

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,200

Open access books available

169,000

International authors and editors

185M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Bioremediation of Wastewaters of Sugarcane Biorefineries

Evrin Özkale

Abstract

In addition to sugar, sugarcane biorefineries emerge as the integration of different sugarcane industries and produce several wastes and wastewaters that are disposed into the environment. In biorefineries and other facilities, these wastes are used to produce several chemicals, including bioplastics and bioethanol. However, these industries use a greater amount of fresh water and their effluents, which have high amounts of solids and are released mostly into water or used for crop irrigation. Inadequately treated and indiscriminately disposing and discharging of effluents to the environment contributes to a greater risk of pollution of soils and waters. Therefore, to minimize the side effects, control the environmental burden, grow the beneficiaries of waste, and sustain a healthy environment for the future, proper management of industrial wastes is important.

Keywords: sugar industry, industrial wastewater, wastewater treatment, biological treatment efforts

1. Introduction

The sugar industry is the most common industry in more than 130 countries, especially in developing countries [1, 2]. Sugarcane processing plants comprise one of the most successful examples of biorefineries in which a wide range of products is obtained from a single raw material of feedstock. Biorefinery is emerging as the integration of the different sugarcane industries based on biomass feedstock.

Harvested sugarcane is used to produce raw sugar in addition to products, such as bagasse (residue from sugarcane crushing), press mud (dirt mud from juice clarification), molasses (final residue from sugar crystallization), and wastewater [3]. Production of ethanol concomitantly is in a greater ratio than to produce only raw sugar or ethanol in many countries around the world [2].

Biorefinery has emerged as the integration of different sugarcane industries based on biomass feedstock. However, ethanol distilleries are growing at an alarming rate across the globe and generate a huge volume of effluent that is disposed/discharged into the environment. These inadequately/non-treated and indiscriminately disposed effluents cause pollution of soil and water resources or deposits [4].

The agricultural industries are the major freshwater consumers and play a major role in polluting water bodies. Freshwater is used in different units of sugar production processes and generates wastewater, which is highly variable both in quantity and

quality. Regarding the processes of the feedstocks, products, and chemicals used in the process, wastewaters/effluents have different characteristics [5, 6].

Different pollution monitoring agencies, such as State and National Pollution Control Boards, have been made compulsory for each industry to set up wastewater treatment plants. In the treatment system, simple treatments of effluent are not effective to the dischargeable limit.

Sugarcane processing plants comprise one of the most successful examples of biorefineries in which a wide range of products is obtained from a single raw material of feedstock. Recently, bioplastic production has emerged as one of the primary interests in the sugar processing industry in which the sugars are converted into lactic acid and polymerized into biopolymer [7].

Wastewaters are treated by several methods, such as adsorbent, electrochemical, anaerobic biological treatment, and biochemical oxidation. However, treated wastewater by these methods are not meeting the discharge limit, therefore requires modification. Electrolysis followed by coagulation is the most effective method of treatment of sugar industry wastewater giving close to 100% reduction of COD as well as the electrochemical process shows 81% COD and 83.5% color reduction at pH 6.0, electrode distance of 20 mm. The combined treatment results show 98% COD and 99.5% color removal at 8 mM mass loading and pH 6 with copper sulfate [8, 9].

The preferred choice for the treatment of effluents is anaerobic degradation because these industries typically generate high-strength wastewater with the potential to recover energy in the form of biogas [10]. Anaerobic treatment processes have been employed to stabilize sewage sludge for more than a century. The application of this process for high-strength industrial wastewater treatment began with the development of high-rate anaerobic reactors (HR). Up-flow anaerobic sludge blanket (UASB) reactor is used in treating sugar wastewater with varying HRT and varying feed concentrations at ambient conditions [11].

