advantages, provided that there is a certain pretreatment (Asato, Zicari, Li, & Zhang, 2014).

Though, they are a potential source for biogas and other valuables, the wines, ethanol and fruit processing industry wastewater are known to contain toxic compounds, which are trapped from farm through processing which include phenolic compounds and heavy metals. Typically, distillery wastewater has phytotoxic, bactericidal, recalcitrant and colored (melanoidins) components, such as phenols, polyphenols as well as heavy metals, which have negative effects on micro-organisms during the latter biodegradation. For instance, Taylor and colleagues (2004) reported that melanoidins are poisonous to various microbes that are active in wastewater remediations that include the genotoxicity, which is because of their oxidation inhibiting characteristic (Taylor et al., 2004).

Further, a recent study by Chandra and Kumar (2017) demonstrated that sugarcane based molasses distillery waste contains androgenic and mutagenic compounds that bring adversative consequence on the river based living organisms (Chandra & Kumar, 2017). In this regard, its conversion to less toxic, biodegradable form rather than the complete mineralization of toxic organic wastes is useful from the resource recovery perspective. Therefore, the detoxification of such wastewater for the subsequent application of any biotechnology is significantly important.

Few studies reported the toxicity reduction potential of a WAO pretreatment of recalcitrant organics at various degrees. Following physicochemical pretreatments of biomass that include WAO, toxicity tests are performed using microbes' and plants' growth potential as an indicator. In fact, a study by Bistan and colleagues (2012) have reported a complete removal of toxic estrogen, which is proved by growing yeast using a catalyst supported WAO at the 230 ^{o}C (Bistan, Tišler, & Pintar, 2012). However, such studies are performed on particular toxic and that undermines the interactive (synergistic or potentiation, additive and antagonistic) effect of the coexisting toxics.

In this regard, wet air oxidation (WAO) as pretreatment can have a considerable relevance. However, the operating parameters, especially at supercritical conditions is reported to be harsh and perhaps uneconomical. For instance, Sushma and coworkers (2018) investigated the integration of the WAO and a biodegradation on different recalcitrant industrial wastewater, which brought the removal of over 98% of COD (Sushma, Kumari, & Saroha, 2018). However, such results are attained using noble metal-based catalysts and on the laboratory scale on synthetic substrate, which may not even work on real scenarios. As a result, the use of an abundant and cheap as well as harmless material supporting WAO brings a paramount importance in industrial wastewater treatment.

Consequently, a material supported WAO of stillage pretreatment is invaluable. Thus, the objective of the current study was to minimize stillage toxicity and enhance its biodegradability in an environmentally friendly manner using iron oxide coated sand (IOCS) supported WAO pretreatment. Subsequently, the toxicity of the pretreated and unpretreated stillage has been tested using *E. coli*.

4.2. Materials and methods

4.2.1.Sampling

The WAO and toxicity evaluation experimental parts of this research were performed at the wastewater treatment laboratory of the department of Biochemical Engineering and Biotechnology of the Indian Institute of Technology in Delhi, India. Thus, a cane molasses distillery stillage sample was brought from a processing industry (Wave Distilleries and Breweries Pvt. Ltd), which is located nearby the capital, Aligarh town, Uttar Pradesh State, India.

Adequate and composite sample was obtained using a clean and tightly closed plastic jar, which reached the laboratory within four hours from factory site; the stock was labelled and kept in a cold room throughout the study period. Later, the tests were performed by grabbing a homogenized subsample using a beaker. The subsample was further subsampled and analyzed while keeping the remaining subsample in a refrigerator all the time.

4.2.2.Iron oxide coated sand preparation

Ferric chloride ($FeCl_3$) was used as a modifier compound in the synthesis of the IOCS. Color of $FeCl_3$ is either dark green or purple-red depending on the angle of viewing. The hexahydrate ferric chloride, used in this study, has a molar mass of 270.3 g/mol, a density of 1.82 g/cm^3 , an odor of like a slight HCl and a structure formula of $[Fe(H_2O)_4Cl_2]\,Cl{\cdot}2H_2O$ (Lide, 2004; Lind, 1967). $FeCl_3$ is soluble in water with measurable hydrolysis occurring at pH 1 (Cotton, 2018).

IOCS was synthesized using an ordinary quartz sand which was obtained from one of the sands and gravel vendors in Addis Ababa, Ethiopia. First, the raw sand was sieved to remove coarse ones and to improve uniformity. After sieving, the sand was reduced to a uniform spherical diameter of 0.5 -1.2 mm using a grinder located at the size reduction laboratory of the Addis Ababa Institute of Technology, Addis Ababa University, Ethiopia. The size-reduced and the relatively uniform sand was soaked in HCl solution (pH 1) for 24 hours to remove dirt and organics. The sand was later washed with distilled water (DW), rinsed and then oven dried overnight at 105 ºC. Afterwards, the analytical grade modifier compound, $FeCl_3.6H_2O$ (2 mol/l) was added, well mixed and the solution-sand mixture was first dried at 105 ºC for two hours and later calcined at the 550 ºC for three hours (Kumar, Gurian, Bucciarelli-Tieger, & Mitchell-Blackwood, 2008). Then a NaOH solution (0.3 mol/g-sand) was added to the already calcined IOCS and the mixture was again oven dried in order to give it a flake nature.

After the sand coating was completed, surface morphological characterization as well as the element analysis was performed using Scanning Electron Microscopy Energy Dispersive X-rays Spectroscopy (SEM EDX) and X-ray Diffraction (XRD). The XRD image and the FTIR for the IOCS were done in the Instrument Laboratory of the Department of Chemistry at Addis Ababa University. The SEM images were also taken using an instrument named INSPECTFS, which is located in the Addis Ababa Science and Technology University, Ethiopia and the SEM, EDX analysis were performed in the central laboratory of Indian Institute of Technology, Delhi.

4.2.3.Experimental setup

The entire experiment that has begun from the sample characterization to toxicity testing has been accomplished as shown on the experimental setup (**Fig. 14).** This study was centered on determininig the impact of IOCS supported WAO pretereatment of the stillage using *E.coli* cultured incubation of media, which was set at a temperature of 37±2 ºC. The effect of each operation was determined 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 analyses performed.

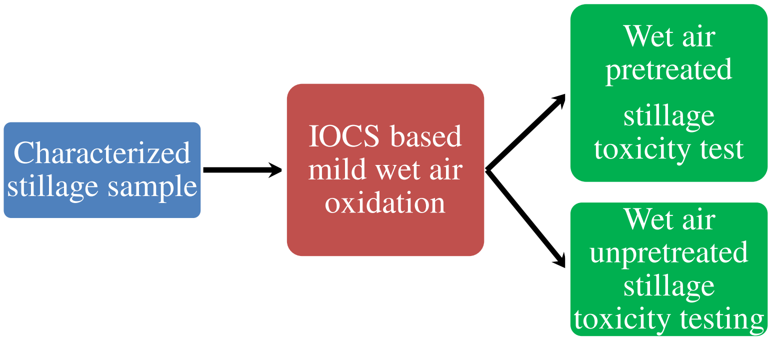


Figure 14 Diagrammatic representation of the experimental setup

4.2.4.The mild wet air pretreatment

In the current study an IOCS supported mild WAO, that is performed on far lower temperature/pressure condition than the conventional system, was applied under atmospheric pressure as a pre-treatment for the cane molasses stillage whereby IOCS is applied at a different dosage. IOCS is used as an adsorbent while the ferric chloride coated over it perhaps brings catalytic effect that is induced during the WAO at ambient condition.

Subcritical WAO of stillage was performed at a temperature ranging 50-70 ºC and at an atmospheric pressure following sample characterization. During the WAO a three-liter volume glass reactor was used. Three of the four necks of the glass vessel were fitted with air bubbler, a thermometer probe and a condenser column. The condenser, which was fitted at the top neck of the glass apparatus, the inlet was supplied with iced water held in a vat into which an injecting pump had been immersed and its top outlet port was fitted with plastic tube for ejecting the reject water into a sink. The air was supplied trough plastic tube from a compressed stream. A rotameter was connected between the air bubbler and the inlet, just outside the vessel to regulate the supply at a measured volumetric flow. The fourth neck was sealed with a stopper that was pulled whenever a sample was drawn for analysis. The fitted reactor had been put on a kettle heater whereby all the system was placed on a bench, in fact with all its accessories.

The temperature was applied at three levels; 50, 60 and 70 ºC while keeping the pressure at one atmosphere. The air flow was maintained at 1.5 liter/minute. The holding time for a batch of wet oxidation was varied between two, four and six hours. Thus, the three variables (use of IOCS, temperature and reaction time) were run at two levels as was designed using the Box-Behnken experiential design method and using Design-Expert® software (Design-Expert 11. Ink). The treated effluent was filtered using meshed sieve (≈0.2mm), decanted and afterwards it was characterized mainly for its COD content and the remaining sample was stored for further experiments. Following the WAO of stillage, the percentage COD reduced was analyzed by taking triplicate samples from each run according to the different temperature, time and the IOCS dosage combinations. Alongside, the biodegradability of the effluent was evaluated to describe the precursor effect of WAO in the following biological treatments.

4.2.5.Toxicity testing

In this study toxicity testing was considered useful to inform the later design and operation of wastewater treatment units whereby determining the biodegradability of the feed is one such advantage. Choi and colleagues (2017) studied that there are a variety of industrial or municipal wastewater toxicity testing mechanisms that include the use of culture microbes, animals and plant samples as indicators (Choi et al., 2017).

The current study applied *E. coli* culture growth to demonstrate the effect of pretreating of the distillery stillage on its biodegradability in terms of toxicity. To do so, an autoclaved (120 ^{o}C for 20 minutes) Luria Bertani broth that consist of tryptone, yeast extract and sodium chloride (NaCl) of 5 g each was added into two 250 ml flasks to grow the *E. coli* solution (five ml), which was isolated earlier as a pure culture. Idalia and Bernardo (2017) noted that E. coli is often preferred to other biometrics in environmental toxicity testing and it is considered as the most important model organism for long before many reasons that include its fast-growing rate, availability of a relatively cheap medium, easy culturing, low sample volume requirement, and a reproducible response (Idalia & Bernardo, 2017).

The flasks were separated as one for the raw stillage and the other for the WAO pretreated with combination of time of 4 hours and temperatures at 60 ^{o}C and with a 3.5% IOCS loading. One ml of the stillage sample (173±8 g-COD/l) was added to respective flasks. In another two test tubes, one for media only and another for stillage sample were kept as control. A batch culture was run at a pH of 7±0.2.

After inoculation, the flasks were kept in a shaking incubator, which was set at 37 ^{o}C and 86 revolutions per minute. Then, samples of one ml were taken from each flask using a pipette and in a biosafety cabinet. The cabinet was UV radiated any time before taking samples for a period of 15-20 minutes. The samples taken were then centrifuged at a relative centrifugal force of 7368.7g for 10 minutes and the supernatant was being expelled while the sediment remained used to be vortexed and diluted to bring uniformly. The consistently diluted sample was then further subsampled for the absorbance or optical density (OD) reading at 600 nanometers (nm) using a spectrophotometer. To maintain the Beer-Lambert law, to keep the detection limit for the OD reading by the instrument, the residue was diluted before taking subsamples for spectrophotometer reading. Every time a sample is tested, it was always performed in duplicate. Regarding accuracy and precision issues, no growth was observed in either of the control Falcon tubes, though the growth of *E. coli* was monitored for 14 days, however, a single sample was taken at the end of the second week after serious of samples during the first week. The incubation of *E. coli* was monitored periodically.

4.2.6.Analytical methods and data analysis

Analysis of the sample parameters was performed per the APHA standard methods guide book (APHA, 1999). Additionally, articles published in reputable journals were also referred. Routinely, pH was monitored using a digital pH and temperature probe (CyberScan pH 510, Thermo Scientific).

Solids analysis was also performed according to the procedure outlined in the APHA's publication (APHA; WWA & WEF, 1999). While performing solids test, measuring cylinder, crucibles, analytical balance, oven, desiccator and furnace were the principal items used. Similarly, the analysis of nitrate & phosphate, COD (Closed Reflux, Colorimetric Method, 5220 D**)**, and color (Methods 2120C) were done using spectrophotometric technique. BOD was determined by titration. Ammoniacal nitrogen (NH_4/NH_3-N) was measured using the Phenate Method (4500-NH_3 F) as outlined in APHA.

4.2.6.1.Standard preparation for the colorimetric chemical oxygen demand determination

The preparation of the standard for the spectrophotometric COD determination was made using a known concentration of the potassium hydrogen phthalate (KPH) and a DW was used as blank. Concentrations of 50, 100, 200, 300, and 400 mg/l were prepared after weighing a known amount of the KHP in a form of the stock solution. To prepare the 1000 mg/l COD standard stock solution, 850 mg dried (120 °C, overnight) KHP was taken and got dissolved in a 1000 ml of DW. Afterwards, OD reading (absorbance) was taken at each point of concentration and a standard curve of absorbance against the concentrations of KHP in mg/l, which was developed with goodness of fit, $R^2 = 0.998$.

4.2.6.2.Biological oxygen demand analysis

The dissolved oxygen (DO) buffer preparation was performed earlier using DW, aerator pump and air diffuser. Before buffer aeration begins, Mg SO_4, Fe Cl_3, Ca Cl_2, phosphate buffer which was comprised of the KH_2PO_4, K_2HPO_4, $NaHPO_4.7H_2O$ and NH_4Cl, one ml/l each, were added to the DW. Afterwards, the solution was aerated for over 12 hours.

Later, the stillage sample was added into two BOD bottles and one BOD bottle was incubated in BOD incubator at 25 °C for 3 days and the other was titrated to determine the DO. To do the titration, one ml of manganese sulphate solution and alkali-iodide-azide reagent were added to the BOD bottle. Afterwards, the bottle was closed and mixed by inverting the mixture several times and a brownish cloud appears in the solution as an indicator of the presence of oxygen. After allowing the brown solution to precipitate out to the bottom, a 2 ml of the concentrated H_2SO_4 was added. Then the bottles were closed and mixed well to dissolve the precipitated back. Finally, a 200 ml of each sample was titrated with a 0.025 N $Na_2S_2O_3$ to a pale-yellow colour. Then a 2 ml of starch indicator was added and the sample instantly turns blue in colour. Titration continues till the sample gets clearer and the readings were noted to calculate the BOD. All based on iodometric methods as described in standard methods, method 4500-O B (APHA; WWA & WEF, 1999).

4.2.6.3The color analysis

Standard methods to determine color that are outlined by APHA (Methods 2120B-D) were considered, of which 2120C was selected in this study since it is recommended for highly colored industrial wastewaters by the same standard. To do so, the sample taken was diluted to the level that it can be read within the transmittance reading capacity of the instrument- the spectrophotometer used. In this study, Spectrophotometer (xenon lamp light source), JENWAY 7305 with a number of selected ordinates of 10 and a spectral bandwidth of 5 nm was applied. The ten ordinates that are used to measure transmittance had different wavelengths ranging between 432 and 646 nanometers.

4.2.6.4 Nitrate and phosphate analysis

In this stud, the NO_3 in the stillage was determined using the rapid colorimetric method. To do so, two reagents (A and B) and a standard were synthesized for the spectrophotometric determination of nitrate by taking the absorbance reading at 410 nm of wavelength. Reagent A was prepared from the mixture of 5 g of salicylic acid ($C_7H_6O_3$) and a 100 ml of concentrated H_2SO_4 whereas reagent B was 2N NaOH. Before conducting the procedure, standard was prepared like for other tests which was, in nitrate case, the determination of a straight line of absorbances versus concentrations of a solution of a 1.37 g of $NaNO_3$ in a liter of DW.

As a procedure, a 100 µl of the sample was taken and mixed with a 400 ml of reagent A and the mixture was incubated at the 25 ^{o}C for 20 minutes. Following incubation, a 9.5 ml of reagent B was added, the mixture was then vortexed and cooled before taking the OD readings at 410 nm (Cataldo, Maroon, Schrader, & Youngs, 1975).

Regarding phosphate analysis, it was performed using the Vanadomolybdophosphoric Acid Colorimetric Method as outlined in APHA (Method 4500-P C). To do so, two solutions (A and B) were synthesized. Solution A was obtained through dissolving a 25 g of ammonium molybdate

($(NH_4)_6Mo_7O_{24}$) in a 300 ml of DW. Solution B was made by dissolving a 1.25 g of ammonium metavanadate (NH_4VO_3) in a 300 ml of boiling water, which get cooled and later mixed with a 330 ml of concentrated HCl solution. Later, the solution was cooled to room temperature and mixed with solution A making up to 1l volume. A standard was prepared by mixing 219.5 mg of hydrous KH_2PO_4 in DW, which was equivalent to 50 µg of PO_4^{3-}—P. The test proceeds by taking a one ml sample and a 0.25 ml reagent and vortex mixing and resting it for 10 minutes at room temperature before taking the OD reading at 470 nm using the spectrophotometer.

4.2.7.Separation weighing and storage

Three types of centrifuges were used in the current study, depending upon 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 third for up to 2 liters (SORVALL, LYNX6000 Centrifuge Thermo Scientific®) volume capacities per cell. Similarly, two different weight scales are used to size samples according to the size required; the one in milligrams to grams unit (Sartorius, Thermo Scientific®) and the other bigger is used in kilogram scale. Regarding sample or subsample storage, small, medium and large (cold room) sized refrigerators are used according to size of the sample. Otherwise, incubators have been used to store at thermophilic and other temperature conditions if not room temperature.

4.2.8.Data analysis

The replicate data collected during various experiments were first registered in a logbook. Later, the data recorded were cleaned and transferred to an Excel sheet in which average values and transformations were computed. Additionally, graphs and tables were also obtained aside from using OriginPro 2016. The data in excel were finally analyzed in Design-Expert® Software (Design-Expert 11. Ink) for optimization in response surface methodology and in R version 3.6.3 (2020-02-29) was used for determining significance, including t-test & Wilcoxon signed-rank test.

