Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 503-510 | ISSN:2251-6727

Exploring Extracellular Hydrolytic Enzyme and Bio Flocculant-Producing Bacterial Isolates for Bioremediation of Vegetable Oil Refinery Effluent.

Gajanan Shinde [a, b], Ajaykumar Jadhav[a]

[a]Department of Microbiology, Government Institute of Science, Aurangabad (MS) India.

[b]Yashwantrao Chavan College of Science, Vidyanagar Karad Dist. Satara (MS) INDIA.

(Received: 04 August 2023 Revised: 12 September Accepted: 06 October)

KEYWORDS

VORE,
BOD,
COD,
Toxicity,
Seed germination.

ABSTRACT:

The process of vegetable oil refining generates an enormous amount of wastewater. This study focused on characterization of vegetable oil refinery effluent (VORE) sample untreated and treated by bacteria for various physicochemical parameters. The sample has high COD and BOD; 12.32g/L and 1.7g/L respectively, this highlights the need for proper treatment. The VORE samples were treated by bacteria producing extracellular hydrolytic enzymes and bioflocculant, identified as *B. licheniformis, S. stutzeri*, *B. amiloliquificance* and, *S. mutabilis.* The bacterial treatment was performed in a separate 250ml flask containing 100ml effluent and inoculated with 2% of respective 24h grown culture. The bacterial treatment achieved a significant decrease in BOD and COD. The treated effluent showed significant increase in seed germination representing a reduction in toxicity in comparison to untreated effluent. This highlights the suitability of present study isolates for VORE treatment with high BOD and COD.

1. Introduction

The major source of vegetable oil is from seeds such as; soybean, sunflower, sesame, coconut, palm, rice bran, and groundnut. India is amongst the largest producers of oilseeds in the world, accounting for 36.56 MT production of nine different oilseeds in the year 2020-21 [1]. The oil production i.e., extraction of oil from seeds is done by physical or chemical extraction using a solvent. Crude oil extracted from seeds must process before human consumption [2]. This process includes degumming, alkali neutralization, bleaching, heating, and deodorization [3], which generates a higher amount of wastewater [4]. Generally, different processes involved in vegetable oil refining used a huge amount of water and generate an equal amount of wastewater, but chemical refining produces 20-30 times higher wastewater than water used in this process [5,6]. A substantial amount of waste is generated including wastewater, organic solid waste, and inorganic residues [7]. In the degumming step, some phosphoric acid is added to separate phospholipids, increasing the phosphorus level in the effluent. In the neutralization step sodium salt of free fatty acid ("soap stocks") were generated and split using H2SO4 in a stoichiometric amount which makes wastewater highly acidic [8]. A typical effluent from vegetable oil contains a higher amount of COD, BOD, TSS, TDS, nickel, oil & grease, fats, soap and sludges which cause deterioration of the environment and human health [6,9,10]. Wastewater generated both quantitatively and qualitatively has a profound impact on ecology, resulting in increased pollution load and concentration. Treatment of this type of effluent is more difficult because of its complex molecular structure [3].

There are numerous physicochemical (skimming of oil, flocculation, coagulation, air floatation) methods available to remove colloidal pollutants [11]. Chemical flocculating agents are the choice of many to remove the pollutant, but flocculating agents like inorganic and organic polymers may be toxic to the ecosystem. In comparison with this, bioflocculant produced by microorganisms are non-toxic, harmless, efficient, and biodegradable [12]. The biological methods are more suitable in terms of generation of secondary pollutant, partial treatment, investment costs. Further use of bacteria to remove pollutants is safe, eco-friendly and inexpensive, five to twenty times less costly than chemical treatments and more economical than physical-chemical treatment methods.

Bacteria are suitable for the degradation of pollutants because of their diverse carbon utilization ability [13]. The bioremediation process mainly depends on microorganisms that attack pollutants enzymatically and convert it to

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 503-510 | ISSN:2251-6727

innocuous products [14]. Thus, very significant in the removal and degradation of chemicals and control of pollution [15].