AD-based biorefineries have great potential to meet the energy deficiency criteria of sustainable development goals in the coming decades. Following the need to increase energy efficiency and diversify the product portfolio derived from sugarcane, anaerobic digestion (AD) may be an old solution with great potential to improve the biorefinery character of the sugarcane industry. Therefore, sugarcane biorefineries and wastewater treatments are required for proper management and for environmental sustainability [1, 12].

2. Characteristics of wastewaters of sugar industry and effluents of ethanol distilleries

The sugar industry is one of the major industries, which has been included in the polluting industries list by the World Bank [13]. In the sugar industry, wastewater is produced from sources primarily due to cane processing during evaporation, crystallization, and refinery, and the other is from cycling, such as in the condensers, chimneys, scrubbers, and refrigeration of turbines [14]. To crush one ton of sugarcane, nearly 2000 l of water is required, which generated nearly 1000 l of wastewater. Moreover, per ton of crushed sugarcane, 0.7 m³ of wastewater is produced due to the high moisture content of the raw material [15, 16]. Also, the wastewater for periodically cleaning procedures, such as cleaning of lime water and SO₂, producing house of the industry is contributed.

A large amount of wastewater as effluent, which has a high amount of solids, BOD₅, COD, chloride, sulfate, nitrate, and magnesium, was also discharged into the lands beside water [17]. The major sources of wastewater in molasses-based distilleries are fermenter sludge, spent wash, and spent less. The fermented wash is the main product of fermentation which is decanted; the remaining sludge is known as yeast sludge (fermenter sludge) and contributes to the pollution load from the distilleries. On the other hand, spent wash is a complex effluent having the strongest organic matter in terms of having high COD (up to 160,000 mg/L), temperature, low acidity (between 3.7 and 4.5), and a high content of dissolved inorganic salts and ash content [4, 18].

Principally, the biological treatment method is effective for highly polluted agro-industrial wastewater from the sugar industries and ethanol distilleries [19]. However, the current anaerobic treatment technologies and the high-rate anaerobic reactors are the most suitable and attractive primary treatment options for high-strength organic effluents, such as sugar industry wastewater and distillery spent wash [20].

More than 25 tons COD of agro-industrial waste (water), on a daily basis, can be converted into 7000 m³CH₄ (80% CH₄ recovery approximately) that accounts for an

Parameter	Sugar Industry	Effluent of ethanol distilleries spent wash	Anaerobically treated effluent quality
Temperature (°C)	29.3–44.3	46.3–56.3	
pH	6.7–8.4	3.9–4.9	7.5–8.0
Electric conductivity	540.3–925.9	3910.0–50,500.00	
BOD ₅	654.6–1968.5	50,000.00–60,000.00	8000–10,000
COD	1100.3–2148.9	110,000.00–190,000.00	45,000–52,000
Chloride	30.5–866.6	6213.6–7475.7	
Total hardness	356.2–2493.1	3100.3–4477.2	
Calcium	365.4–468.0	8000–8500	
Magnesium	214.8–341.0	816.3–1828.1	
Total solids	2452.3–3050.6	91,876.9–150,300.9	70,000–75,000
Total dissolved solids	1480.2–1915.1	13,000.0–88,265.00	30,000–32,000
Total suspended solids	220.3–790.7	3611.1–150,000.00	
Nitrates	0.4–0.9	2.40–32.9	
Organic- N	24.3–36.4	75.2–400.7	
Ammonia- N	0.0–4.2	10.9–18.1	
Total Nitrogen	11.1–40.6	85.8–1355.3	
Phosphate	1.2–9.6	2500–2700	1500–1700
Sulfate	21.5–51.7	803–6050.5	
Oil and greases	88.7–134.4	3.3–202.1	

Table 1. Characteristics of wastewater of sugar industry and effluents of ethanol distilleries and some quality parameters of anaerobically treated effluent (mg/L) [1].

energy equivalent of about 250 GJ/d working with a modern combined heat power (CHP) gas engine, due to 40% of efficiency, a useful 1–2 MW electric output can be achieved. By this conversion, CO₂ emission reduction (ton CO₂/m³.y), is based on coal-driven powerplant (**Table 1**) [21, 22].