4.3.Results and discussion

4.3.1.Characterstics of molasses ethanol distillery stillage

In this study, the spectrophotometric color category, the hue, based on the dominant wavelength identified (580 nm) is found to be yellowish orange with a 60% purity (APHA; WWA & WEF, 1999). The stillage sample in this study showed the far higher total COD concentration which was over 172,000 mg/l when compared with literature reports (Wilkie et al., 2000). It also contains over 23,0000 mg/l of solids that may be due to the vacuum techniques used during liquor distillation thereby concentrating the bottom higher than the normal distillation system. Disproportionately, the amount of phosphate in this stillage sample was far higher compared to nitrate, which could be due to the excessive use of diammonium phosphate to foster yeast growth before seeding the fermentation vat (Oosterkamp et al., 2016) (**Table 12**). Following the stillage characterization, a mild IOCS based WAO of stillage was performed under ambient conditions.

4.3.2.Iron oxide coated sand characterization

Positioning the diffraction detector at 2θ, the intensity count per second was plotted using OrigionPro 2016 and spreadsheet (**Fig. 15 and 16**). The result showed that the sand had no amorphous material in each sample.

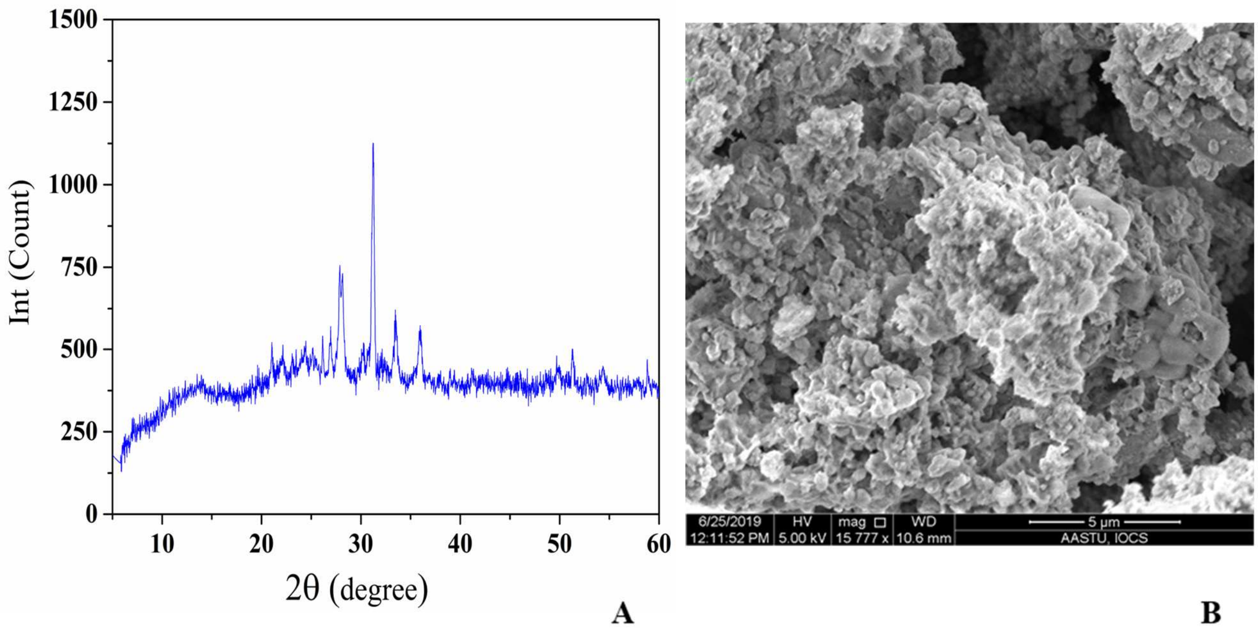


Figure 15 X-ray diffraction (A) and scanning electron microscopy (B) of iron oxide coated sand

Table 12 Physicochemical characteristics of stillage 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/l)	TSS (g/l)	TDS (g/l)	VSS (g/l)	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%							

Further, the SEM EDAX elemental analysis of the IOCS showed the composition and quantity of each element with the respective percentage (**Table 13**). The concentration of iron on the coated sand was found to be 65±3 mg/g-sand.

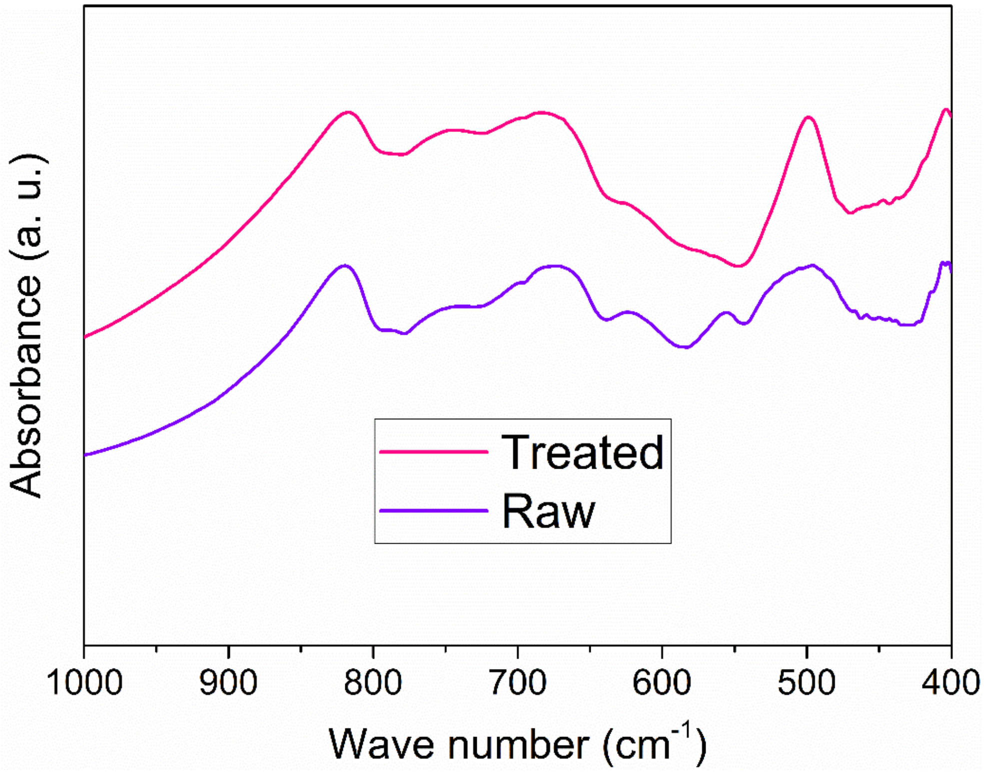


Figure 16 Fourier Transform Infrared Spectroscopy of raw and iron oxide coated sand

The FTIR analysis for the raw sand & IOCS was performed in a frequency range of 4000 to 400 cm^{-1} and a resolution of one cm^{-1}. It appeared that the treatment of sand lifted entirely the absorption status at each wave number, which might have improved the intensity/function of the treated sand significantly compared to the raw sand. The treated sand is more absorbing due to the increase surface area and pore volume brought by the attachment of iron. However, the structure appears to be consistent for both. The highest steep decline and increase in absorbance was read around 1000 wave number for both; the depression in absorbance being at 4.3 and 28.8 for the raw and treated sand respectively. Whereas, the peak in absorbance was read around 23.5 and 51.5 for the raw and treated sand respectively (**Fig. 2**).

Table 13 Elemental composition and proportions of the iron oxide coated sand

Element	Weight %	Atomic %
Oxygen	40	60
Sodium	6.1	6.4
Magnesium	0.4	0.4
Aluminum	4	3.6
Silicon	15.8	13.6
Chlorine	2.8	1.9
Potassium	1.6	1
Calcium	2.9	1.7
Iron	26.4	11.4

4.3.2.Mild iron oxide coated sand based wet air oxidation of molasses distillery stillage

By the mild WAO average removals of COD ranging between 18.5 and 27.9 percent were obtained. Though there were COD removals in the runs without the use of IOCS, it could be due to the fact that the oxidation of the less complex organics in the stillage brought the reduction in COD which is not desired in terms of retaining the degradability. Contrarily the COD reductions in the use of IOCS would have been brought due to the detoxification of the complex organics (**Table 14**). Coating and calcining the sand brings more pore size and specific surface area due to the attachment of the iron compound with durability whereby the provision of contact surface and resilience are among the desirable properties of a catalyst (Hansen, Kwan, Benjamin, Li, & Korshin, 2001; Lo, Jeng, & Lai, 1997; Öztel, Akbal, & Altaş, 2015). IOCS was opted for many reasons of which its abundance, its cheap availability even as waste material released as a by-product of iron rich groundwater aeration, chlorination and sand filtration, its remarkable biocompatibility especially for the subsequent AD treatment and magnetic property are few to mention (Devi et al., 2014; P. Xu et al., 2012).

Table 14 Chemical oxygen demand reduction after wet oxidation of stillage at different time, temperature and iron oxide coated sand loading

Temperature (°C)	Time (hrs)	IOCS (%)	Responses	
			CODr (mg/l)	% CODr
60	2	0	43667	19
60	4	3.5	62778	27
50	4	7	58334	25
50	4	0	74334	22
50	2	3.5	42778	19
70	4	0	54334	24
60	2	7	64556	28
60	4	3.5	62778	27
60	4	3.5	62778	27
70	2	3.5	64111	28
60	6	7	48111	21
60	4	3.5	62778	27
70	4	7	57889	25
60	6	0	57445	25
60	4	3.5	62778	27
50	6	3.5	55667	24
70	6	3.5	55223	24

Based on the actual coding of the temperature, time and IOCS variables as factors and the COD removal in mg/l as a response in stillage WAO, a factor response analysis has been performed using the Design Expert program. It was done to find the optimum removal of the COD while identifying the actual factor at the same time. The time of WAO were varied at 2, 4 and 6 hours and the temperature were varied narrowly between 50 and 70 °C. Accordingly, a temperature of 60 °C, a retention time of 4 hours and the percent IOCS loading of 3.5% brought an optimal COD removal. The decrease in COD removals from four hours to six hours may be due to leach back and formation of intermediate degradation products appearing due to thermal decomposition of hemicelluloses that solubilizes with time, which include the formation of low molecular weight organic compounds such as lactic acid, glycerol and acetic acid. (**Fig. 17**).

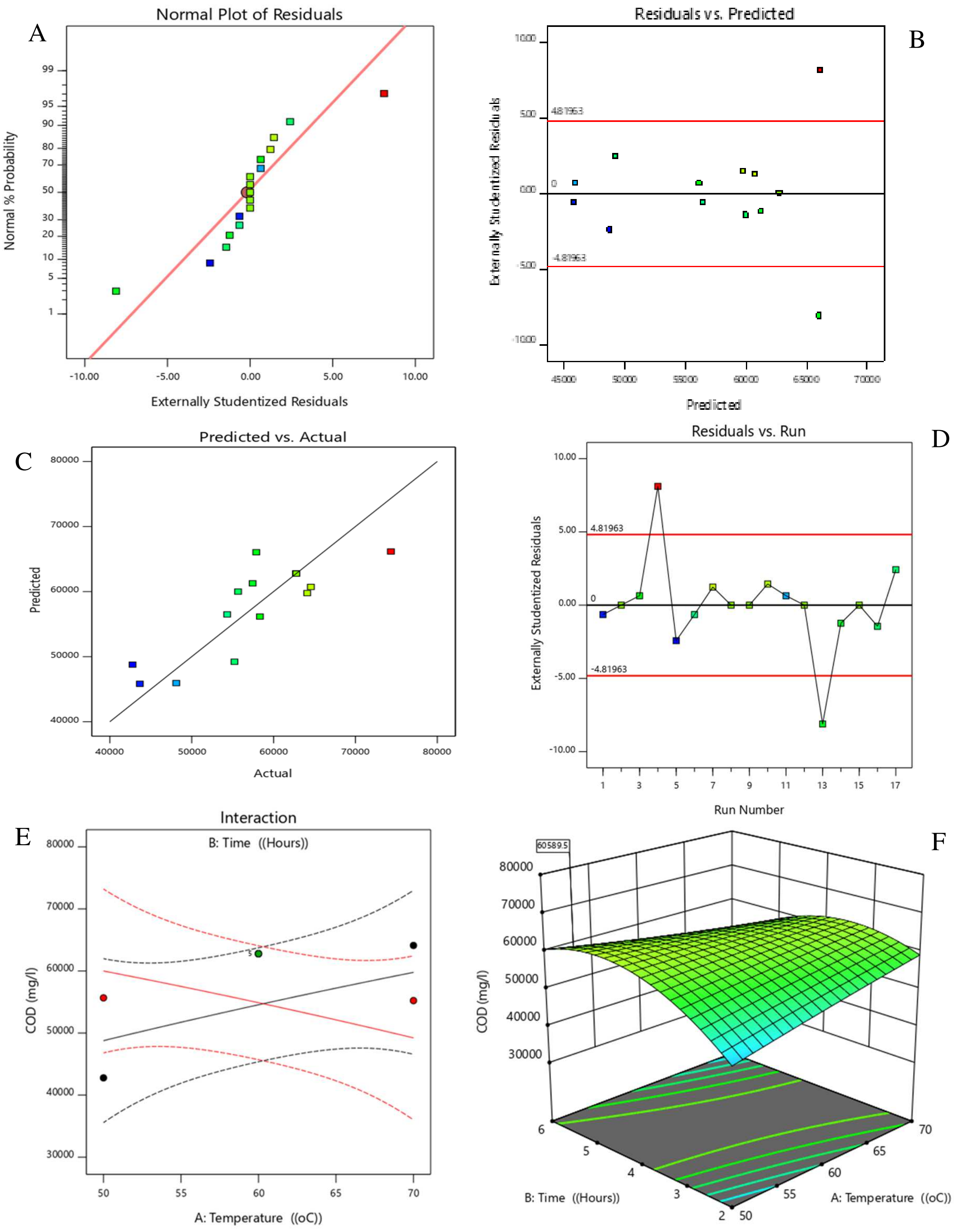


Figure 17 Diagnostic and numerical graphs: normal probability plot (A), residual versus predicted B), predicted versus actual (C), residuals versus run (D), variables' interaction (E) and model graph (F) of the wet air oxidation experiment

Based on analysis of variance (ANOVA), diagnostics and fit summary, it is shown that, the model can best be predicted in the quadratic model with a predicted residual square value of -3.5571. A negative predicted R^2 implies that the overall mean may be a better predictor of your response than the current model. In fact, the model can predict the removal of the COD at 72% fitness. The sum of squares for the model was 7.310E+08 (lack of fit=2.911E+08) while its mean square is 8.122E+07 (lack of fit=9.704E+07) with a p-value of 0.1948 and F-value of 1.95. Indeed, this large F-value can have a 19% chance of occurring due to noise resulting in the insignificance of the model for a $p\text{-value} < 0.05$. A near significant interaction (p-value= 0.0516) between contact time and the IOCS has been reported by the model whereby a significant effect was seen by the power of the former (p-value=0.0374).

Thus, based on the original unit of each factor, the following actual equation can be used to predict about the response for a given level of each factor. The wet air pre-treatment reduces part of the COD in the stillage while at the same time detoxifying and solubilizing the non-biodegradable fraction. Indeed, the IOCS based mild wet air pre-treatment in this study is motivated by its detoxification effect through various mechanisms whereby the recalcitrant constituents in the stillage are converted to bio-amenable organics so as to exploit the bio-recovery potential of stillage.

COD removal (mg/l) = -32381.9 + 938.675 * temperature (°C) + 36305.2 * time (hrs) + -3364.86 * IOCS (%) + -272.212 * temperature (°C) * time + 139.679 * temperature (°C) * IOCS + -1079.39 * time (hrs) * IOCS + -2.77625 * temperature ^2(°C) + -2013.91 * time ^2 (hrs) + -104.296 * IOCS ^2 (%)

Regarding the possible mechanisms in WAO degradation of complex organics different proposals are reported depending on the contexts of diverse processes, which include the adsorption/desorption/ oxidation. In fact, there is a big difference between the supercritical and subcritical WAO operation conditions. Unlike the supercritical operation, which completely mineralizes organics, a subcritical WAO process changes complex organics as presented in the following equation

$$Organics + O_2 \xrightarrow{gives} CO_2 + H_2O + RCOOH$$

Thus, short chain organic acids such as acetic acid make up the major fraction of residual organic compounds (Belkadhi et al., 2017; Corp, 2011). However, many experimental investigations revealed that chain reactions could occur during WAO process (Debellefontaine & Foussard, 2000).

Further, as air is supplied at high temperatures of an aqueous solution, the form in which oxygen participates in chemical reactions is complex. The elevated temperatures necessarily result in the formation of oxygen radicals, $O^{\cdot}$, which in turn can react with water and oxygen to form peroxides, H_2O_2, and ozone, O_3, so that these four species $O^{\cdot}$, O_2, O_3, and H_2O_2 are all capable of participating in the oxidation of phenol or other ingredients.

In phenol oxidation, especially at elevated temperatures and in the presence of water, oxygen is capable of inducing three different oxidation reactions. It can replace oxygen in the aromatic ring to form a dihydric phenol or quinone. Oxygen is also capable of attacking carbon to carbon double bonds to form carbonyl compounds, and also in oxidizing alcohols and carbonyl groups to form carboxylic acids. Devlin and Harris (1984) investigated that under the condition of excess oxygen, phenol breakdown is quite rapid whereby different degradation pathways are reported (Devlin & Harris, 1984).

However, WAO operated at high temperature and pressure is quite an aggressive process resulting in a process related safety and technoeconomic feasibility issues. Conversely, Zou and coworkers (2007) reported that WAO is a mild and economical process when it is operated under subcritical conditions and even more favored at ambient conditions (Zou, Li, & Hung, 2007). In this regard, several catalyst with their pros and cons are applied in conducting a subcritical WAO including use of noble metal catalysts (Mondal, Mondal, & Roy, 2016), ferrous sulphate (Ganesh M. Bhoite & Prakash D. Vaidya, 2018b), CuO (Sriprom et al., 2015), and graphene oxide (D. Zhang et al., 2014). In this study a hexahydrate $FeCl_3$ sand coating was applied as catalyst.