2. Material and methods

Sample collection

The effluent sample was collected in a pre-sterilized plastic bottle from an edible oil refinery located in Aurangabad (MS, India). The samples were immediately processed for physicochemical analysis and the remaining sample was otherwise stored at 4°C.

Physicochemical characterization of the effluent sample

Physicochemical parameters such as pH, temperature, color, electrical conductivity (EC), alkalinity, acidity, total dissolved solids (TDS), total solids (TS), nitrate, total phosphorous, carbonate, bicarbonate, total hardness, chloride, sulphate, oil and grease, chemical oxygen demand (COD), biological oxygen demand (BOD) were analysed by using standard methods of APHA [16]. Protein and carbohydrate concentrations in the VORE sample were estimated by Lowry's method and Molish test respectively.

Biological treatment to effluent

Bacteria capable to produce extracellular hydrolytic enzymes (Cellulase, Protease, Amylase, Lipase) were selected for further treatment. Screening for hydrolytic enzymes was performed using respective agar mediums [17,18,19,20]. The pH of VORE sample was highly acidic (1.53), before biological treatment, the pH was adjusted to pH 7.0 by using 1N NaOH. A total 2% of 24h grown bacterial broth culture was inoculated in 100 ml VORE sample in 250 ml Erlenmeyer flask and kept on a rotary shaker at 60 rpm for 48h. After treatment the effluent sample was filtered to remove bacterial biomass and then subjected to physicochemical analysis and toxicity assessment.

Bioflocculant producing ability of selected bacterial isolates

All selected bacterial isolates were subjected to evaluation for bioflocculant production using a medium containing (g/L): glucose 10, peptone 1, $MgSO_47H_2O$ 0.3, K_2HPO_4 5, KH_2PO_4 2, pH 7.0. The medium was prepared in double distilled water and sterilized at 121°C for 15 min. The bacterial cultures were inoculated in production media and incubated on a rotary shaker at 120 rpm for 48h at room temperature. The supernatant was employed as a bioflocculant after incubation.

Flocculating activity assay was carried out in accordance with Aljuboori et al., [21] with some modification. Two gm of kaolin clay was suspended in 1 L of deionized water and pH was adjusted to 7.0 using 1 M NaOH or HCl. A total 198.5 ml of kaolin suspension, 1 ml of cell free extract and 0.5 ml of CaCl2 (10 mM) was added in a 500-mL beaker. The mixture was stirred at 200 rpm for 1 min, slowly stirred at 60 rpm for 5 min and allowed to stand for 10 min. Then the absorbance of the supernatant was measured at of 550 nm, with heat-inactivated bioflocculant was used in a control experiment. The flocculating rate was calculated according to the following formula:

$$Flocculating\ rate\% = \left(\frac{A550 - B550}{A550}\right) \times 100$$

Where A550 and B550 are the absorbance of the control and sample suspension respectively.

Toxicity assessment by seed germination

Germination study was performed by using wheat seeds by watering with untreated and treated effluent by standard roll towel method of ISTA [22]. Seeds were surface sterilized with 0.1% $HgCl_2$ for 5 min and washed with sterile distilled water. A total of 10 seeds were placed in germination paper, then rolled and placed in a Petri plate. The watering of germinating sets were done by 10 ml of four different concentrations (diluted in distilled water) of the untreated effluent sample (25%, 50%, 75%, 100%), along with treated (without dilution) and control (tap water). After 48h the seed germination was observed and germination percentages were calculated using the formula mentioned below.

$$Seed\ germination\ \% = \left(\frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sown}\right) \times 100$$

3. Result and Discussion

The selected isolates in this study were identified based on morphological, biochemical, and 16S rDNA sequencing and sequences submitted to GeneBank with accession as; OM977118 *Bacillus licheniformis strain GACE1,* OP649739 *Stutzerimonas stutzeri strain GAPS1,* OQ780768 *Bacillus amiloliquificance strain UAS2,* MW575257 *Streptomyces mutabilis strain GAA1.* Further hydrolytic enzyme-producing ability was detected (Table no.1).