3. Wastewater management and biological treatment

The most common ways of wastewater management in the sugar industry and biorefineries are fertirrigation, bio-compost, and concentration by evaporation (incineration). However, these conversions are difficult to manage since the huge volumes of wastes produced during the sugar production and biorefinery processes are costly. Also, prior to producing bio-compost, BOD₅ and COD of the distillery spent wash should be anaerobically digested (biomethanation). As the industrial waste is converted into organic-rich manure, not only the problems of waste disposal and pollution are solved but also the soils are replenished and renovated. However, raw distillery spent wash has to be subjected primarily to anaerobic digestion (biomethanation) treatment to decrease BOD₅ and COD and other pollutants before combining with the press mud to produce bio-compost (**Figure 1**) [23, 24].

There is a growing interest in biological treatment systems as a common procedure for wastewater treatment to eliminate solids, nutrients, and organic matter since the various conventional physicochemical methods have been tested and are found as inefficient and had some drawbacks for the treatment of sugar industry wastewaters.

In the usual procedure, biological wastewater treatment processes use microorganisms to utilize wastewater pollutants for their growth and metabolize the organic substrate in the wastewater to gain their energy and into metabolic wastes, such as CO₂ and water [25]. Biological wastewater treatment processes are beneficiary alternatives

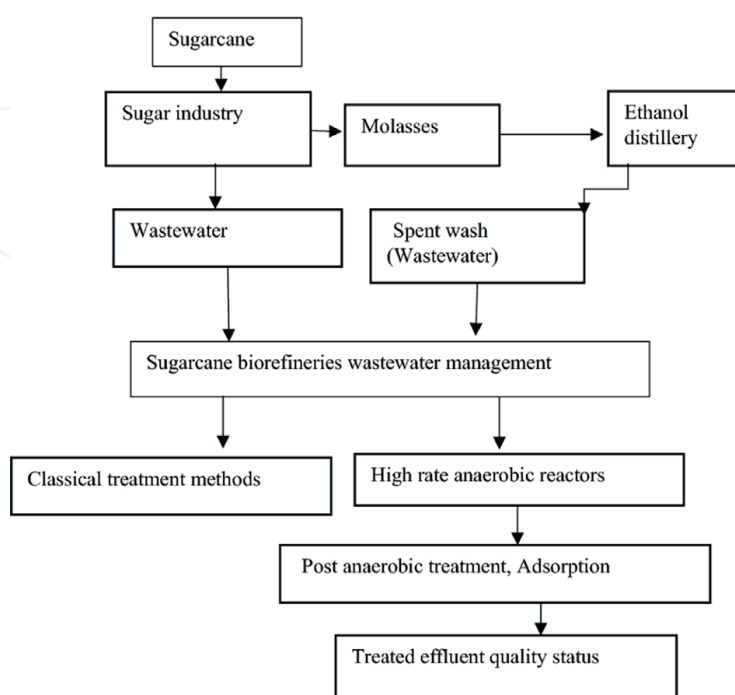


Figure 1. Sugar industry wastes and wastewater management and biological treatment [1].

to conventional treatments. Methods, such as anaerobic bioremediation, employed to remove various pollutants from several industrial wastewaters can be applicable to wastewaters of sugar industries and ethanol distilleries spent wash. Additionally, the removal of pollutants and toxic materials from industrial wastewater is increasingly shifting from the use of conventional approaches to the implementation of the advanced bioremediation processes of high-rate anaerobic conditions [26].