Thus, the $FeCl_3$ would have played a catalytic role as a sturdy Lewis acid, which form a joint product with the Lewis base and activates organic substrates which prompts phenol oxidation. Typically, the phenol oxidative dimerization followed by the coupling (*ortho* positions) and the resulting of an intermediate of a stable radical cation as identified by Wang and colleagues (2009) from using $FeCl_3$ as a catalyst (Kailiang Wang, Lü, Yu, Zhu, & Wang, 2009).

Further, Kuo and Lin (1983) studied that coupling and the combination of the radicals, oxidation of intermediates with $FeCl_3$ as a catalyst, reduction by Fe^{2+} and dehydration would have been involved in the mechanism (Kuo & Lin, 1983). Regardless of the temperature effect, the mechanism involved can propose the fact that the $FeCl_3$ catalyzed WAO. For instance, a study by Jun Lu and coworkers (2001) suggested a related mechanism in the use of $FeCl_3$ $6H_2O$ for oxidation of hantzsch 1,4-dihydropyridines while explaining the environmentally friendly nature of oxidation yields (Lu, Bai, Wang, Yang, & Li, 2001). Therefore, the oxidation of stillage could possibly have reduced its toxicity through breakdown of recalcitrant organics, which was evidenced by the following toxicity testing results. Additionally, the catalytic role of the IOCS may not merely happen as surface activity as in the heterogeneous phase, it could also be taken place in homogeneous condition, which could be brought due to the leaching of the Fe from the surface of the IOCS. The latter could even possibly enhance the degradation rate as evidenced by Bento and colleagues (Bento, Emídio, Hammer, & Nogueira, 2019).

4.3.3. Post wet air oxidation toxicity testing on stillage

The stillage after the mild WAO pretreatment at atmospheric condition, four hours of holding time and 3.5% IOCS loading was toxicity tested. Based on the spectrophotometric OD reading taken at 600 nanometers of wavelength, a divergent *E. coli* growth was observed from day one of sampling between treatments that continued to vary widely up to the final OD testing. The *E. coli* grown with the WAO pretreated stillage showed higher density as an indicator of biomass boom when compared to the *E. coli grown* with the unpretreated stillage feed (**Fig. 18**). In fact, the *E. coli* growth measured in time intervals showed a statistically significant difference between treatments (**Fig. 19**). To prove that a statistical analysis of the data obtained was performed using R version 3.6.3. (2020-02-29).

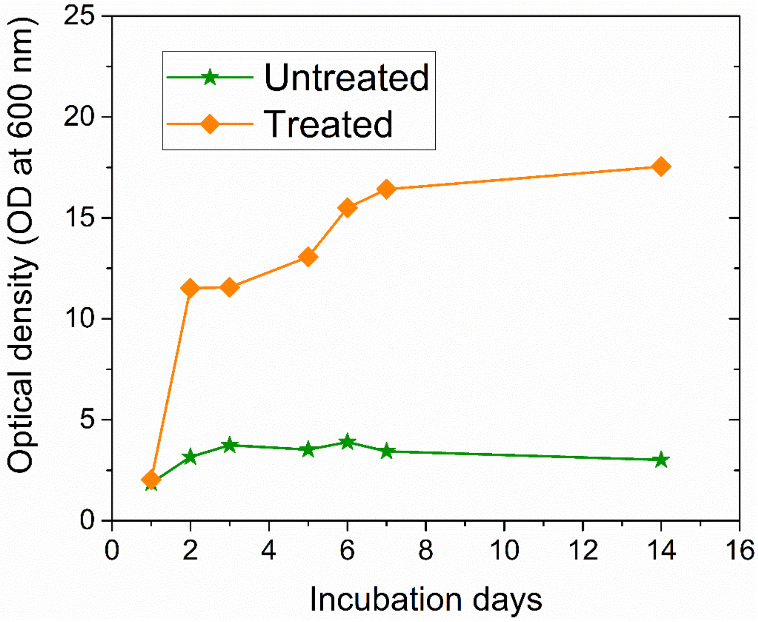


Figure 18 Average optical density of the pretreated and untreated stillage without dilution

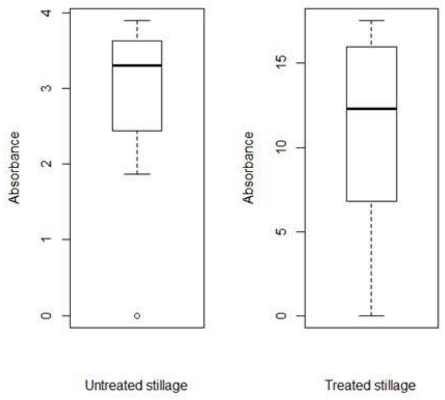


Figure 19 The box plot for E. coli growth pattern between treatments in stillage feed

Further, the description the data shows that the IOCS based WAO pretreated sample displayed a sample variance of 42.8 while the untreated is 1.67. This unequal variance and the limited sample points together does not permit the application of the central limit theorem for a t-test. Yet, the two groups were compared using the Wilcoxon signed-rank test. Accordingly, data on the growth of the bacteria using selective media were significantly different (*p*-value = 0.02) with a significantly higher density and hence growth by the IOCS based WAO pretreated stillage containing culture. The lowest absorbance read by the latter was even higher (mean ± SD = 2.83±1.3) than the highest optical density reading of the former (mean ± SD = 10.95 ± 6.5). Therefore, pretreating the toxic stillage with an IOCS based WAO can significantly reduce toxicity and enhance the biodegradability and hence its bioresources recovery potential (**Fig. 18**).

In another perspective on the toxicity testing, the same procedure was followed except the reduction of broth to one gram, which was again diluted in a 50 ml of DW. It was performed to see if dilution of substrate would be a confounding factor in the toxicity testing. Accordingly, the growth was monitored and optical density is taken using a same spectrophotometer at 600 nm for nine days (**Fig. 20**).

Based on the data points, the absorbance taken for the IOCS based WAO pretreated stillage was always higher even though the degree of variability between the non-pretreated (Variance = 0.04) and the pretreated stillage (Variance = 0.07) was a little lower relative to the undiluted sample. Yet the two groups are still significantly different on average (*p-value* = 0.03), the mean ± SD absorbance for the untreated is approximately 0.29±0.20 while it is 0.54±0.26 for the pretreated stillage.

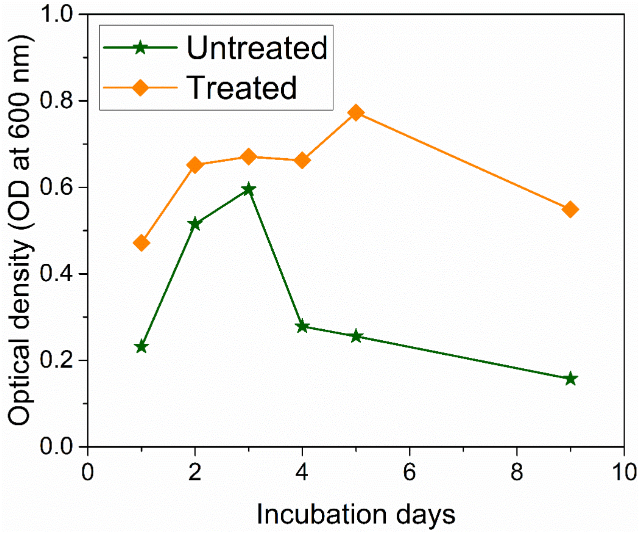


Figure 20 Average optical density of wet air pretreated and untreated stillage with dilution

Thus, despite the dilution effect that has lowered the reading value for the second cohort of toxicity test, the two groups still showed a statistically significant difference in absorbance. This implies, cell growth was consistent that the toxicity was significantly lower for the IOCS based WAO pretreated stillage when compared to the untreated stillage (**Fig. 20**). Consequently, dilution of stillage had no effect on the toxicity testing outcome before and after pre-treatment.

Regarding possible ways of reduction in the toxicity of the stillage, the mechanisms discussed under the WAO pretreatment section with respect to COD reduction and phenol degradation could similarly justify. For instance, Toda and colleagues (1989) studied that the oxidative linking of phenols & metal salts like $FeCl_3$ is quicker and extra competent, especially if it is used in a catalytic amount (Toda, Tanaka, & Iwata, 1989). In this regard, the adsorptive catalytic roles by the IOCS that is performed through a heated aeration at atmospheric pressure would have reduced the toxicity of the distillery stillage via phenolics removal. In fact, when the solution is too concentrated, the ferric oxidizes the phenols, leading to the formation of free radicals and oxidative phenolic coupling.

Though improved biodegradability due to WAO pretreatment does not hold true for every kind of wastewater, most wastewater from food and other industries including phenolic wastewater and pesticides demonstrated a reduced toxicity at various degrees and hence heightened biodegradability as reported by Nigus and coworkers (Nigus et al., 2010). Even though, different toxicity bioassays have been followed in prior studies, the current finding agrees with a review performed by Levec and Pintar (2007). The review and analysis reported regarding the lowering of toxicity by a huge magnitude due to WAO pretreatment (Levec & Pintar, 2007).

Thus, the reduction in the toxicity and hence the enhanced biodegradability of the stillage due to WAO pretreatment cook the stillage for the subsequent integration to biological treatments. In this regard, study by Gallipoli and coworkers (2019) have evidenced the limitation in the energy and economic efficiency of various biotechnologies, including the anaerobic digestion, due to the nature of biomass feed whereby pretreatments are suggested in enhancing biodegradability of the feed towards its economic and environmental feasibility (Gallipoli, Gianico, Montecchio, Pagliaccia, & Braguglia, 2019). The application of such abundant, cheap as well as environmentally friendly materials for biomass pretreatment is invaluable including the use of iron salts that even concur with the later biodegradation. Furthermore, the repeated application of IOCS proved to have an enduring efficiency. Lo and colleagues (1997) investigated the possibility of the frequent recovery of an IOCS, including after treatment by the use of dilute acid (Lo et al., 1997). In addition to the recoverability advantage, Hansen and colleagues (2001) studied that IOCS is inexpensive, is nontoxic, and can be synthesized from locally available materials (Hansen et al., 2001). To this end, the results of the current study would contribute to the energy and economic sustainability of ethanol distilleries. In addition to the improved efficiency of biotechnologies in recovering energy and valuables from stillage, enhancing biodegradability ultimately adds to the possibility of meeting industrial wastewater discharge limits set by the respective authorities.

4.4.Conclusions

The IOCS based mild WAO of molasses ethanol stillage has demonstrated a statistically significant reduction in toxicity while improving its biodegradability. Such detoxification due to the application of a WAO near ambient temperature and atmospheric pressure together with the relative abundance of sand and iron can efficiently pretreat such complex and noxious molasses ethanol stillage sustainable. In turn, the pre-treatment can help boost the performance of the subsequent biodegradation, especially with respect to improved bio amenability and energy recovery operations. Even though frequent recovery of IOCS after wastewater treatment is already reported in another study, the characterization of IOCS after WAO is recommended for further studies.

CHAPTER FIVE

The current chapter is based on the publication on objective three of the thesis as can be referred as: Gebreeyessus GD, Sreekrishnan TR, Mekonnen A, Chebude Y, Alemayehu E. Efficient anaerobic digestion of a mild wet air pretreated molasses ethanol distillery stillage: a comparative approach. *Heliyon*. 2020;6:e05539. https://doi.org/10.1016/j.heliyon.2020.e05539.

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

Abstract

The effect of a mild, WAp 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.

Keywords: Biogas, Chemical oxygen demand, Scoria, Stillage, Wet air pretreatment

5.1. 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 by-product 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 (Arasteh, Pakfetrat, & Roozbeh, 2020; Davarnejad & Azizi, 2016; Enos, 2020; Getachew D Gebreeyessus, Mekonnen, & Alemayehu, 2019).

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 (Barta et al., 2010). For instance, Beltran and coworkers tested the combined effect of stillage AD and ozonation (Fernando J. Beltrán, Álvarez, Rodríguez, García-Araya, & Rivas, 2001), Malik and colleagues reported the effect of combining AD and aerobic degradation, that even preceded by WAO (Malik et al., 2014). However, after all these efforts the COD removal 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 (Sharafi et al., 2019).

Despite its perturbing pollution potential, distillery stillage can still be used as a feed to bioenergy systems and other valuable by-product making (Asato et al., 2014; Barta et al., 2010; Chowdhary et al., 2018). 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 (Lucas Tadeu Fuess & Marcelo Loureiro Garcia, 2014; Wilkie et al., 2000). Consequently, a significant number of studies are recommending the feasibility of an integrated or coupled approach that include stillage pre-treatment to enhance the performance on recovery and discharge limits.

In fact, the success and the economic viability of anaerobic conversion depends on its pre-treatment, the nature of the feed and the scale of operation (Gallipoli et al., 2019). 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.

5.2.Methodology

5.2.1.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 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 (**Fig. 21A**) and images obtained during the BMP test (top) and the batch AD (bottom) of stillage (**Fig. 21B**). 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.

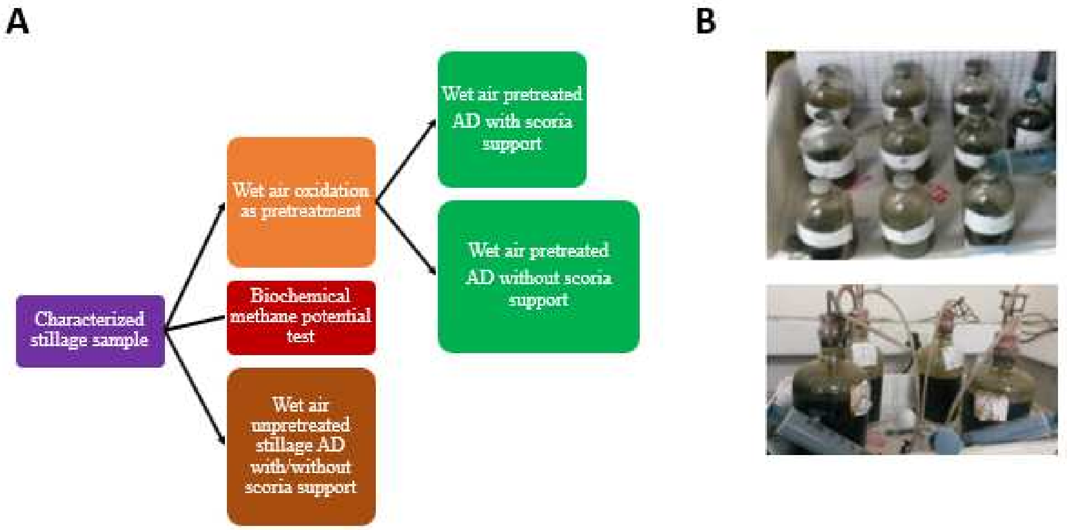


Figure 21 Diagram of the sequential experimental unit operations (A) and the setups (B)

5.2.2.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 hours at the 60 °C with 3.5% IOCS loading. The WAO was done at atmospheric pressure and on a 1.5 l/minute aeration rate, in which the pretreated stillage was also kept in refrigerator till usage in AD.

5.2.3.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. Contrary to the use of synthetic supports in AD, use of scoria appears non-bio-toxic, abundant & helpful in stabilizing pH.

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 (**Fig. 22**). 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.

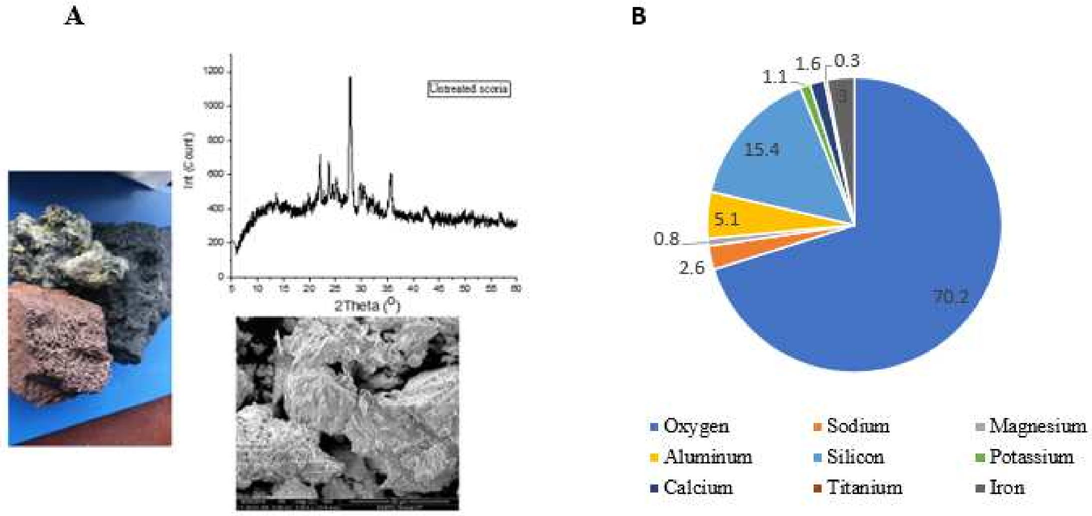


Figure 22 The x-ray diffraction, scanning electron microscopy image (A) and atomic percentage (B) of raw scoria

5.2.4.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 BMP test was conducted in a batch reaction time of 45 days following the characterization of the stillage. The BMP test was aimed at quantifying the ultimate practical methane yield that can possibly be recovered through AD, while at the same time evaluating the degree of conversion of the COD into biogas (Braguglia, Gianico, Gallipoli, & Mininni, 2015; Chan, Guisasola, & Baeza, 2020; Gallipoli et al., 2019).