The agriculture-based industrial effluents like VORE usually comprise a high level of organic material, cellulose,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 503-510 | ISSN:2251-6727

hemicellulose, lignin, oil, and fatty acids [23, 24, 25, 26]. Microorganisms with the potential of hydrolytic enzymes could be useful to treat such types of effluent and successfully used for utilization and treatment of agriculture and industrial waste [27, 28, 29, 30].

VORE sample composition varies with the extraction method but is generally constituted of water from different processes of extraction and various organic compounds (sugar, lipids, pectin, organic acids) and minerals [31]. Soapstock splitting is one of the important steps, it may use to produce fatty acids, animal feed, and soap products. The splitting of soapstock is usually done by sulphuric acid [32]. The excess use of sulphuric acid makes effluent highly acidic. The pH of studied effluent sample was also highly acidic, similar to VORE characterized by Decloux et al., [8] and Aslan et al., [33]. The VORE sample of this study has 0.7536 g/L of oil and grease, while the permissible limit is 0.05 g/L. The other primary parameters of effluent like BOD 1.7 g/L, COD 12.32 g/L, protein and carbohydrate suggest the suitability of biological treatments using hydrolytic enzyme-producing microorganisms.

Wastewater with high amounts of fatty materials is readily biodegradable and these types of effluents are acquiescent to biological treatment [34]. Biological treatment suitable for edible oil refinery effluent was suggested by Mkhize et al., [35], with 75% reduction in COD and 90% removal of oil and suspended solids by anaerobic/ aerobic process. As per Tay [36], 95% of reduction in BOD was achieved by activated sludge process. The findings of the present study showed a significant reduction in COD and BOD by treatment with selected bacterial isolates. The 96%, 95%, 89% and 93% reduction in COD and 74%, 89%, 81%, and 89% reduction in BOD were shown by *B. licheniformis S. stutzeri*, *B. amiloliquificance*, and *S. mutabilis* respectively This showed highest reduction in BOD and COD by *S. stutzeri* followed by *S. mutabilis* treatment.

Bioflocculant production

Besides this, the isolates of the present study showed bioflocculant production. VORE contains high Nitrogen and phosphorus concentrations, suspended solid content, organic and inorganic matter. Bacteria can utilize these toxic substances, and thus useful for the treatment of such wastewater [37].

Table 1: Enzyme producing ability of bacterial isolates

Sr. no.	Isolate	Cellulase	Protease	Amylase	Lipase
1	*B. licheniformis*	+	+	+	+
2	*S. stutzeri*	+	+	+	-
3	*B. amiloliquificance*	+	+	+	+
4	*S. mutabilis*	+	-	+	+

Table 2: Physicochemical analysis of VORE sample

Sr. No.	Parameter	Result
1	pH	1.53
2	Temperature (^{o}C)	30
3	Colour	Brownish
4	EC(S/m)	3040000
5	TDS(g/L)	29.6
6	TS(g/L)	31.2
7	Total hardness(g/L)	1.18
8	Sulphate (g/L)	13.13
9	Carbonate(g/L)	ND*

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 503-510 | ISSN:2251-6727

10	Bicarbonate(g/L)	ND*
11	Chloride(g/L)	3.699
12	Nitrate (g/L)	0.052
13	Total phosphorus (g/L)	0.2289
14	Oil and grease (g/L)	0.7536
15	COD (g/L)	12.32
16	BOD (g/L)	1.7

ND*- Not Determine

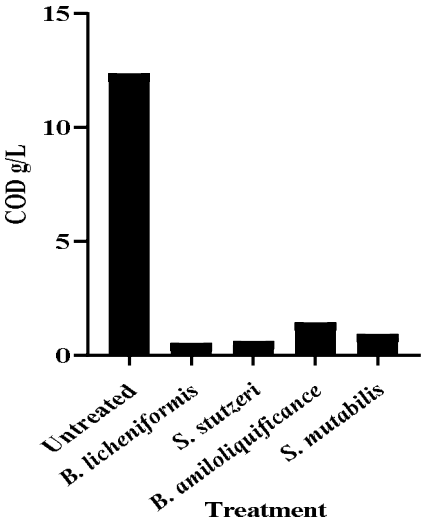

Figure 1. COD of VORE sample (untreated and treated)