In recent years, fermentation reactors have been the preferred method for treating sugar industry effluent, encompassing technologies, such as anaerobic fixed bed (UAFB), up-flow anaerobic sludge blanket (UASB), anaerobic downflow stationary fixed film (DSFF), aerated fixed film (AFF), and anaerobic batch reactors [27]. High-rate anaerobic reactors are the most suitable treatment option for high-strength organic effluents and have characteristics, such as high loading capacity, better stability, resistance to inhibitors, and low sludge production. One disadvantage of the fermentation methods is that the anaerobic process is characterized by low nitrogen and phosphorus removal rates. The fluidized active filling (FAF) method was presented as an innovative solution to treat sugar industry effluent in anaerobic reactors. It was shown that the use of the FAF-reactor could improve phosphorus removal rates [27].

The molasses distillery wastewater has similarities with sugar industry wastewater in composition, but the distillery spent wash is high-strength wastewater. Recent studies have proven the suitability of anaerobically processing sugarcane vinasse on technical, economic, and environmental bases. The exploitation of AD plants within the sugarcane biorefinery has provided reliable data to encourage energetic exploitation and economic feasibility of sugarcane vinasse.

There is a continuous search to combine clean energy supply with the exploitation of bioenergy sources to meet global energy demands on an environmentally friendly basis. On this basis, sugarcane biorefineries have a central role by providing sugar, ethanol, and bioelectricity [28].

The extensively used technology—UASB technology—is efficient and commonly used for also the sugar industry effluent and distillery spent wash. UASB is effective among the high-rate anaerobic digestion methods, but its limitations are found, such as longer HRTs, long periods of startup, and the wash-up of the sludge, in the bioreactor [17, 29].

Even though this treatment technology is very promising for the wastewater treatment sector, it was observed that its efficiency is somehow lower as compared to conventional biological treatment systems [30]. Therefore, the application of this treatment option is not feasible enough for the high strength of the sugar industry and ethanol distillery wastewater, yet. The maximum BOD₅ reduction (86%) occurred reported at a BOD₅ loading rate of 2.74 kg/m³ and a digestion temperature of 50°C [17].

The energy returned on energy invested (EROEI) ratio associated with sugarcane-derived 1G ethanol is often reported to exceed a value of 6.5. The effective EROEI ratio obtained for ethanol production should be lower than 2.0 without considering the contribution of bagasse burning [31].

Applications apart from electricity generation may also comprise energetically efficient alternatives to exploit biogas focusing on biomethane (bioCH₄) production. BioCH₄ in which the maximum methane content is 96.5% (v/v), may be used *in loco* diesel replacement and/or injected into the gas grid. Previous studies indicated that bioCH₄ production is the most attractive approach (compared to electricity production) on economic bases for vinasse-fed AD plants [32].

Using the anaerobic processing of vinasse tends to increase the EROEI in ethanol-producing plants by less than 20.0%, whilst investing in biogas production from raw feed-stocks leads to an approximately 400% increase in the sugarcane biorefinery. Different designs for AD plants have been applied to vinasse in large-scale distilleries for enhancement of energy recovery. The application of chemicals to raw vinasse is a key factor for obtaining high treatment performances and specific composition characteristics, such as high carbohydrate concentrations, low pH, and absence of alkalinity provide favorable conditions for rapid vinasse acidification. Sodium bicarbonate has been frequently applied as an alkalizing component in bench-scale AD systems for vinasse treatments [32–34]. Sodium hydroxide and calcium carbonate have also been commonly used in treating vinasse prior to methane production. However, the use of high chemical doses negatively affects the economic favorability of scaling up AD plants. Urea application has also been tested to support alkalinity to methanogens and to increase nitrogen levels in the treated vinasse thereby increasing its fertilization potential. However, the potential of accumulation of ammonia within the reactors could lead to inhibitory effects over methanogenic populations, thus impairing the bioenergy recovery from vinasse [35].

The combined use of low dosages of chemicals for the recirculation of the effluent may offer the most suitable alternative for the alkalization of full-scale AD plants on an economic and environmental basis. Further studies on alkalizing strategies for vinasse are under consideration as an approach to improve both the economic and environmental performances of biodigestion plants [32, 36].