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 (Filer, Ding, & Chang, 2019). Though the results were normalized to standard temperature and pressure (STP) conditions (0 ºC and 1 atmosphere), the tests were performed at 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.

5.2.5.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.

5.2.6.Analytical methods and the data analysis perspectives

The analysis of the sample parameters was mostly performed according to the APHA standards guide book (APHA, 1999). 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 (APHA; WWA & WEF, 1999) 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).

5.2.6.1.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.

5.2.6.2.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 ^{o}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.

5.2.6.3.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, due to its versatility and ease of operation, in these testing at a detection temperature of around 90 ^{o}C. The oven temperature or the GC-injector temperature was always kept around 80 ^{o}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 seconds gives the methane value, which was taken as percentage methane.

5.2.6.4.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.

5.2.6.5.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 hours.

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) (American Public Health Association, 1999). 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.

5.2.6.6.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.

5.2.6.7.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 g 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 ^oC for 20 minutes. 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 minutes at a room temperature before taking the OD reading at 470 nm using the spectrophotometer.

5.2.6.8.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 liters (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.

5.2.6.9.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.

5.3.Results and discussions

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

Following characterization of the stillage sample (**Table 13**), a biochemical methane potential test was run. Accordingly, 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 standard temperature and pressure **(Fig. 23)**. The methane yield in this BMP test is significantly lower compared to other organic substrate sources and the obvious theoretical yield (Chan et al., 2020; Filer et al., 2019; Sunada et al., 2012). As many other studies argue, including Janke and colleagues, the lower yield is related to the complex nature of the substrate as well as the additional effect contributed by the ethanol process technology (Janke et al., 2015).

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 (Lucas Tadeu Fuess & Marcelo Loureiro Garcia, 2014). Therefore, this low BMP result in combination with the high post-AD COD (see Table) suggests that stillage pre-treatment is a necessity. In fact pre-treatment of stillage could enhance its degreadability and helps to tap the ultimate methane potential (Gallipoli et al., 2019; Jönsson & Martín, 2016).

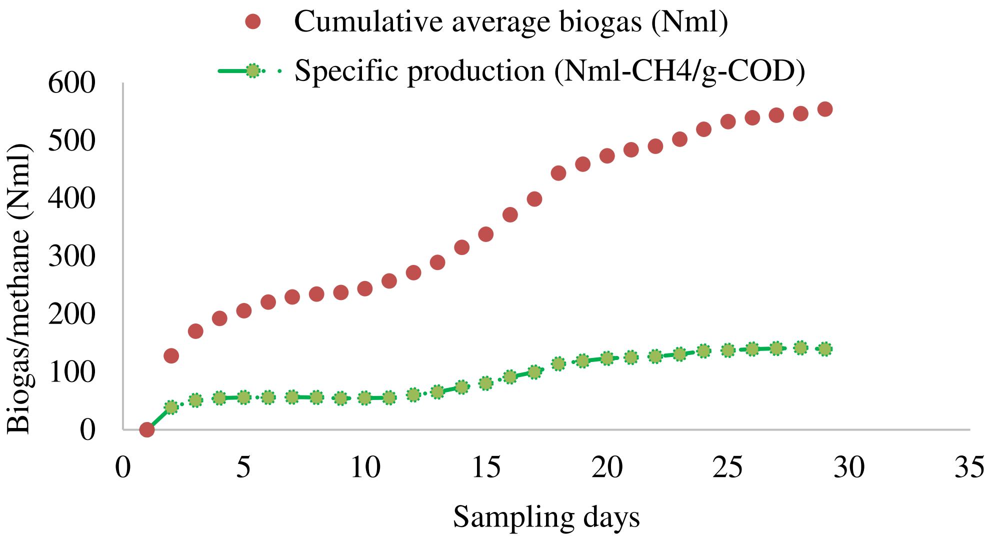


Figure 23 Averaged cumulative biogas and specific methane produced

Indeed, the post BMP analysis of stillage showed a significant reduction in volatile solid (VS) and an almost similar pH compared to the inoculum; however, the soluble COD (CODsol) remained was still higher (19.5 g/l) when compared with the inculum (5.2 g/l). This high residual CODsol is attributed to the poor degreadability 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 significantly contrary to the COD, this latter case may further strengthen the issue of the existence of some recalcitrant organics in the system (**Table 15**).

Table 15 Characteristics of stillage post biochemical methane production (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)	-

5.3.2. The stillage batch anaerobic digestion experiment

In the current study, stillage had been pretreated using an IOCS based WAO, which was performed at atmospheric pressure and mild temperature. The pre-treatment 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 pre-treatment, while both were also contrasted to 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 principally aimed at regulating pH.

5.3.2.1. Biogas and methane production of raw versus wet air pretreated stillage

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 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 raw coarse scoria as support. The scoria used possibly helped in substrate shock minimization and thereby the attainment of a relatively optimal pH in the digesters (**Fig. 24 and 25**). 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, which is 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.

The application of scoria might have helped improve the efficiency of the AD in several ways. One is the physical provision of space also known as cavities for biofilm attachment and extracellular polymeric substance formation as scoria is a highly porous and vesicular igneous rock formed several major and minor minerals. The other possible influence of the support material is the leach and hence the availability of nutrients such as Fe, K, and phosphate from the hematite, biotite and apatite minor minerals in scoria. These nutrients again help foster microbial metabolism and the anaerobic oxidation of organic matter including the effect of Fe on the increase of peroxidase enzymes (de Albuquerque, Silva, de Macedo, Gonçalves, & Rocha, 2019; Epstein, 2003; Merino, Kuzyakov, Godoy, Cornejo, & Matus, 2020).

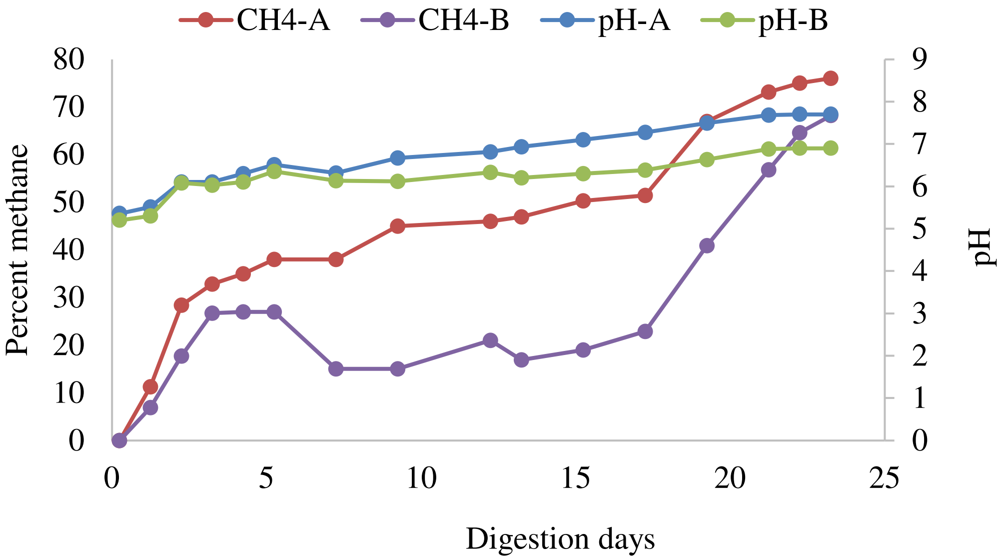


Figure 24 Methane content between stillage batch anerobic digestion using scoria (CH_4-A) and without using scoria (CH_4-B) as well as pH

Other previous studies also support the proposed effect of such related 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 (Amin, Rahimi, Bina, Heidari, & Mohammadi Moghadam, 2014). A related study also revealed the detoxification effect of scoria used as an adsorbent in the treatment of tannery wastewater (Aregu et al., 2018). Similarly, this result of improved biogas and methane yield from the use of scoria support in AD might have resulted in reduced toxicity effect of stillage. 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 (**Fig. 25**). In fact, the other biogas components, mainly CO_2 and hydrogen were also detected during the analysis.

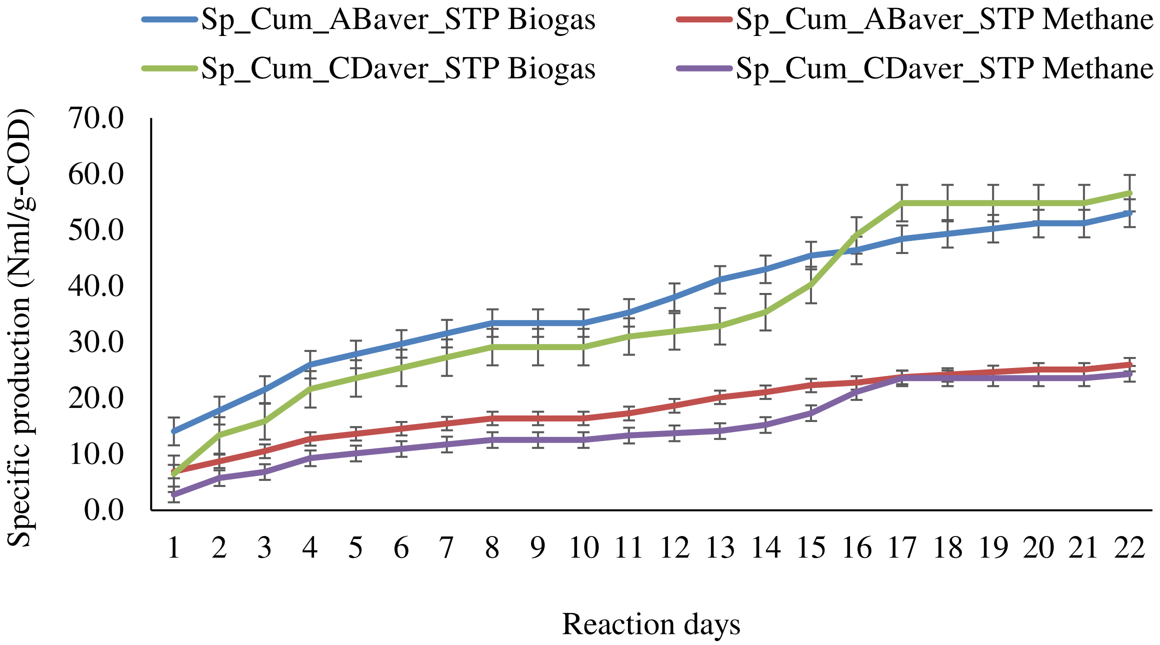


Figure 25 Variation of normalized specific biogas and methane yield against scoria support

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 = 4.276e-05) as well as the biogas produced (p-value = 0.029) showed a significant difference on average where the difference is more prominent in the case of methane yield. The degree of variability in both cases was closer; however, the average production of percent methane and 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 to consider the effect of other related minerals.

In another look, the batch AD results showed that the WAOp stillage better extracted the ultimate methane or biogas potential of the cane molasses ethanol stillage as compared to the one without pre-treatment. Interestingly, 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 pre-treatment, 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 heretofore. 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 (Lucas Tadeu Fuess & Marcelo Loureiro Garcia, 2014) (**Fig. 26, Table 16**).

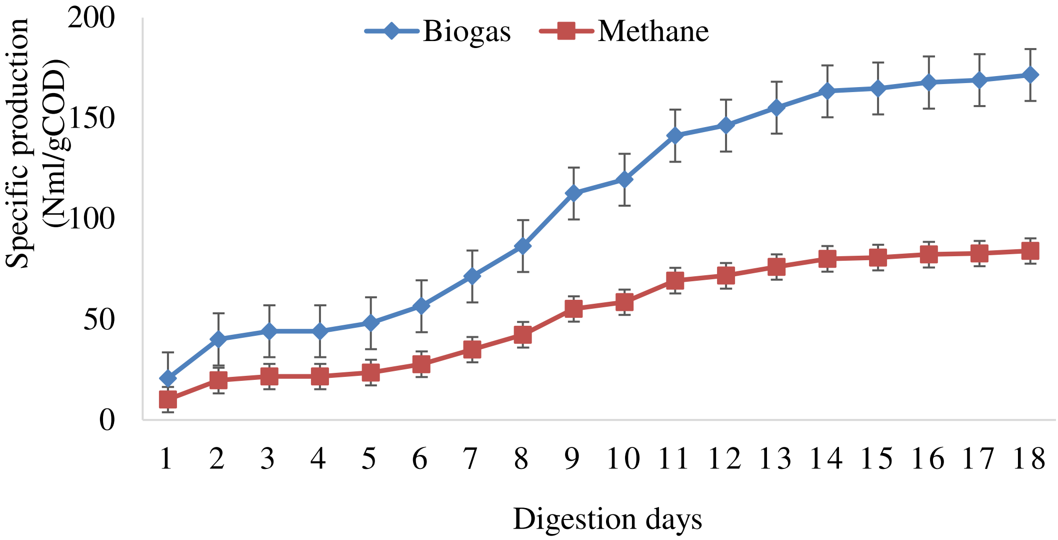


Figure 26 Normalized biogas & methane yield of a wet air pretreated stillage

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 (Grady Jr, Daigger, Love, & Filipe, 2011; Sarker & Møller, 2013). 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, pre-treatment applied and process stability whereby the latter was also monitored in the current study.

Table 16 Post anaerobic digestion characteristics of stillage with respect to pre-treatment (mean ± SD)

Parameter	With scoria	Without scoria	Scoria & 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

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 in AD 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 here dropped to 5.3 in the first two days, it later recovered itself to be over 6 within the first five days without even a chemical addition to the desired range where process instability was not a significant problem. The AD of organics passes through the hydrolysis, acidogenesis, acetogenesis and methanogenesis stages during biogas production. These stages have implications in process stability and yield.

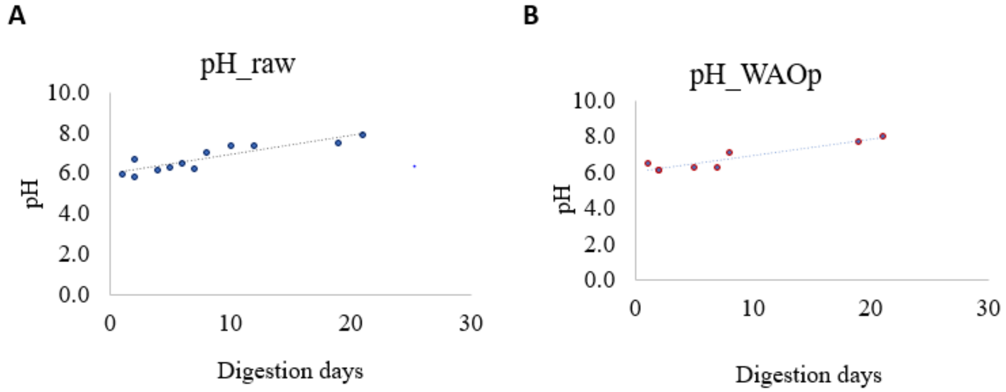


Figure 27 Averaged pH results of the digesters fed with raw stillage (A) and a pretreated stillage (B)

Both reactors showed a close reading in pH (**Fig. 27**). 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 pre-treatment, 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.

5.3.2.2.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.

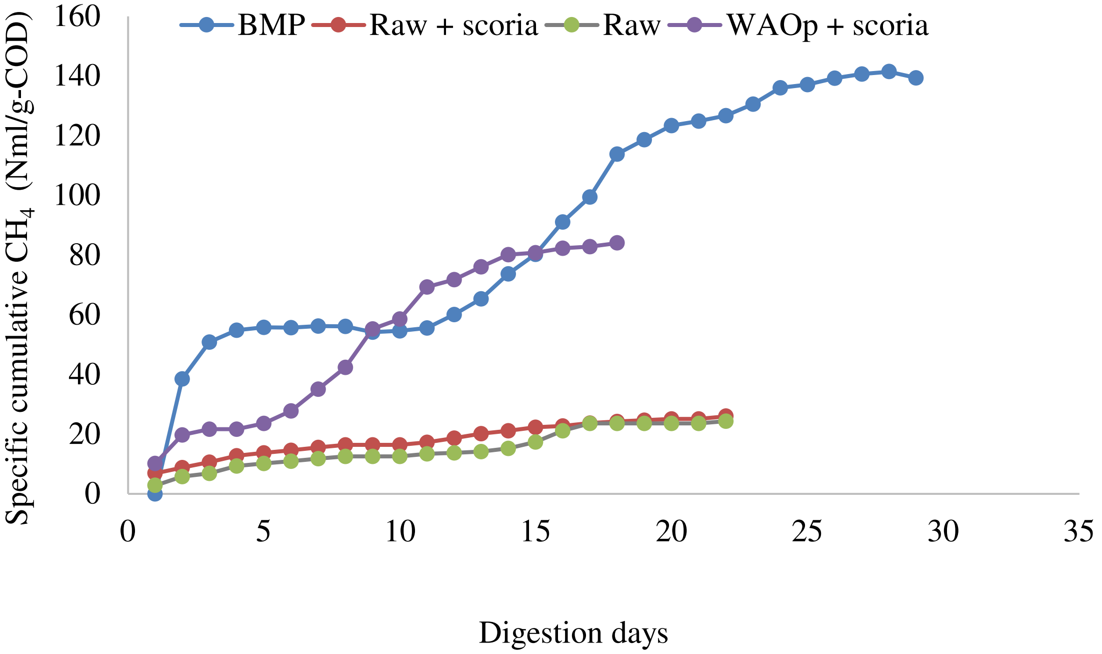


Figure 28 Comparative efficiency in methane recovery of treatments and the ultimate biochemical methane potential of stillage

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 pre-treatment, 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 (**Fig. 28**).

5.3.3.Color removal

With any treatment, the color change observed was from yellowish orange to greenish yellow, which would be due to the interference of the microbial activities and related process issues, 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 17**). 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.

5.3.4.The biochemical oxygen demand in raw and treated stillage

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 18**).