The selected bacterial isolates of this study showed substantial bioflocculant production (Fig. 3). It is comparable with bacteria isolated from soil *B. cereus* and *B. thuringiensis* showed 76.3% and 75% flocculating activity respectively [38]. Besides this, the isolates of the present study showed bioflocculant production. VORE contains high Nitrogen and phosphorus concentrations, suspended solid content, organic and inorganic matter. Bacteria can utilize these toxic substances, and thus useful for the treatment of such wastewater [37]. The selected bacterial isolates of this study showed substantial bioflocculant production (Fig. 3). It is comparable with bacteria isolated from soil *B. cereus* and *B. thuringiensis* showed 76.3% and 75% flocculating activity respectively[38].

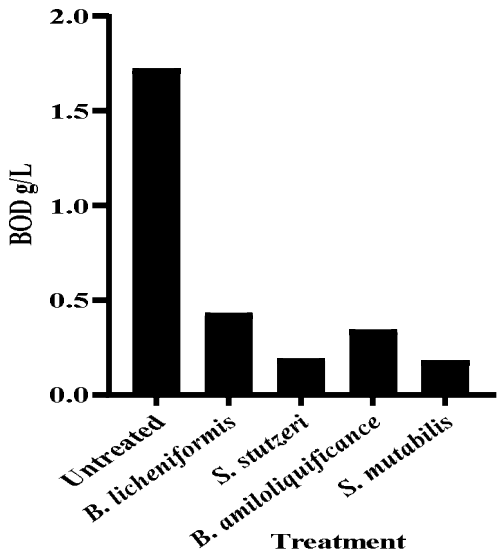

Figure 2. BOD of VORE sample (untreated and treated)

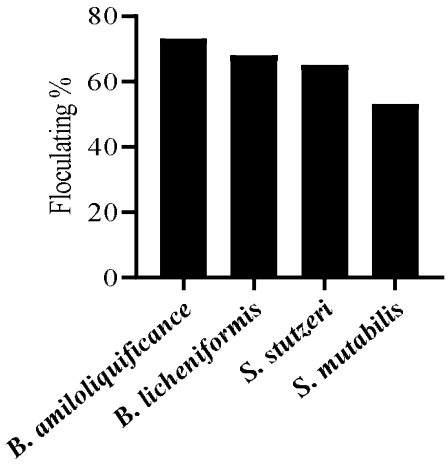

Figure 3 Bioflocculant production by selected bacterial isolates.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 503-510 | ISSN:2251-6727

Toxicity assessment of VORE by seed germination

Germination of seeds depends on internal seed balance factor and external environmental factors like temperature, humidity, light, and oxygen concentration [39]. Substances from effluent like oil may shawl to seeds and prevent oxygen, water, nutrient and gas exchange [40, 41, 42]. Some amount of essential micronutrients are needed for the germination of seeds and for plant growth, but the extreme level can be toxic [43] and significantly reduce seed germination [44, 45].

Oil and nutrients concentration in collected VORE sample was higher than permissible limit, and inhibit the germination of wheat seeds. In present study no germination was reported for watering with untreated effluent. Even after dilution of 1:3 (Untreated VORE: Distilled water) only 10% seeds were able to germinate, this indicates dilution is not an effective solution to reduce the toxicity of such types of effluent.

The selected isolates produces bioflocculant, this can utilizes toxic substances, thus responsible for reduction in toxicity of wastewater. This was evident by increased seed germination percentage with watering by treated wastewater. The germination study highlights those selected isolates significantly reduced the toxicity of the effluent.