On the other hand, AD alone cannot remove all the pollutants of sugarcane biorefinery wastewater. As post-treatment, another treatment technology is highly recommended for the removal of less biologically degradable organic compounds in terms of the treatment efficiency, cost, and ease of operation as well as social acceptance. As a potential candidate for post-anaerobic treatment of sugarcane biorefinery wastewater, adsorption treatment technology is considered mostly [1].

In recent years, the integration of microalgal cultivation with other processes for achieving inexpensive nutrient and energy use has become an important issue. Integrating mixotrophic microalgae into wastewater treatment is a cost-effective and feasible method for biofixation of CO₂ [37, 38]. The principal advantage of incorporating microalgae into wastewater treatment is the generation of O₂ through photosynthesis and is necessary for heterotrophic bacteria to biodegrade carbonaceous material. The potential of the fuel gas and the wastewater of a sugar factory to support microalgae growth for biofuel and biofertilizer production has also been evaluated. In addition to removing pollutants, the cultivation of microalgae in conjunction with wastewater treatment provided lipids that can be converted into biodiesel.

4. Conclusions

Sugarcane processing industries are highly water-intensive processes. Proper water conservations and management of cane industrial wastewater are highly important for sustainable freshwater uses.

Conventional treatment methods are challenging in the elimination of pollutants. Recently high-rate anaerobic digestion has been recognized as a safe and effective treatment, particularly for wastewaters of the organic saturated sugar industry and ethanol distilleries. Although economic and environmental assessments are still required to fully subsidize the demonstration of the advantages of the AD-based

biorefinery, the results of the studies encourage more efficient renewable systems. The proper management of vinasse is standing as one of the major issues facing sugarcane-based ethanol biorefineries due to environmental concerns.

The anaerobic treatment of wastewater is an important field of research where improvements and new developments are needed to overcome the limitations of the system. Technological solutions allowing the increase of the technological efficiency of anaerobic methods of wastewater treatment are still under investigation.

IntechOpen


IntechOpen

Author details

Evrin Özkale
Manisa Celal Bayar University, Manisa, Turkey

*Address all correspondence to: evrimmiko@gmail.com

IntechOpen

© 2022 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Fito J, Tefera N, van Hulle SWH. Sugarcane biorefineries wastewater: Bioremediation technologies for environmental sustainability. *Chemical and Biological Technologies in Agriculture*. 2019;**6**:6. DOI: 10.1186/s40538-019-0144-5
- [2] Fito J, Tefera N, Demeku S, Kloos H. Water footprint as an emerging environmental tool for assessing sustainable water use of the bioethanol distillery at Metahara sugarcane farm, Oromiya region, Ethiopia. *Water Conservation Science Engineering*. 2017;**2**:165-176
- [3] Nandy T, Shastry S, Kaul SN. Wastewater management in a cane molasses distillery involving bioresource recovery. *Journal of Environmental Management*. 2002;**65**:25-38
- [4] Fito J, Tefera N, Van Hulle SWH. Physicochemical properties of the sugar industry and ethanol distillery wastewater and their impact on the environment. *Sugar Technology*. 2019;**21**:265-267. DOI: 10.007/s12355-018-0622-z
- [5] Agarwal MK, Kumar M, Ghosh P, Kumar SS, Sigh L, Vijay VK, et al. Anaerobic digestion of sugarcane bagasse for biogas production and digestate valorization. *Chemosphere*. 2022;**295**:133893. DOI: 10.1016/j.chemosphere.2022.133893
- [6] Klein BC, Chagas MF, Watanabe MDB, Bonomi A, Filho RM. Low carbon biofuels and the new Brazilian National Biofuel Policy (RenovaBio): A case study for sugarcane mills and integrated sugarcane-microalgae biorefineries. *Renewable and Sustainable Energy Reviews*. 2019;**115**:109365. DOI: 10.1016/j.rser.2019.109365
- [7] Sriroth K, Vanichsriratana W, Sunthornvarabhas J. The current status of sugar industry and by-products in Thailand. *Sugar Technology*. 2016;**18**:576-582
- [8] Fuess LT, Klein BC, Chagas MF, Rezende MCAF, Garcia ML, Bonomi A, et al. Diversifying the technological strategies for recovering bioenergy from the two-phase anaerobic digestion of sugarcane vinasse: An integrated technoeconomic and environmental approach. *Renewable Energy*. 2018;**122**:674-687
- [9] Konde KS, Nagarajan S, Kumar V, Patil SV, Ranade E. Sugarcane bagasse based biorefineries in India: Potential and challenges. *Sustainable Energy and Fuels*. 2021;**5**:52-78. DOI: 10.1039/d0se01332c
- [10] Breitenmoser L, Gross T, Huesch R, Rau J, Dhar H, Kumar S, et al. Anaerobic digestion of biowastes in India: Opportunities, challenges and research needs. *Journal of Environmental Management*. 2019;**236**:396-412
- [11] Surendra KC, Sawatdeenarunat C, Shresta S, Sung S, Kumar SK. Anaerobic digestion-based biorefinery for bioenergy and biobased products. *Industrial Biotechnology*. 2015;**11**:2. DOI: 10.1089/ind.2015.0001
- [12] Liu Z, Wei L, Liu Y. A sustainable biorefinery to convert agricultural residues into value-added chemicals. *Biotechnology for Biofuels and Bioproducts*. 2016;**9**:197. DOI: 10.1186/s13068-016-0609-8
- [13] Tiwari A, Sahu O. Treatment of food-agro (sugar) industry wastewater