Table 17 Summary of color characteristics and pH of raw molasses stillage and after subject to biochemical treatments

		Color properties			
Sample	pH	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 and scoria	7.70	570	Greenish yellow	94.6	40

Table 18 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	4.3	132500 (3536)	0.57	
After WAO	7.2	1.6	169500 (6000)	0.73	
After AD of raw	9.4	7.6	12000 (2400)	0.40	CODsol
After AD of WAOp	9.3	8.3	0	0.00	CODsol
Blank (seeding)	9.5	8.5	1 (0)	n/a	

The application of WAO pre-treatment 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 pre-treatment. On the other hand, WAO improved the biodegradability of the stillage as it has influenced its bioamenability due to the reduction of 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 pre-treatment 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 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 (Cesaro & Belgiorno, 2015). 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.

5.4. Conclusion

The application of an IOCS based mild WAp 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. It is suspected that remaining melanoidins and even phenols, including acids, would be the cause of color in the untreated COD. The direct testing of phenols in stillage before and after treatment is recommended for future research.

CHAPTER SIX

This chapter based on a publication regarding objectives four of the research as can be referred as: Getachew Dagnew Gebreeyessus, Andualem Mekonnen, Yonas Chebude, Perumal Asaithambi, Trichur Ramaswamy Sreekrishnan, Esayas Alemayehu (2021). Effect of Stillage Pretreatment During a Coupled Scoria-Supported Anaerobic Digestion Followed by Aerobic Degradation. Air, Soil and Water Research, Volume 14: 1–9.
https://doi.org/10.1177/1178622121991810

Effect of stillage pretreatment on a coupled scoria supported anaerobic digestion and aerobic degradation

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 the 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 were removed within eight hours of retention time. Despite the color, the removal of organics in stillage due to integrating WAp, continuous anaerobic digestion and aerobic degradation was successful. The pretreatment and hybrid technique also appear as a promising technique toward the sustainable management of stillage, thereby meeting discharge limits set for the ethanol industry by regulators.

Keywords**:** Chemical oxygen demand, organic loading rate, pretreatment, scoria, stillage

6.1. 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 (Aliyu & Amadu, 2017; Im et al., 2019; Little, McGivern, & Kerins, 2016; Rehm et al., 2020; Satterthwaite, McGranahan, & Tacoli, 2010). However, the mass production of ethanol has got pros and cons regarding the water, energy and environment nexus worldwide (Lazarova, Choo, & Cornel, 2012; Sheehan et al., 2003; C. Zhang, Chen, Li, Ding, & Fu, 2018). 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 ensure the sustainability of such sectors, especially in the developing countries. For every liter of ethanol an average of thirteen liters of stillage is produced as a bottom product (de Oliveira Bordonal et al., 2018; Getachew D Gebreeyessus et al., 2019; Smeets, Junginger, Faaij, Walter, & Dolzan, 2006).

In spite of 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 COD & BOD falls far above the discharge limits set by the regulatory authorities for ethanol industries following conventional treatments to discharge their effluent to nearby environmental media (Fernando J Beltrán, Álvarez, Rodríguez, García-Araya, & Rivas, 2001; Noukeu et al., 2016; Wilkie et al., 2000). In this regard, various stakeholders revealed their concern in minimizing the environmental footprint of ethanol industries, while at the same time improving the energy self-sufficiency through recovery of byproducts thereby ensuring sustainability. Due to the cost and techno-complexity of other management alternatives, stillage AD remains preferable as it is robust, simple and feasible technique (Anderson & Baumberg, 2006; Chanthawong & Dhakal, 2016; Longati, Lino, Giordano, Furlan, & Cruz, 2019).

In spite of 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 (Pradeep et al., 2015). Those AD tests on real ethanol wastewater remained at an efficiency of around 80% of COD removal, (Espana-Gamboa et al., 2011; Kharayat, 2012; Luo, Xie, & Zhou, 2009) whereby most of the studies were even performed under batch mode (Fernando J. Beltrán et al., 2001; Wilkie et al., 2000). Few studies have been tested on the continuous mode and the improved performance of AD was reported under fixed film digestion (Kharayat, 2012). The fixed film AD used some media to pack the digesters with some synthetic medium which would help attach biofilm (Eskicioglu & Ghorbani, 2011; Eskicioglu, Kennedy, Marin, & Strehler, 2011; Getachew D Gebreeyessus et al., 2019).

Though there are emerging AD techniques for an improved stillage COD reduction (Sayedin, Kermanshahi-pour, & He, 2019), 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 (Apollo, Onyango, & Ochieng, 2013; Mikucka & Zielińska, 2020a; Padi & Chimphango, 2020). Therefore, the current study evaluates the effect of stillage pretreatment on the 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.

6.2.Methods and materials

6.2.1. Experimental setup

In the current study, the experiments were run in sequence as depicted in Figure one. The stillage brought from the molasses ethanol factory was first characterized. Following that, subsample of the stillage was mildly wet air pretreated in a four necked glass vessel using air at a flow rate of 2 liters/minute and a temperature of 60 °C. The mild and IOCS based (3.5% by weight) wet air pre-treatment was conducted for a period of four hours. After WAO of the stillage, the sample was moved onto a continuous AD alongside of the raw subsample of stillage. Later, a predetermined soluble COD containing (CODsol) reject water from the AD was moved to the aerobic degradation bottle of one-liter volume glass reactor. The reactor was sparged with air supplied from an external electric pump. Both the AD and aerobic degradation were performed at mesophilic temperatures (35±2 °C) (**Fig. 29**).

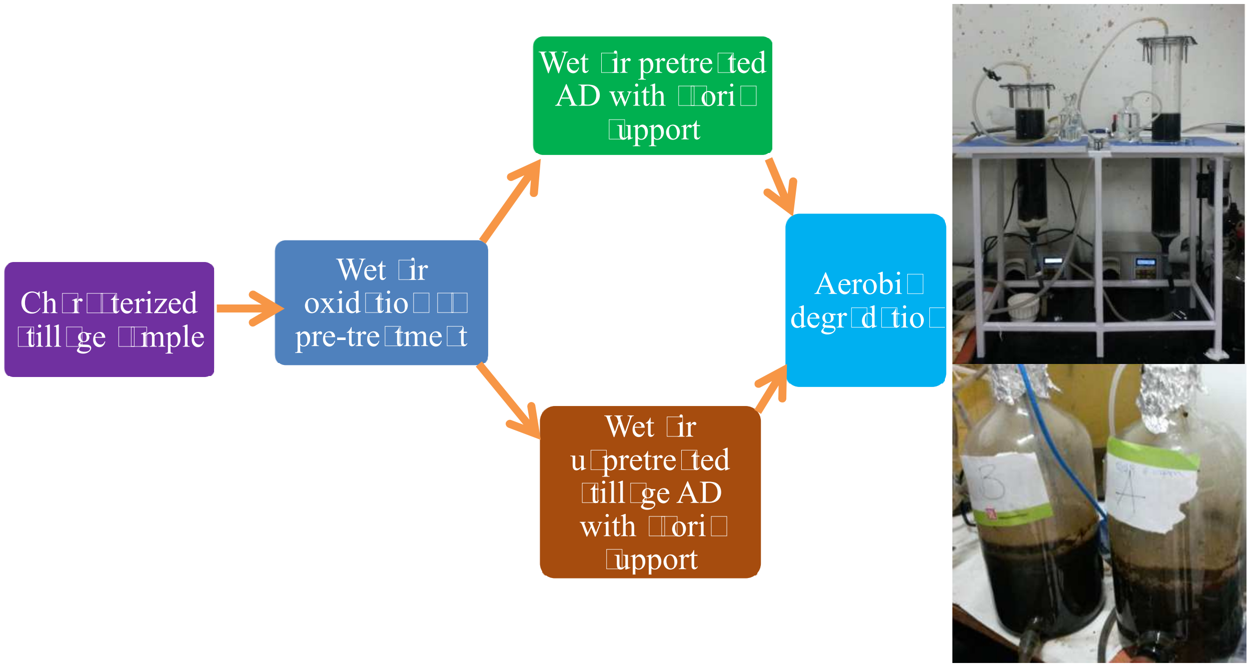


Figure 29 The diagrammatic and photographic setup of the experiments

The experiments on biogas yield and COD removal have been conducted on a semi-continuous up-flow anaerobic digester. The aim of the semi-continuous system experiment was to evaluate the performance of the COD removal from the stillage feed. Anaerobic digesters of two liters working volumes were constructed in the lab using acrylic sheets, plastic funnels, silicon tubing, clamps, pumps, and a multipurpose sealant among other materials. The digesters construction includes those accessories assembled, including the feed tank, electric pumps fixed around 150 revolutions per minute, feed inlet, gas exit and effluent ports on which the silicon tubes are fitted, and the beakers to collect the exiting effluent as well as the upright table to fix the digesters erected. Both digesters were packed with coarse scoria, a vesicular mineral rock produced in volcanos. The anaerobically treated stillage was further moved to polishing aerobic degradation. The aerobic polishing was performed using one-liter volume batch glass reactors connected to electric pump and sampling ports (**Fig. 29**).

6.2.2. Sampling

6.2.2.1.Scoria sampling

Scoria, which is a volcanic rock prevailing in the rift valley region of Ethiopia has been brought from two locations whose coordinates are taken as indicated in figure one (**Fig. 30**). The scoria has been broken down to a relatively uniform coarse size and was washed with tap water before packing.

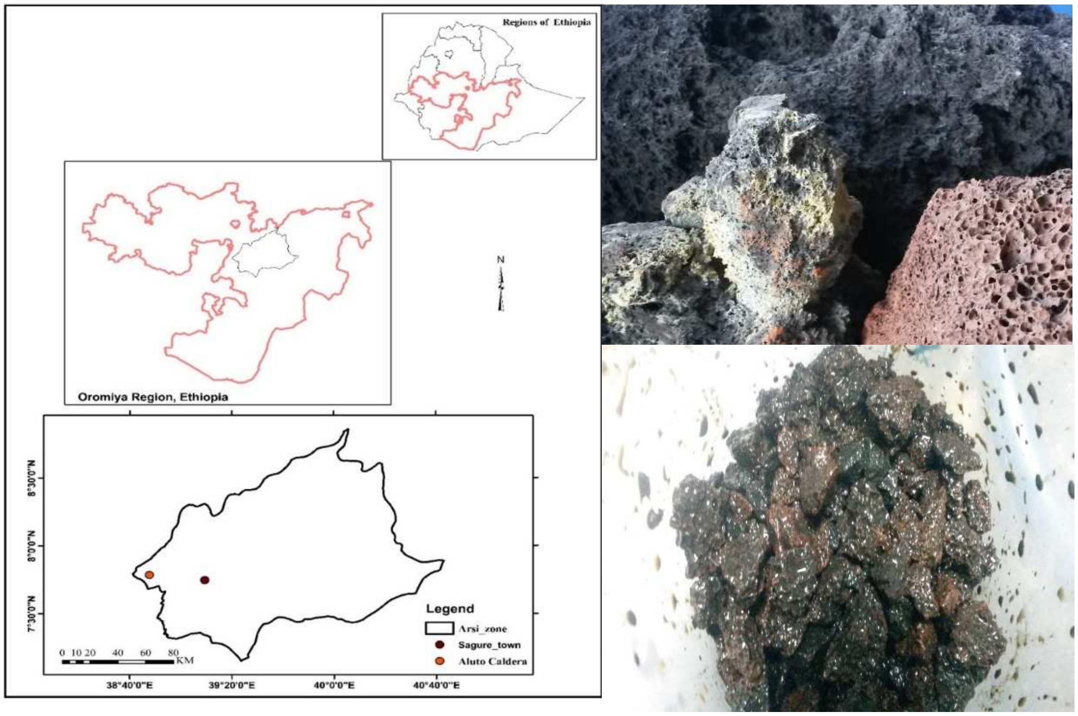


Figure 30 Location of (left), raw (top-right) and used (bottom- right) scoria sample used for the anaerobic digester packing

Characteristically, the raw scoria has been examined for its morphology and elemental composition using x-ray diffraction (XRD), scanning electron microscopy (SEM) and SEMEDEX. The SEMEDX analysis of the scoria was performed on SwiftED3000 to quantify all elements whose analysis took an acquisition time of 60 seconds, a process time of four minutes and an accelerating voltage of 15kV. No coating was applied. By composition the top four elements are oxygen (70%), silicon (15%), aluminum (5%) and iron (3%). (**Fig. 31**).

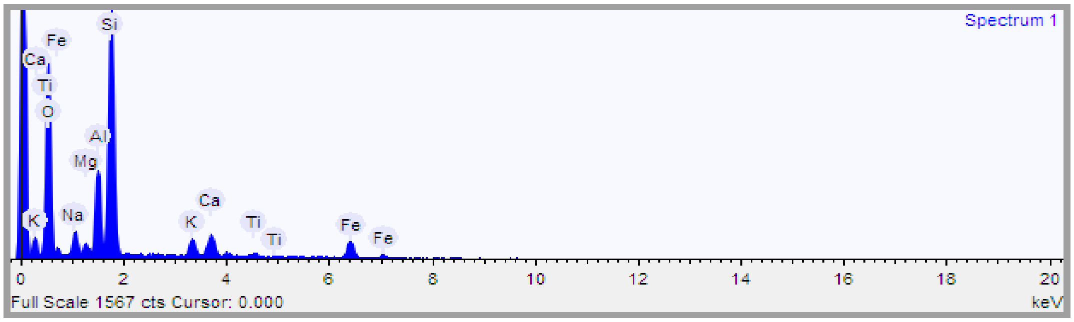


Figure 31 Spectrum of elemental composition of the raw scoria (instrument output)

The XRD image read at 2 thetas showed the non-amorphous nature of the rock. 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 (U. Epa, 2011). A further analysis of the scoria using the Fourier Transform Infrared Spectroscopy (FTIR) is presented (**Fig. 32**).

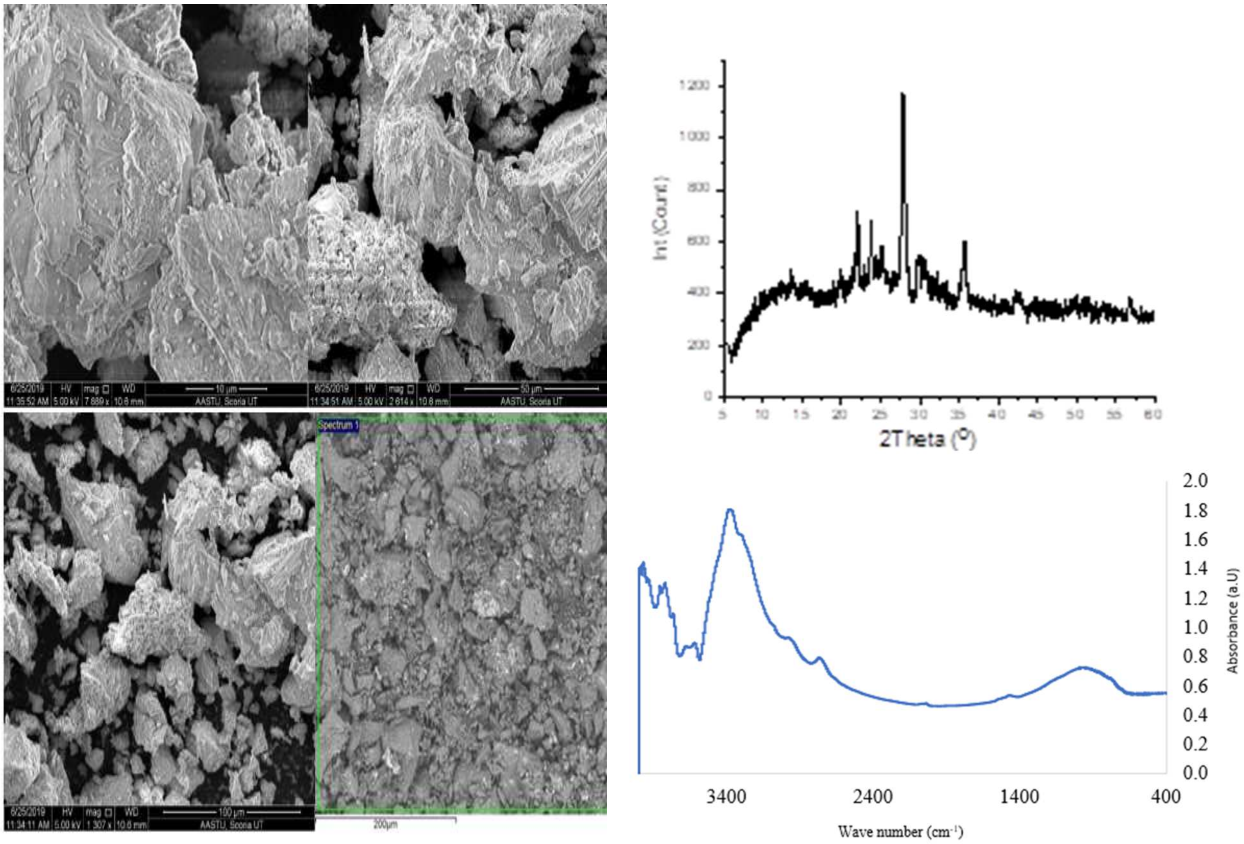


Figure 32 X-ray diffraction (right top) and scanning electron microscopic taken at 10, 50, 100 and 474.1µm resolutions (left) as well as the Fourier Transform Spectroscopy (right bottom) of scoria

6.2.2.2.Stillage sampling

A real molasses ethanol stillage sample was brought from Aligarh, Uttar Pradesh State, India. The sample has been car transported in plastic container within four hours to the working laboratory where it was further subsampled, analyzed and got cold stored.

6.2.3. 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 19**), the other was brought from a sewage AD process operating at a place in Delhi, India.