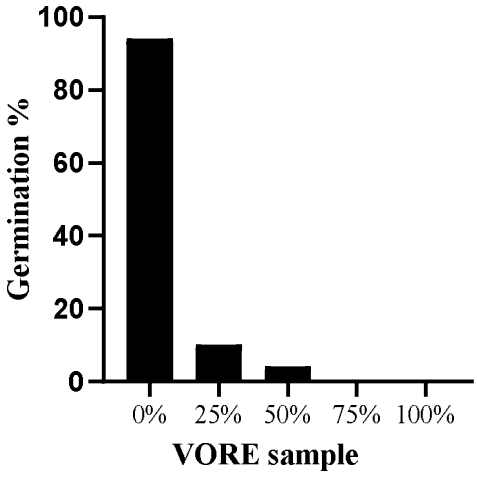

Figure 4. Seed germination in control (0%) and untreated VORE sample

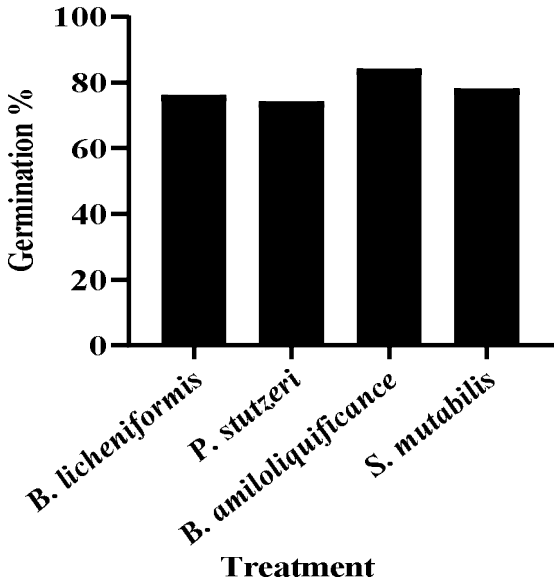

Figure 5. Seed germination in VORE sample after bacterial treatment

4. Conclusion

Edible oil refinery effluent treatment is a challenging process because of the chemical and physical characteristics of the effluent [46]. Physicochemical characteristics of the collected VORE confirmed complex nature. The pH of the effluent was highly acidic in nature, along with EC, nitrate, total phosphorous, total hardness, chloride, sulphate, oil, and grease content were higher than the permissible limit. Highly acidic wastewater restricts the growth of bacteria and negatively affects wheat seeds' germination. The COD and BOD of the effluent were 12.32g/L and 1.7g/L respectively, which makes the effluent very toxic. Toxicity study by seed germination suggests that dilution of effluent cannot solve the toxicity problem, and needs a proper treatment before discharge. The bacteria producing hydrolytic enzymes and bioflocculant can utilize toxic components and thus helps in the remediation of VORE. Biological treatment of VORE by bacterial isolates of present study significantly reduced BOD and COD along with toxicity confirmed by seed germination on wheat seeds. This suggests the potential of isolates for further treatment development.

Acknowledgement

GM Shinde gratefully acknowledges CSIR, New Delhi (India) for SRF. The authors are thankful to NCMR, Pune (India) for 16S rDNA analysis, and the Director,

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 503-510 | ISSN:2251-6727

Government Institute of Science, Aurangabad for providing the facility.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