with copper metal and salt: Chemical oxidation and electro-oxidation combined study in batch mode. *Water Resources and Industry*. 2017;**17**:19-25. DOI: 10.1016/j.wri.2016.12.001

[14] Ingaramo A, Heluane H, Colombo M, Cesca M. Water and wastewater eco-efficiency indicators for the sugar cane industry. *Journal of Cleaner Production*. 2009;**17**:487-495

[15] Memon AR, Suhail Ahmed S, Abdul Khaliq A. Sugar industry effluent characteristics and chemical analysis. *Journal of Applied Emerging Science*. 2006;**1**:156-157

[16] Solomon SK. Environmental pollution and its management in sugar industry in India: An appraisal. *Sugar Technology*. 2005;**7**:77-81

[17] Mohana S, Acharya BK, Madamwar D. Distillery spent wash: Treatment technologies and potential applications. *Journal of Hazardous Materials*. 2009;**163**:12-25

[18] Basu S, Mukherjee S, Kaushik A, Batra VS. Integrated treatment of molasses distillery wastewater using micro filtration (MF). *Journal of Environmental Management*. 2015;**158**:55-60

[19] Pant D, Adholeya A. Biological approaches for treatment of distillery wastewater: A review. *Bioresource Technology*. 2007;**98**:2321-2334

[20] Rajeshwari KV, Balakrishnan M, Kansal A, Lata K, Kishore VVN. State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. *Renewable and Sustainable Energy Reviews*. 2000;**4**:135-156

[21] Fuess LT, Zaiat M. Economics of anaerobic digestion for processing

sugarcane vinasse: Applying sensitivity analysis to increase process profitability in diversified biogas applications. *Process Safe Environment Protection*. 2018;**115**:27-37

[22] Fuess LT, Kiyuna LSM, Ferraz ADN Jr, Persinoti GF, Squina FM, Garcia ML, et al. Thermophilic two-phase anaerobic digestion using an innovative fixed-bed reactor for enhanced organic matter removal and bioenergy recovery from sugarcane vinasse. *Applied Energy*. 2017;**189**:480-491. DOI: 10.1016/j.apenergy.2016.12.071

[23] Ghulam S, Khan MJ, Usman K. Effect of different rates of pressmud on plant growth and yield of lentil in calcareous soil. *Sarhad Journal of Agriculture*. 2012;**28**:8-11