Table 19 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

6.2.4. Analysis and equipment

Standard methods were followed in the determination of stillage parameters, which include BOD and COD, before and after treatment, mainly performed based on the American Public Health Association outline (APHA; WWA & WEF, 1999). However, other standard procedures were also referred from related peer reviewed journals. During analysis, prior calibration of instruments, which include the pH meter and the spectrophotometer (JENWAY 7305) have also been performed. A thermal Conductivity Detector (TCD) based gas chromatography (GC) was used for the analysis of biogas methane (5700 NUCON). The GC carrier gas used was hydrogen performed at a detection temperature of around 90 ^{o}C. The oven temperature or the GC-injector temperature was always kept around 80 ^{o}C. Additionally, weighing (Sartorius, Thermo Scientific®), pH measuring (CyberScan pH 510, Thermo Scientific), 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 instrument FTIR Nicolet iS05.

6.2.5. Data analysis

Collected meticulous data was first entered in excel and later statistical analyses for significance using paired t-test and Wilcoxon Signed-Rank test were performed in R, version 3.6.3. 2020-02-29. The results obtained were also discussed by contrasting them with existing evidence.

6.3.Results and discussion

6.3.1. 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 to be above 232,000 mg/l with a BOD of 133,000 mg/l. However, the AD was started at a lower OLR of 120 mg/l CODsol by diluting the original sample.

6.3.1.1. The startup periods

Following the completion of digester construction and setup, old stillage batch AD inoculum of 900 ml was shared between reactors A & B each packed with the 400 ml scoria by volume on day one (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. Additionally, as a natural material it could not have a negative effect for the microbes over other synthetic support materials. Moreover, its abundance as a local rock and the possibility of nutrient leaching makes it promising substance to use.

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 two weeks by emptying the old content with entirely new one. Thus, on the 16th of November 2019, the incoula was 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 the reactors were 1176 mg/l and 1155 mg/l for reactors A and B. 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 by 999.5 ml of distilled water. From the day 16-11-19 on, 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, process stability in AD behaves as a function of substrate type, system susceptibility to xenobiotics, the mode of operation and temperature; either continuous or batch or thermophilic or mesophilic, and pH (Getachew D Gebreeyessus & Jenicek, 2016). Thus, towards 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 four days during the second week that also followed poor methane generation with 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. Generally, a stability in pH has been monitored for over 40 days (**Fig. 33**).

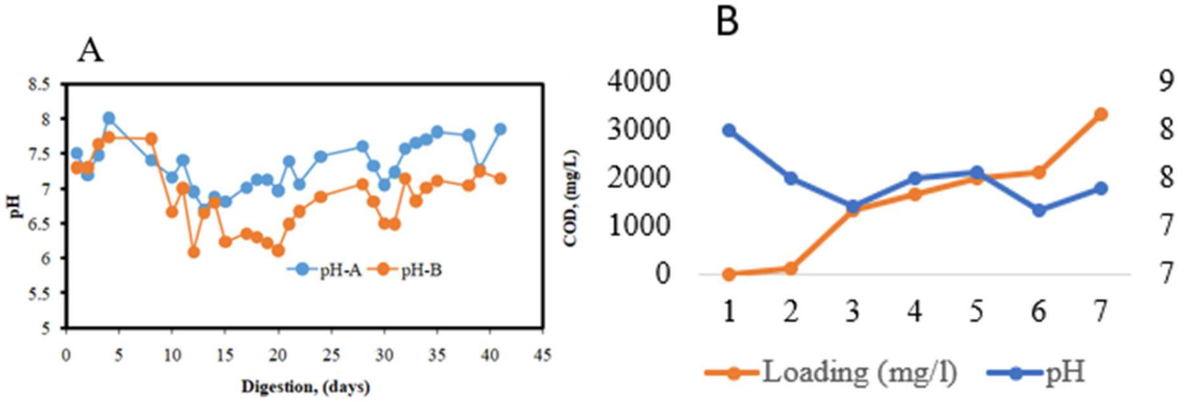


Figure 33 pH profile in the digester (A) and pH versus loading rate (B) profile in the continuous anaerobic digesters

The slightly lower pH on the second day of the pseudo-steady operation of the reactor ‘A’ was associated with the relatively higher initial COD and the subsequent hydrolysis and acidogenesis activities happening in the system. Though the slight variation in pH between the two systems persisted nearly all the time, the pH of both systems was maintained between six and eight during the entire start up period. Maximums of 8.0 and 7.7 and minimums of 6.7 and 6.1 pH have been recorded for the digester ‘A’ and ‘B’ subsequently. However, the pH monitored showed a significant difference between reactors ‘A’ and ‘B’ (p-value = 0.00) based on a t-test performed in R. Despite their major disparity in pH, the two digesters were within desirable ranges of pH except the slight increase recorded by 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 as well as feed were changed over time.

6.3.1.2. Chemical oxygen demand removal efficiency variation with feed pre-treatment

Average values from both digesters were taken for nearly two months after establishing pseudo-steady state conditions whereby OLR was varied between two to three 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 two 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 as well as the use of undiluted samples for the COD analysis. Despite the accumulating COD, the pH of both reactors was on a slight increase that 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 as well as 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 the recalcitrant organic content fit to the application of WAO. The WAp was important in lowering the solids while at the same time minimizing the toxic and recalcitrant nature, thereby enhancing biodegradability of the stillage that would help in the later biological treatment stage.

After giving some time to run both systems, the two reactors were compared against feed type for COD removal efficiency with respect to the OLR applied. Upon establishing a steady operating condition, the OLR and the COD removal efficiency in percentage is compared between the WAOp and the raw feed. The averaged organic/stillage loading rate against the CODsol removed has been compared between the wet air pretreated (CODr (mg/l) _WAO) and the unpretreated (CODr (mg/l)) for which the graph shows a relatively better performance of the earlier. Furthermore, the two systems showed inverse relation in COD removal against loading from 1667mg/l-d upwards.

Further, based on a simple linear regression modelling of the COD removal a visible difference between the WAOp feed and the raw feed has been observed; which is CODr = 0.022x OLR + 2.7381 and CODr = 0.0167xOLR + 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-value = 0.036) with an average of 13% difference in COD removal.

Despite the decline on the average COD removal at 1667 mg/l OLR, the WAOp 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 fed showed steep decline in COD removal with such an increase in OLR (**Fig. 34**). Though the data obtained is 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.

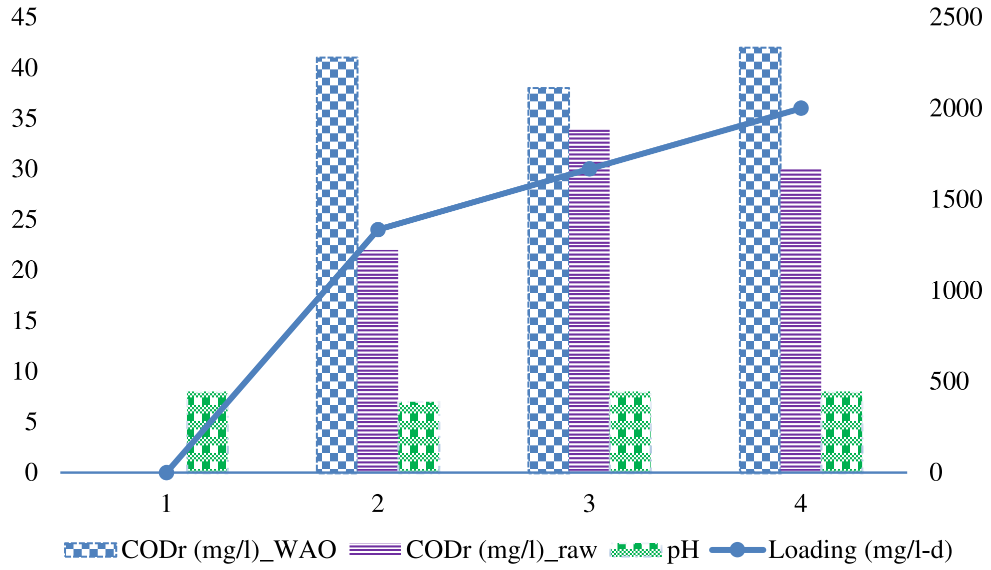


Figure 34 Stillage anaerobic digestion average pH and COD removal difference between wet air pretreated (CODr (mg/l) _WAO) and the raw (CODr (mg/l) _raw)

Though the focus of the current study was to see the effect of the stillage pre-treatment 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 OLR, ranging between 37% and 40% on average. However, a relatively better percent methane (up to 42%) was frequently recorded by the digester which was fed with a WAOp stillage. In a related experiment, the reject water from the AD testing was moved to a polishing treatment, the aerobic degradation. The pH trend convincingly shows decline initially following a steep increase in OLR due to the subsequent acidogenesis while it increased back as the system self-adjusts and continued with little variability with OLR.

6.3.2.Aerobic batch digestion of the post anaerobic digested pretreated stillage

Though there is a relatively recent attraction in AD, aerobic degradation is 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 by-products, including the endogenous respiration. In such schemes, the rate of oxygen consumption is stoichiometrically linked the organic utilization rate to cell mass buildup in the system (Metcalf & Eddy, 2003). Aerobic systems are good for their shorter hydraulic retention time. Though, the process demands many variables with a higher biomass yield resulting in a huge mass of sludge.

The mechanical supply of air/oxygen, nutrient system supplement as well as diversification of the consortia of organisms as degrading crew among other environmental factors is used to be a process performance determinant. 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 other hand, the application of either of the two systems alone is less efficient, especially in the treatment of complex industrial waste including distillery stillage (Getachew D Gebreeyessus & Jenicek, 2016). Therefore, the coupled application of both systems is reported to have a desirable effect on the degradation of stillage including the application of different pre-treatments. The aerobic degradation potential of the stillage after the WAOp AD is performed in the current study to see if the final removal is desirable to meet discharge limits, which is becoming widely and increasingly stringent.

Aerobic reactors, glass bottles of one-liter 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 eight days. During those experimental days, the COD removal of the feed, including the glucose concentration supplement, was monitored along with pH and DO (**Fig 35**).

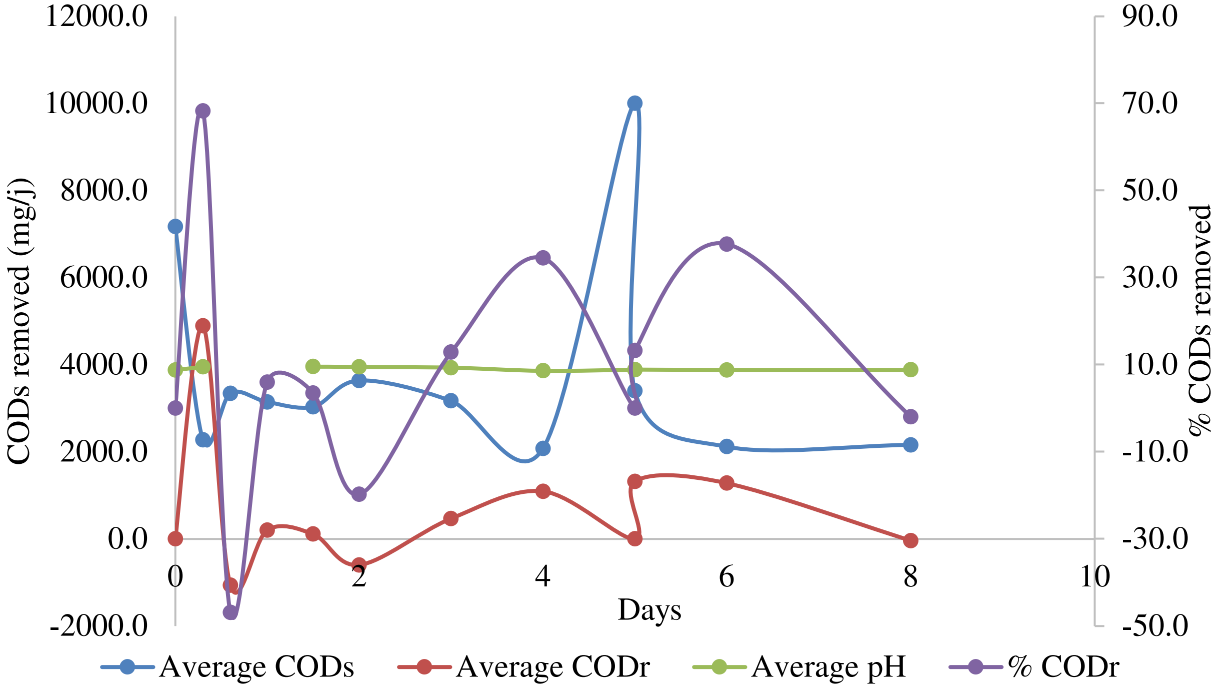


Figure 35 Soluble chemical oxygen demand removal efficiency and pH profile in the aerobic degradation of the wet air pretreated and biomethanated molasses stillage

Even though there were drops in DO up to 2-3 mg/l in the systems during the initial and when glucose supplement was performed, mostly read DO was around 6 mg/l. Though the optimal pH was around 7 and a pH of 6-9 is tolerable (Metcalf & Eddy, 2003), there were times when the pH of the systems went over 9, especially during the first three days of the experiment. Consequently, regulating the rise in pH was done by adding more inoculum on the 3rd day and by using a 2 % HCl solution, which was added to a volume of 5-10 ml to 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 eight hours. In fact, the final corrected COD after aerobic degradation is still around 2278 mg/l; however, it is far better when compared to the removal of the same, which was obtained from prior studies and of course that is far better than the same COD measured after the AD was completed (Mikucka & Zielińska, 2020a). During the aerobic test, the least COD measured is attained within four days. Besides, it has to be noted from the graph that the negative removals were representing the effect of the glucose solution added on the 5th 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 (**Fig. 35**).

The aerobic degradation has improved the overall average COD removal efficiency of the integrated treatment in the current study. Thus, the elimination efficiency has brought above 90%, which is very much closer to the set distillery wastewater (DWW) discharge limit set by the authorities, by considering the concentrated stillage alone without mixing another component wastewater in the sector. More significantly, the current integrated biological treatment has completely removed the BOD of the stillage feed. In Ethiopia, the discharge limits are set in a way that the waste treatments are efficient enough for the effluents to be able to discharge into the natural water streams. Like most other developing nations, 250 mg-COD/l and 60 mg-BOD/L effluent or less are set for the ethanol industries.

Despite the existing reality of the local river pollution, which is already becoming open channels of wastewater of either industrial, municipal or agricultural origin because of the absence of well laid infrastructure for sewage management, industrial discharge limits can have alternatives. For instance, the discharge limits can be set at two 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, the former being at a relatively high level. In such cases, discharge limits are set at two stages whereby linking to sewage can easily be met.

Considering the huge water portion in the entire DWW, this degree of reduced COD level along with total BOD removal can meet the discharge limits on its own even without the need to join other municipal waste streams as far as COD and BOD are concerned. Moreover, it is not only pollution minimization but the recovery of water also has to be given large emphasis as the sector is part of a major water consuming category, that is the industry and agriculture, that consume 90 % of the overall global freshwater (Supply, Programme, & Organization, 2015). Such removal efficiency is superior compared to reports based on coupled treatment studies (Beltran et al., 2001; Fernando J. Beltrán et al., 2008; Kharayat, 2012).

6.4.Conclusions

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 eight 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 organics removal, especially with WAp. 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.

CHAPTER SEVEN

This chapter deals with the adsorption trial conducted to test if acid treated scoria could remove color and COD as mentioned in objective five of this thesis

Treated scoria adsorption efficiency trial on a wet air pretreated anaerobically digested molasses ethanol stillage

7.1. Introduction

The efficient management of industrial organic wastes including stillage cannot be achieved using a single technology. The integration of physicochemical and biological techniques can have the potential for a sustainable management of stillage through enhanced recovery thereby adhering to the stringent discharge limits (Lin & Luque, 2014; Mikucka & Zielińska, 2020c; Mussatto, 2016). This polishing alternative treatment could even help regenerate water to a natural sink.

Adsorption of pollutants on to organic and inorganic materials with and without pre-treatments have been explored by many, which include the application of waste materials to heavy metals removal (Kim & Kim, 2020; Mohajeri, Selamat, Abdul Aziz, & Smith, 2019; Nsami & Mbadcam, 2013). Adsorption as a physical, chemical phenomenon has been considered based on a synthetic phenol wastewater removal performed on a lab scale using different adsorbents, which includes the use of scoria. A recent study has reported H_2SO4 treated scoria showed maximum phenol removal as the acid treated adsorbent was applied on a range of 0.01-0.07 mg/ml. The same study stated the resulted adsorption had followed a pseudo-second order kinetics (Sharafi et al., 2019). Thus, it was upgraded in the current study to real sample, which is known to have phenols as nonbiodegradable matter in it. This was assumed to catch the recalcitrant organics after the AD of the stillage as an integrated treatment approach to it.

Integrated stillage treatment approaches are frequently recommended as a single technology couldn’t manage it effectively. Therefore, the application of polishing treatment, including adsorption could be an alternative to be integrated. In this regard, looking into available and cheap resources would be worth testing with some surface property modification. Thus, the AD effluent was subject to scoria adsorption parallel to the polishing aerobic degradation performed.

The batch adsorption experiment in the current study was performed based on the phenolic adsorptive effect of acid treated scoria, a vesicular brown/black colored volcanic rock, which is obtained from two location in the rift valley area of Ethiopia. Varying the adsorbent dose and the contact time, the batch test performed showed a reduction of recalcitrant organics in terms of the CODsol removed per unit volume of the stillage sample. Though, the effluent from the batch adsorption bottles does not meet the stringent stillage discharge limits set by regulators in itself. However, the significant reduction of the CODsol in the effluent following batch scoria adsorption of a biochemically pretreated molasses ethanol stillage can either be mixed with other wastewater stream within the same ethanol factory or it can alternatively be mixed to municipal sewerage systems.