1. Government of India, Department of Food and Public Distribution, 2021. (https://dfpd.gov.in).
2. Dumont, M.J. and Narine, S.S., 2007. Soapstock and deodorizer distillates from North American vegetable oils: Review on their characterization, extraction and utilization. *Food Research International*, **40**(8), 957-974.
3. Rajkumar, K., Muthukumar, M. and Sivakumar, R., 2010. Novel approach for the treatment and recycle of wastewater from soya edible oil refinery industry—an economic perspective. *Resources, Conservation and Recycling*, **54**(10),752-758.
4. Kale, V., Katikaneni, S.P.R. and Cheryan, M., 1999. Deacidifying rice bran oil by solvent extraction and membrane technology. *Journal of the American Oil Chemists Society*, **76**, 723-727.
5. Wolf, H. and Hamilton, R., 2000. Edible Oil Processing. Sheffield Academic Pres, England, 1-275.
6. Hung, Y.T. and Kaya, D., 2020. Treatment of vegetable oil refining wastes. *Evolutionary Progress in Science, Technology, Engineering, Arts, and Mathematics*, 1-80.
7. Ngoie, W.I., Oyekola, O.O., Ikhu-Omoregbe, D. and Welz, P.J., 2020. Valorisation of edible oil wastewater sludge: bioethanol and biodiesel production. *Waste and biomass valorization*, **11**, 2431-2440.
8. Decloux, M., Lameloise, M.L., Brocard, A., Bisson, E., Parmentier, M. and Spiraers, A., 2007. Treatment of acidic wastewater arising from the refining of vegetable oil by crossflow microfiltration at very low transmembrane pressure. *Process Biochemistry*, **42**(4), 693-699.
9. Rodríguez Mateus, Z., Agualimpia Valderrama, B.E. and Zafra, G., 2016. Isolation and molecular characterization of microorganisms with potential for the degradation of oil and grease from palm oil refinery wastes. *Chemical Engineering Transactions,***49**, 517-522.
10. Vincent, C.S., Nwuche, C.O., Mayel, M.H. and Eze, S.O.O., 2022. Production, Partial Purification, and Characterization of Lipase from Aspergillus niger and Its Application in Treatment of Vegetable Oil Effluent. *Biomolecular Engineering*, **7**(1), 1-7.
11. Pathe, P.P., Nandy, T., and Kaul, S.N., 2000. Wastewater management in vegetable oil industries. *Indian Journal of Environmental Protection*, **20**(7), 481-492.
12. Yin, Y.J., Tian, Z.M., Tang, W., Li, L., Song, L.Y. and McElmurry, S.P., 2014. Production and characterization of high efficiency bioflocculant isolated from Klebsiella sp. ZZ-3. *Bioresource technology*, ***171***, 336-342.
13. Vasileva-Tonkova, E. and Galabova, D., 2003. Hydrolytic enzymes and surfactants of bacterial isolates from lubricant-contaminated wastewater. *Zeitschrift für Naturforschung C*, **58**(1-2), 87-92.
14. Karigar, C.S. and Rao, S.S., 2011. Role of microbial enzymes in the bioremediation of pollutants: a review. Enzyme Res, 805187(11), doi:10.4061/2011/805187.
15. Praveen Kumar, G.N. and Bhat Sumangala, K., 2012. Decolorization of azo dye Red 3BN by bacteria. International Research Journal of Biological Sciences, 1(5), 46-52.
16. APHA, 2017. Standard Methods for The Examination of Water and Wastewater 23rd. American Public Health Association, American Water Works Association, Water Environment Federation.
17. Liu, X.D. and Xu, Y., 2008. A novel raw starch digesting α-amylase from a newly isolated Bacillus sp. YX-1: purification and characterization. Bioresource Technology, 99(10), 4315-4320.
18. Patel, A.R., Mokashe, N.U., Chaudhari, D.S., Jadhav, A.G. and Patil, U.K., 2019. Production optimisation and characterisation of extracellular protease secreted by newly isolated Bacillus subtilis AU-2 strain obtained from Tribolium castaneum gut. Biocatalysis and agricultural biotechnology, 19, p.101122.
19. Gupta, P., Samant, K. and Sahu, A., 2012. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. International Journal of Microbiology, 578925, https://doi.org/10.1155/2012/578925.
20. Boonmahome, P. and Mongkolthanaruk, W., 2013. Lipase-producing bacterium and its enzyme characterization, Journal of Life Sciences and Technologies, 1(4), 196-200.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 503-510 | ISSN:2251-6727