[24] Analia A, Juliana MS, Costa SD, Colin VL, Fuentes MS, Sergio Antonio C, et al. Actinobacteria: Current research and perspectives for bioremediation of pesticides and heavy metals. *Chemosphere*. 2017;**166**:41-62

[25] Kharayat Y. Distillery wastewater: Bioremediation approaches. *Journal of Integrative Environmental Sciences*. 2012;**9**:69-91

[26] Igwe JC, Abia AA. A bioseparation process for removing heavy metals from waste water using biosorbents. *African Journal of Biotechnology*. 2006;**5**:1167-1179

[27] Debowski M, Zieliński M. Technological effectiveness of sugar industry effluent methane fermentation in a fluidized active filling reactor (FAF-R). *Energies*. 2020;**13**:6626. DOI: 10.3390/en13246626

[28] Fuess LT, Cruz RBCM, Zaiat M, Nascimento CAO. Diversifying the portfolio of sugarcane biorefineries:

Anaerobic digestion as the core process for enhanced resource recovery. *Renewable and Sustainable Energy Reviews*. 2021;**147**:111246. DOI: 10.1016/j.rser.2021.111246

[29] Acharya BK, Mohana S, Madamwar D. Anaerobic treatment of distillery spent wash—A study on upflow anaerobic fixed film bioreactor. *Bioresource Technology*. 2008;**99**:4621-4626

[30] Yasar A, Tabinda AB. Anaerobic treatment of industrial wastewater by UASB reactor integrated with chemical oxidation processes; an overview. *Polish Journal of Environmental Studies*. 2010;**19**:1051-1061

[31] Chiriboga G, de la Rosa A, Molina C, Velarde S, Carvajal CG. Energy return on investment (EROI) and life cycle analysis (LCA) of biofuels in Ecuador. *Heliyon*. 2020;**6**:e04213. DOI: 10.1016/j.heliyon.2020.e04213

[32] Fuess LT, de Araujo Junior MM, Garcia ML, Zaiat M. Designing full-scale biodigestion plants for the treatment of vinasse in sugarcane biorefineries: How phase separation and alkalization impact biogas and electricity production costs? *Chemical Engineering Research and Design*. 2017;**119**:209-220

[33] Ferraz ADN Jr, Koyama MH, Araújo MM Jr, Zaiat M. Thermophilic anaerobic digestion of raw sugarcane vinasse. *Renewable Energy*. 2016;**89**:245-252

[34] Siqueira LM, Damiano ESG, Silva EL. Influence of organic loading rate on the anaerobic treatment of sugarcane vinasse and biogas production in fluidized bed reactor. *Journal of Environmental Science and Health, Part A*. 2013;**48**(13):1707-1716. DOI: 10.1080/10934529.2013.815535

[35] Bonc MA, Formagini E, Santos LS, Marques RD, Paulo PL. Application of urea dosing for alkalinity supply during anaerobic digestion of vinasse. *Water Science and Technology*. 2012;**66**(11):2453-2460. DOI: 10.2166/wst.2012.476

[36] van Haandel AC. Integrated energy production and reduction of the environmental impact at alcohol distillery plants. *Water Science and Technology*. 2005;**52**(1-2):49-57

[37] Chen Y, Ho S, Nagarajan D, Ren N, Chang J. Waste biorefineries- integrating anaerobic digestion and microalgae cultivation for bioenergy production. *Current Opinion in Biotechnology*. 2018;**50**:101-110

[38] Silva MA, Barbosa GH, Codato CB, Mattos LFA, Bastos RG, Kieckbusch TG. Heterotrophic growth of green microalgae *Desmodesmus subspicatus* in ethanol distillation wastewater (vinasse) and lipid extraction with supercritical CO₂. *Journal of Chemical Technology and Biotechnology*. 2017;**92**:573-579