7.2. Methods and materials

7.2.1 Materials and equipment

Different material, equipment, reagents and chemicals have been used to carry out the adsorption test in the current investigation, which includes the centrifuges, weighing scales, grinder, sieve, H_2SO_4, distilled water, glass bottles, COD reagents and chemicals, filters, spectrophotometer, falcon tubes and a shaker incubator. These lists of equipment were basically used during scoria pre-treatment, stillage sample preparation for a batch adsorption and during analytics that include CODsol analysis.

7.2.2. Scoria pre-treatment

Earlier in this study, scoria has been physicochemical pretreated principally involving sieving, size reduction, acid washing and acid soaking to enhance its surface activity (**Fig. 36**). The whole purpose of size reduction was to increase the surface area of the adsorption material, the scoria, so that pollutants can be deposited over it. Likewise, acid pre-treatment removes impurities and organic materials so that adsorptive interference can be minimized and surface-active adsorbents can be exposed sufficiently (Guo et al., 2019; Sharafi et al., 2019).

Material properties have been characterized using XRD, SEM coupled with elemental composition analysis and Fourier transform spectroscopy (FTIR). The XRD image of the intensity count at two thetas, especially between 25-35, showed a structured or crystalline, nature of the material even without pre-treatment, however there has been a slight difference in intensity count in favor of the treated scoria. The later difference shows the enhanced exposure of nature of the material due to the acid treatment as humic and other impure materials have been removed (**Fig. 36-A, B**). Later, the surface property of the scoria has been examined using a SEM at different magnification. By comparison, the surface of the treated scoria showed smoothness than the raw scoria (**Fig. 36-C, D**).

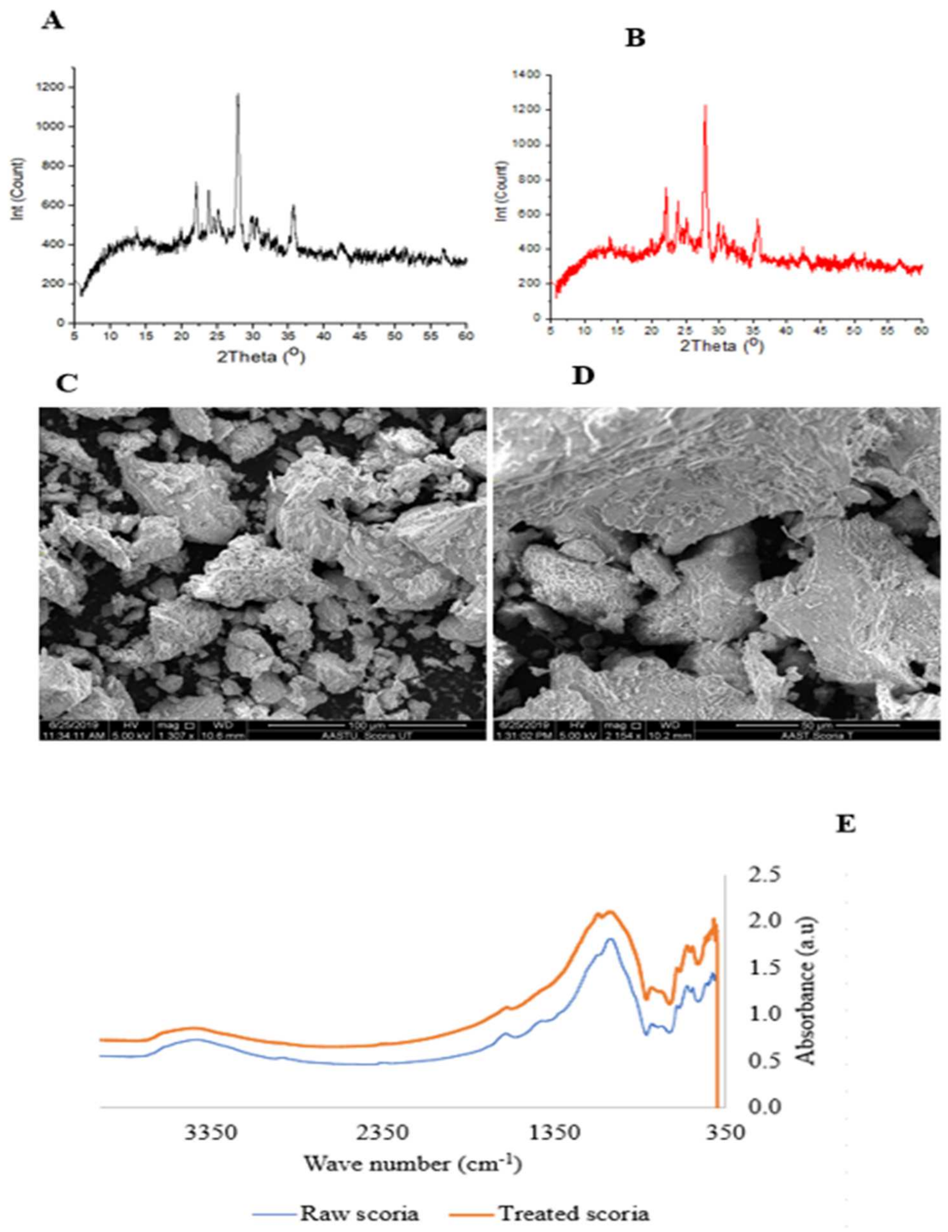


Figure 36 The x-ray diffraction, scanning electron microscopy and Fourier transform spectroscopy for the raw (A, C, E) and the treated (B, D, E) scoria

The SEM coupled elemental analysis also showed variability in elemental composition between the raw and treated scoria. Though both of them revealed same composition with nearly same magnitude, which include magnesium and titanium, slight difference in the relative elemental compositions such as iron, oxygen, calcium and silicon have been seen (**Table 20**). The FTIR image showed a peak in absorbance around a 1000 wave number for both treated and raw scoria.

Yet again the intensity of absorbance was higher for the treated scoria as compared to the raw, which is proving the same thing with that of the XRD image (**Fig. 36-E**).

Table 20 Relative composition of the elements in raw and treated scoria

	Treated scoria		Raw scoria	
Elements	Weight %	Atomic %	Weight %	Atomic %
Oxygen	56.451	71.012	54.569	70.212
Sodium	2.865	2.508	2.877	2.576
Magnesium	0.884	0.731	0.883	0.748
Aluminum	6.629	4.944	6.654	5.076
Silicon	23.021	16.496	20.976	15.374
Potassium	1.751	0.901	2.162	1.138
Calcium	2.264	1.137	3.145	1.615
Titanium	0.989	0.416	0.661	0.284
Iron	5.147	1.855	8.073	2.976

7.2.3. Stillage sample conditioning and the test procedures

The biomethanated stillage has also been conditioned, the wet air pretreated stillage sample after batch AD was decanted and centrifuged at 800 revolutions per minute (rpm) for 30 minutes to remove the suspended solids. The treated scoria was weighted using standard lab balance (Sartorius Quintix$^{®}$) according to the design of experiments. A 100 ml each of the 2.5times diluted decant sample was poured to batch test glass bottles of 130 ml volume to which the weighed scoria was also added at 0.03 mg/ml, 0.075 mg/ml and 0.12 mg/ml doses respectively and only stillage is run as control, all in duplicate **(Fig. 37)**. Afterwards, the test bottles were kept in a shaker, which was set at 170±5 revolutions per minute.

Figure 37 Photograph images of the batch adsorption testing trials performed

7.2.4. Analytics

7.2.4.1. Sample preparation

Samples were taken at 3, 7.5, 12- and 24-hours' time interval for CODsol analysis while the pH was being recorded at the same time. The samples taken in a falcon tube were centrifuged at 9000 rpm for 10 minutes whereby subsamples of 1ml from each bottle was transferred to other falcon tubes containing 9 ml of a distilled water into which each subsample was diluted to 10 times. Later, the dilute samples were subsampled to 2.5 ml volume and were passed through a filter of 0.2 µm pore diameter using syringes by following the standard methods (APHA; WWA & WEF, 1999) .

7.2.4.2. Chemical oxygen demand analysis

To do the CODt as well as the soluble fraction the chemical digestion method was applied (USEPA, 1993). Hence, the stillage sample is mineralized by adding a concentrated H_2SO_4 and $K_2Cr_2O_7$ solutions into those COD vials containing the stillage sample and are oxidized in DRB 200 digester which is set at a temperature of 150 °C for 2 hours. Next, the absorbance of the sample was measured at the 600 nm, which works based on a change in color of the potassium dichromate. A spectrophotometer, DR3900 (HACH LANGE), was used to measure the absorbances as a function of the amount of the COD.

7.2.5. Data analysis

The data obtained was first entered in to a lab logbook, which later was processed and finalized and entered into spreadsheet for presenting into tables, graphs and further numerical analysis. The data constructed was then contrasted, discussed and interpreted based on related studies.

7.3 Results and discussion

Adsorption can have any of the physical, chemical and electrostatic mechanisms to take place whereby the chemical adsorption can be expected if the adsorbent is treated with chemicals that affect its pH especially with the non-ionic organic solutes including hydrolysis (Moreno-Castilla, 2004; L. K. Wang, Leonard, Goupil, & Wang, 1975). There was relatively highest pH with a fairly stable condition (SD±0.08) at scoria dosage of 0.03 mg/ml which could have implications in the adsorption, despite the acid treatment, these relatively higher pH, even at each dose of the scoria. In fact, pH might have also impacted the adsorption of COD onto the treated scoria due to electrical adsorption of the cationic complex organics. Contrarily, the low pH would have enhanced the adsorption of anionic organics whereby sulfates and chlorides can be mentioned as possible targets in distillery stillage as they appear in significant concentration which could have a reversing effect. Generally, studies also showed that phenol adsorption declines sharply above solution pH 7 (Mohana et al., 2013; Moreno-Castilla, 2004). Despite the narrow gap observed, the least pH is recorded at a scoria dosage of 0.12mg/ml and after 24 hours of adsorption for all the doses (**Table 21**).

Table 21 Average pH of the subsamples taken at intervals of time against adsorbent dose

	Contact time (hrs)			
Scoria dosage (mg/ml)	3	7.5	12	24
0.03	8.57	8.51	8.55	8.39
0.075	8.55	8.41	8.35	8.18
0.12	8.45	8.36	8.225	8.14
Only stillage	8.59	8.65	8.46	8.49

In the current study, almost all the removals were noted at 3 hours of contact time for all the doses, which are 42.8, 42.8 and 50.4 percent for 0.03, 0.075 and 0.12 mg/ml respectively. Removal rate was maximum at 0.03 mg/ml for 3hrs and lowest for all doses of scoria at 7.5 hrs. From 7.5 hours until 24 hours of contact time, the 0.12 mg/ml dosed tests showed slight improvement in removal.

The simple linear regression model for the COD removal as a function of time is shown as % CODr = 1.3856t + 28.232 (**Fig. 38**). The mechanism by which the scoria adsorbs organics include physical adsorption and chemisorption of the organics to base active sites.

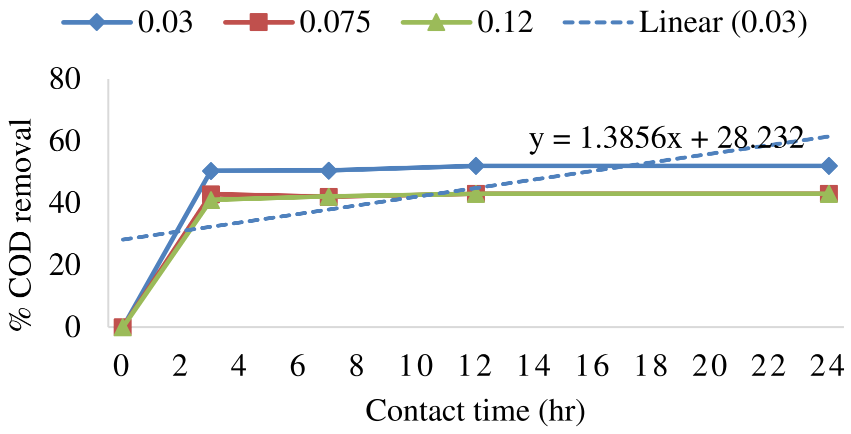


Figure 38 The adsorptive percent chemical oxygen demand removal against contact time at each scoria dosage

Based on the laboratory data acquired, the removal of CODsol by adsorption was not that significant to trigger the discharge of the effluent to a natural water body while meeting the standards set by the regulators. However, the sample taken in this study was only distillery bottom that the entire distillery wastewater was far diluted by content which will certainly affect the concentration of the COD perhaps to a level low enough to be discharged to community or municipal wastewater, if not to natural water bodies (**Fig.38**). It has to be considered that the application of such abundant and cheap natural materials is worth minimizing the cost of treatment when compared with the artificially aerated biodegradation units.

The purpose of this study was to test the integration effect of different stillage treatment technologies, including adsorption, in view of the efficient COD removal. Thus, the COD of stillage after AD did not vary as a study factor, rather contact time and adsorbent dosage were varied causes difficulty in kinetic modelling. However, the scoria adsorption isotherm for removing COD in stillage was modelled using linearized/non linearized technique (Table 22) with respect to contact time and adsorbent dosage. The COD adsorbed (q_e in mg-COD/g-scoria) was calculated using the famous equation:

$$q_e = \frac{(C_o - C_e)\mathrm{V}}{m}$$

where C_0 and Ce (mg P/L) are the initial and equilibrium phosphate concentrations, respectively, V (L) is the volume of the solution and m (g) is the mass of the adsorbent.

Table 22 Adsorption data for different adsorbent dose and contact time

	Scoria dosage (mg/ml)					
	0.03		0.075		0.12	
Time (hr)	Ce	qe	Ce	qe	Ce	qe
3	7154	242306	8250	82307	8510	49279
7.5	7139	242787	8365	80769	8351	50601
12	6923	249999	8221	82692	8221	51682
24	6923	249999	8221	82692	8221	51682

According to table 22, a significant removal of the COD in terms of Ce/qe was high during the first three hours of contact time and adsorbent dosage of 0.03 mg/l while there as been a slight increase until 12 hours at the same dosage. Contrarily, removal was lower with time until 12 hours for the doses at 0.075 and 0.12 mg/l, which could be due to the possible desorption while shaking the solution. Obviously, the cumulative graph for the adsorptive COD removal is increasing with time for all the doses. The trend line, however, fitted better for the adsorbent doses at 0.03 and 0.075 mg/l when compared to the adsorbent dose at 0.12 mg/l (**Fig.39 A-C**). The non-linear fit for the adsorbent dosage at 0.03 mg/l did fit well with the pseudo- Langmuir model (**Fig.39 D**). Despite that, modelling the isotherm needs evaluation at varying pollutant dose.

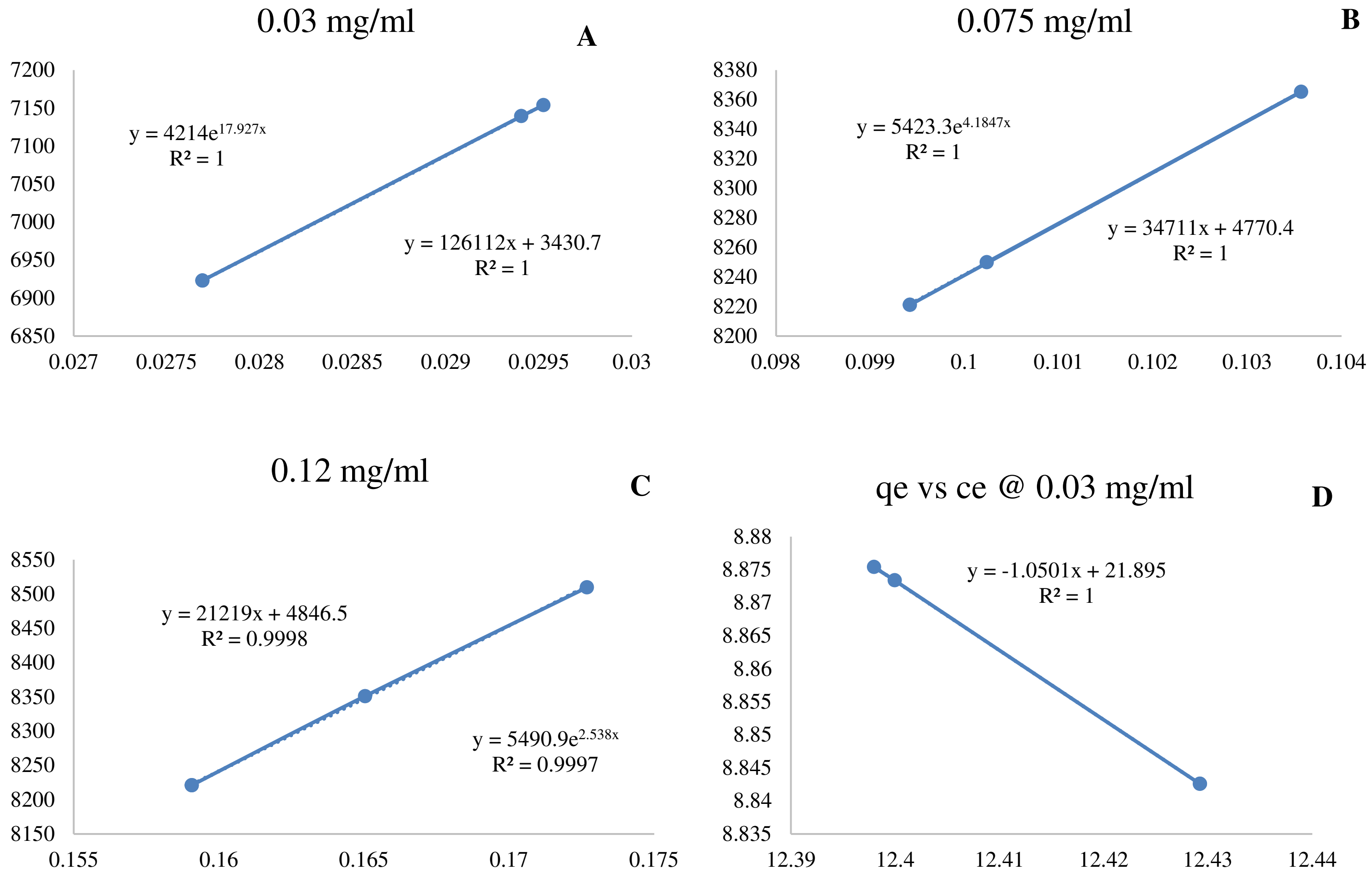


Figure 39 Linear (A-C) and non-linear pseudo-Langmuir fitting (D), isotherm models at different adsorbent dose and contact time

7.4. Conclusions and recommendation

The final COD of the stillage did not go well to the lowest level as expected; which was recorded at 7500 mg/l. Therefore, the discharge limit by standard authorities has been met for BOD after integral of treatments but not for the COD, which suggests the need for merging of other wastewater with the treated stillage will probably lower COD to the standard, then only it will allow to meet the set standard. Thus, further study on adsorbent surface modifiers or even doses are recommended.

CHAPTER EIGHT

Summary and recommendations

The rationale behind this research project was the issue of the sustainable management of the natural resources, including water courses that are being polluted by the discharges of industries like the distilleries. Hence, the focus was on the minimization of water pollution & maximization of resource recovery from such wastewater. In this regard, the increasing distillery stillage water pollution and inefficiency of the existing stillage treatment technologies have been identified as pollution problems and technological limits. The stillage discharged without treatment brings terrestrial and aquatic ecosystem toxicities thereby affecting biodiversity as well as productivity. The application of a single treatment technology is reported inefficient to manage stillage. For example, stillage physicochemical treatments as well as the multiple effect evaporation and incineration are either associated with the huge investment and operating costs or result in secondary waste instead of recovery.

Thus, the objective of the current study was to control stillage water pollution through integrated efficient treatment and recovery. To do so, materials preparations, treatments and lab scale application on a real stillage sample using wet oxidation, AD, adsorption and aerobic digestion technologies were performed using design of experiments and standard analytics. These has involved the application of a mild IOCS based wet air oxidation pretreatment, scoria supported AD and subsequent aerobic polishing or a treated scoria-based adsorption. In fact, measuring the magnitude and complexity of ethanol stillage in itself was a first step in this study whereby the quantitative characterization of molasses and molasses stillage in Ethiopia was determined. In Ethiopia the annual production of potable ethanol from cane molasses jumped from where it was 3000 m^3 before 2000 to above 33000 m^3 in just two decades, which alone has the potential to a discharge of over 431,000 m^3 stillage. The emerging practice on fuel ethanol production further lifted the stillage discharge in the country to 260 million liters annually with almost all discharged without proper treatment.

Following stillage quantitative characterization and materials syntheses, the effects of an IOCS based mild, wet air stillage pre-treatment followed by AD of with or without scoria support and a polishing scoria-based adsorption or aerobic biodegradation has been examined on a lab scale if such integration can efficiently treat distillery stillage. The efficiency of treatment and recovery were evaluated based on COD removals and biogas or methane production as well as quality. As a result, the application of the IOCS based mild WAO pre-treatment has significantly minimized the toxic nature of the molasses stillage based on a subsequent *E.coli* incubation test thereby contributing to a significantly improved efficiency of the AD. The AD efficiency has been witnessed by the comparable significant COD removal and hence a quality biogas recovery to an almost complete degradation of the BOD in a short time contrary to the AD of the non-pretreated stillage. Hence, the WAO pre-treatment has enhanced the degradability of the stillage.

The overall average treatment efficiency achieved from the WAOp stillage AD and adsorption in the current study reached 96% in terms of COD removal. In spite of the complex and heavily solid nature of the raw stillage obtained, a complete BOD & 96% COD removal attained on a real sample is promising when compared to previous studies. Moreover, the bio-energy recovery, which has converted 92% of the COD in the stillage into biogas, as well as the nutrient contained in the final AD sludge from this third generation complex and less biodegradable biomass was promising. However, there was still undegraded COD in the stillage even after the AD, which was over 13000 mg/l. This remaining COD needs to be fixed, especially from the point of view of discharge regulations that are getting stringent with time. Indeed, with such effluent quality, it can be discharged into the municipal sewers or be diluted alternatively with the significant other wastewater streams from distilleries and get discharged to a natural water body. Moreover, the significant reduction in the toxic nature of stillage before the AD has resulted in the substantial recovery of biogas with a speedy tapping of its ultimate potential. Unlike other earlier study reports, the usuals intensification of color of stillage after AD was not observed in the current study, which was another opportunity.

In addition, the remaining nonbiodegradable COD from the integrated treatment effluent ultimately settles in the receiving water or the ecosystem as it is less sensitive to degradation and will not impact on the DO of the receiving water and hence will not affect aquatic lives. As a matter of fact, this final stillage can even be discharged together with other municipal wastewater collection, which naturally contains the degradable organics. Thus, an almost completely degradable domestic waste or specifically sewage can compensate for the remaining nonbiodegradable COD if it is discharged combined to meet discharge limits like the Ethiopian and most environmental protection authorities are demanding the level of an effluent to be 250 mg/l or less COD and a BOD of 60 mg/l or less.

In spite of the huge removal of COD by the WAO and AD of stillage, further minimization was sought motivated by the discharge limit that are getting stringent using batch adsorption trial and a polishing aerobic degradation. The adsorption trial has reduced the effluent soluble COD further to 7500 mg/l and the aerobic degradation to 2278 mg/l. Following the result from the integration of WAp, AD and adsorption stillage treatment, it was proposed to go trying aerobic final oxidation, if it could replace adsorption and if an almost 100 % removal of the COD would be possible. However, the COD removal towards meeting discharge limits was remarkable pushing the removal of the COD to 98.7% due to the integration of AD and aerobic digestion. With the artificial aeration of the batch aerobic degradation the COD removal was significant during the first eight hours that was about 68%.

Despite further degradation of the COD during aerobic degradation, the pH increase, the acclimatization and co-substrate feed were challenges. Moreover, the cost implication of the aerobic system may be a challenge to its application.

A further evaluation of the effect of stillage pretreatment on the OLR and COD removal efficiency of a scoria packed continuous AD was investigated. Upon establishing a pseudo steady state conditions, the assessment showed a relatively better COD removal and increased OLR of the WAOp stillage AD compared to the non-pretreated one, which was around 40% and 2000mg/l-d.

In conclusion, using an integrated treatment approach this study significantly contributes to the proper management of the ever-increasing distillery wastewater problem from the ethanol industry in order for it to become less polluters energy self-sufficient. However, there were few limitations which need to be addressed in further studies. These include the examination of the biofilm formed on the scoria support from the AD in terms of composition as well as the study of the possible leaching of nutrients. In addition, considering possible other scoria surface modification towards efficient adsorption is recommended. Further modelling and pilot scale studies are also suggested to extend the finding to a commercial scale application. In fact, practical challenges, which include the high initial investment and training of operating staff especially in managing flow systems need to be considered during planning.

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Appendices

Annex I Pros and cons of advanced oxidation technologies

Table 23 Comparison of Advanced Oxidation Technologies (Sushma et al., 2018)

AOP	Advantage	Disadvantage
General AOPs	Rapid process, small carbon footprint, no sludge. Potential to reduce toxicity and complete mineralization of organic materials No concentration of wastes for further treatment	Cost, Quenching of excess H_2O_2 Complex chemistry needs to be tailored to specific applications
Fenton's oxidation	Very effective for treatment of toxic industrial wastewater	Separation of iron from the treated solution is required and generated sludge has to be disposed of carefully
Ozonation	Ozone has high oxidation potential of 2.07 compared to hydrogen peroxide (1.78) and chlorine (1.36) Combination of ozone with other oxidizing agents such as hydrogen peroxide, UV light is used for the treatment of wide range of toxic organic pollutants	At high effluent flow rate and organic load, these processes are less useful Ozone is a harmful gas; the unreacted ozone requires an ozone destruction unit which increases the cost of the process
Photocatalysis	A good technology in which organic compounds breakdown take place with the help of UV radiation energy	Photocatalysts are active only under UV-irradiation which contributes only 5% intensity in solar energy while other absorbing visible light are not stable during this process, therefore, unsatisfactory photocatalytic efficiency due to the insufficient solar light

		absorption, low surface area of photocatalysts make this process less useful
WAO	Is an eco-friendly process compared to other AOPs like ozonation and hydrogen peroxide oxidation which use harmful and expensive oxidizing agents Efficient technique for treating wastewater with high organic loading (10–100 g/L of COD) which is too dilute for incineration and toxic for biological treatment WAO does not generate NOx, SO2, HCl, dioxins, furans, fly ash	Requirement of high temperature and pressure and corrosive environment (low pH due to carboxylic acids intermediates formation during reaction)

Annex II The true color of raw molasses stillage and following treatments

The true color of stillage has been checked visually and as per the standard test before any treatment and after each treatment. Among the standard methods to determine color that are outlined by APHA (Methods 2120B-D), 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 5nm (**Table 23**).

Table 24 Summary of color characteristics of molasses stillage subject to biochemical treatments

Sample	pH	Dominant wave length (nm)	Color properties		
			Hue	Luminance (%)	Purity (%)
Raw stillage	6.04	580	Yellowish orange	65	60
WAO pretreated	4.12	570	Greenish yellow	81.6	40
Original	3.96	570	Greenish yellow	93.1	40
After AD with scoria	6.8	570	Greenish yellow	91.3	60
After AD without scoria	6.7	570	Greenish yellow	94.3	60
After AD with WAO & scoria	7.7	570	Greenish yellow	94.6	40
After AD with WAO & scoria and adsorption	8.5	550	Greenish yellow	97.1	20

With any treatment the color change was from yellowish orange to greenish yellow, which would be due to the interference of the microbial activities and some process issue including addition of innocula, dilution effect as well as pH. The major range in color parameters was seen in percent purity followed by percent luminance while the dominant wavelength showed very close values, varying between 550 nm and 580 nm, among treatments (**Table 23**). Visually, there was negligible difference in color before and after as well as among treatments. The lowest purity was obtained from the sample after adsorption study followed by the WAO pretreated and anaerobically digested stillages. The intensity of light emitted per unit area as expressed in percent luminance in this study generally increased following treatments which may signal the decreased effect of molasses stillage towards light transmission when it is released to aquatic systems.

Annex III The biochemical oxygen demand tests on the raw and treated stillage samples

BOD tests are used to identify the degradable organic content of the stillage and evaluate the degradation efficiency of the different treatment technologies applied. The tests followed the standard methods as outlined by APHA (5210C) (APHA; WWA & WEF, 1999).

Figure 40 Biological oxygen demand buffer preparation, titration and color analysis

The BOD of the stillage was tested several times. Initially, the BOD as characterization parameter was performed and was found to be around 132500 mg/l. Afterwards, BOD test was conducted following WAO, AD of the raw stillage, AD of the WAO pretreated stillage, aerobic digestion of the WAO pretreated stillage (**Table 24**).

Table 25 Summary of the biological demand analysis tests

	DO_aver (mg/l)		BOD_ave (mg/l)		BOD/COD	
Sample	Initial (mg/l)	Final (mg/l)		SD		Remark
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	7.6	12000	2400	0.40	CODsol
After AD of WAOp	9.3	8.3	0		0.0	CODsol
After aerobic digestion of the WAOp	9.2	8.2	0	0	0	CODsol
Blank (Seeding)	9.5	8.5	1	0	n/a	

Annex IV The schema of the wet air oxidation installation

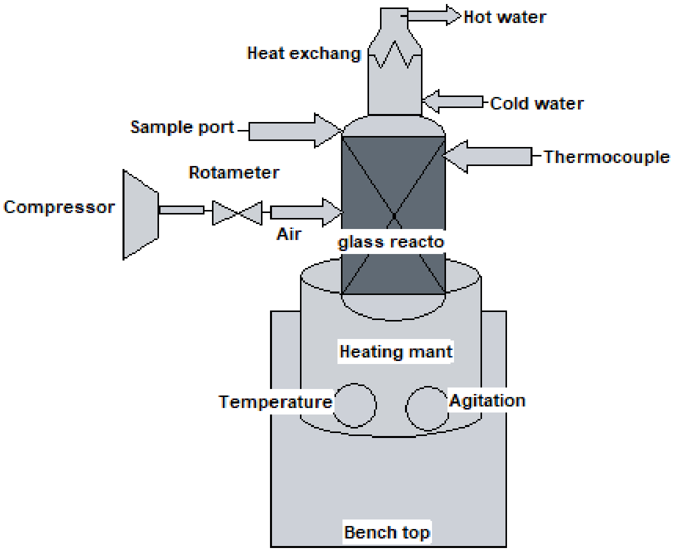

Figure 41 Schematic presentation of the wet air oxidation installation (Supplementary material in the publication)

Annex V Certificates of conference presentations/speaker acceptance

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120 WALL STREET, 23 FL.
NEW YORK, NY
10005 USA

July 15, 2020

Getachew Gebreeyessus
Kotebe Metropolitan University
Addis Ababa, Ethiopia

Dear Getachew Gebreeyessus:

The AIChE cordially invites you to attend this year's 2020 AIChE Annual Meeting taking place November 15 – 20, 2020.

Your paper, "*Enhanced Recovery from Anaerobic Digestion of Molasses Distillery Stillage with Wet Air Pretreatment and Scoria Support*", has been accepted for presentation at the 2020 AIChE Annual Meeting.

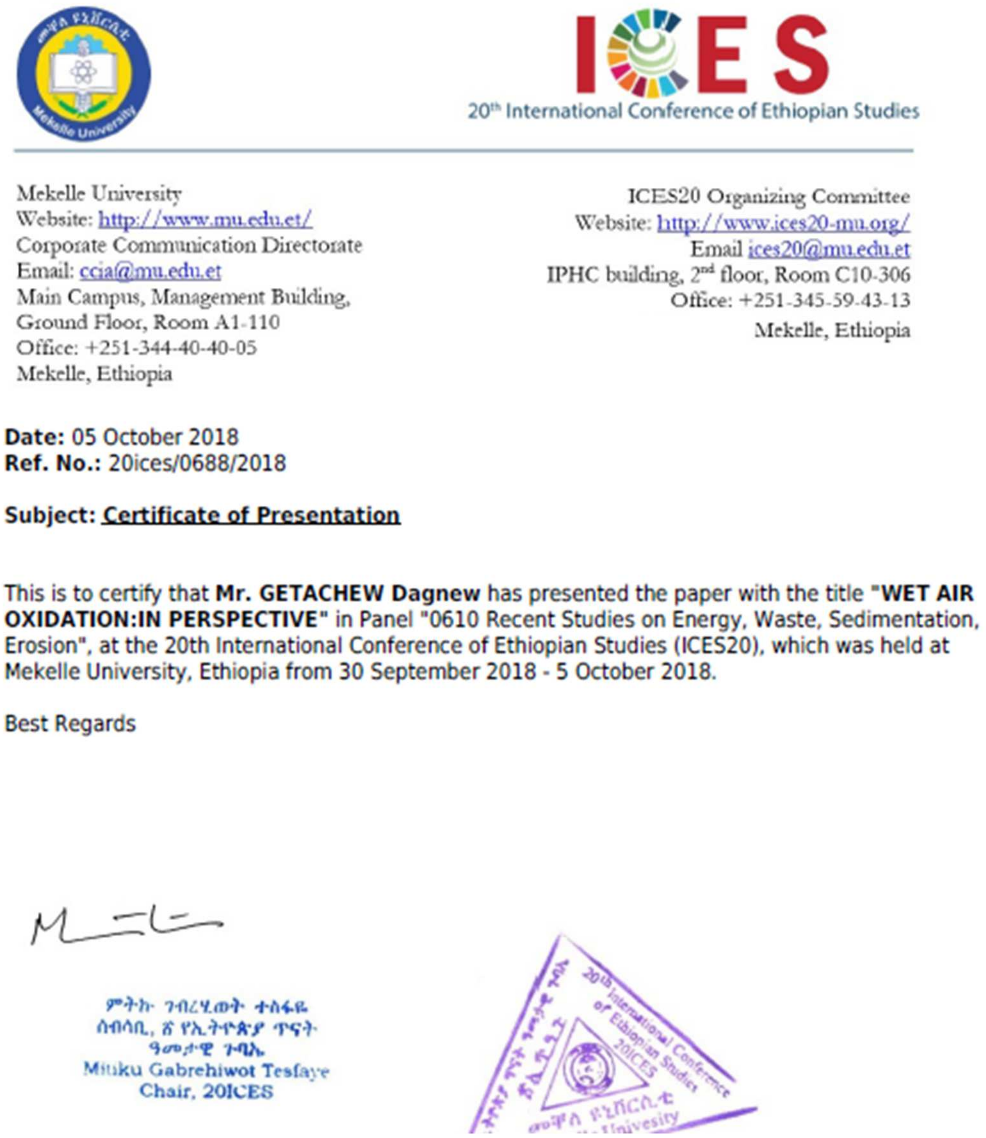
The AIChE Annual Meeting is accomplished by participation of all who attend the conference. We are delighted that you intend to join us and it is our belief that your participation will be beneficial and invaluable to the meeting and the dissemination of knowledge.

It is our stated wish that you be allowed to attend this meeting for the interchange of information and numerous learning opportunities you will encounter at this meeting of the American Institute of Chemical Engineers. All available conference information can be found at http://www.aiche.org/annual.

Thank you very much.

Regards,

Jeffrey W. Wood
Meetings Director
American Institute of Chemical Engineers
120 Wall Street, 23rd Floor



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Date: 05 October 2018
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Subject: Certificate of Presentation

This is to certify that **Mr. GETACHEW Dagnew** has presented the paper with the title **"WET AIR OXIDATION:IN PERSPECTIVE"** in Panel "0610 Recent Studies on Energy, Waste, Sedimentation, Erosion", at the 20th International Conference of Ethiopian Studies (ICES20), which was held at Mekelle University, Ethiopia from 30 September 2018 - 5 October 2018.

Best Regards

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