21. Aljuboori, A.H.R., Uemura, Y., Osman, N.B. and Yusup, S., 2014. Production of a bioflocculant from Aspergillus niger using palm oil mill effluent as carbon source. Bioresource technology, 171, 66-70.
22. ISTA, (International Seed Testing Association), 1985. International Rules for Seed Testing. Seed Science and Technology, (13): 307-520.
23. Baharuddin, A.S., Hock, L.S., Yusof, M.Z., Rahman, N.A.A., Shah, U.K., Hassan, M.A., Wakisaka, M., Sakai, K. and Shirai, Y., 2010. Effects of palm oil mill effluent (POME) anaerobic sludge from 500 m3 of closed anaerobic methane digested tank on pressed-shredded empty fruit bunch (EFB) composting process. African Journal of Biotechnology, 9(16), 2427-2436.
24. Al-Shorgani, N.K.N., Shukor, H., Abdeshahian, P., Nazir, M.Y.M., Kalil, M.S., Hamid, A.A. and Yusoff, W.M.W., 2015. Process optimization of butanol production by Clostridium saccharoperbutylacetonicum N1-4 (ATCC 13564) using palm oil mill effluent in acetone–butanol–ethanol fermentation. Biocatalysis and Agricultural Biotechnology, 4(2), 244-249.
25. Azman, N.F., Abdeshahian, P., Al-Shorgani, N.K.N., Hamid, A.A. and Kalil, M.S., 2016. Production of hydrogen energy from dilute acid-hydrolyzed palm oil mill effluent in dark fermentation using an empirical model. International Journal of Hydrogen Energy, 41(37), 16373-16384.
26. Martinez-Burgos, W.J., Sydney, E.B., Medeiros, A.B.P., Magalhães, A.I., de Carvalho, J.C., Karp, S.G., de Souza Vandenberghe, L.P., Letti, L.A.J., Soccol, V.T., de Melo Pereira, G.V. and Rodrigues, C., 2021. Agro-industrial wastewater in a circular economy: Characteristics, impacts and applications for bioenergy and biochemicals. Bioresource Technology, 341, 125795
27. Rosa, D.R., Cammarota, M.C. and Freire, D.M.G., 2006. Production and utilization of a novel solid enzymatic preparation produced by Penicillium restrictum in activated sludge systems treating wastewater with high levels of oil and grease. Environmental Engineering Science, 23(5), 814-823.
28. Leal, M.C., Freire, D.M., Cammarota, M.C. and Sant'Anna Jr, G.L., 2006. Effect of enzymatic hydrolysis on anaerobic treatment of dairy wastewater. Process Biochemistry, 41(5), 1173-1178.
29. Rosa, D.R., Duarte, I.C., Saavedra, N.K., Varesche, M.B., Zaiat, M., Cammarota, M.C. and Freire, D.M., 2009. Performance and molecular evaluation of an anaerobic system with suspended biomass for treating wastewater with high fat content after enzymatic hydrolysis. Bioresource Technology, 100(24), 6170-6176.
30. Shinde, G.M. and Jadhav, A.G., 2021. Characterization and production of groundnut-shell degrading cellulase of Streptomyces mutabilis. Bulletin of Environment, Pharmacology and Life Sciences, 10, 153-159.
31. Denaro, R., Cappello, S. and Yakimov, M. M., 2010. Vegetable oil wastes. Handbook of Hydrocarbon and Lipid Microbiology.
32. Barbusiński, K., Fajkis, S. and Szeląg, B., 2021. Optimization of soapstock splitting process to reduce the concentration of impurities in wastewater. Journal of Cleaner Production, 280, 124459.
33. Aslan, S., Alyüz, B., Bozkurt, Z. and Bakaoglu, M., 2009. Characterization and Biological Treatability of Edible Oil Wastewaters. Polish Journal of Environmental Studies, 18(4), 533-538.
34. Eroglu, V., Ozturk, I., San, H.A. and Demir, I., 1990. Comparative evaluation of treatment alternatives for wastewaters from an edible oil refining industry. Water Science and Technology, 22(9), 225-234.
35. Mkhize, S.P., Atkinson, B.W. and Bux, F., 2000. Evaluation of a laboratory-scale biological process for the treatment of edible oil effluent. Water SA, 26(4), 555-558.
36. Tay, J.H., 1990. Biological treatment of soya bean waste. Water Science and Technology, 22(9), 141-147.
37. Luo, L., Zhao, Z., Huang, X., Du, X., Wang, C.A., Li, J., Wang, L. and Xu, Q., 2016. Isolation, identification, and optimization of culture conditions of a bioflocculant-producing bacterium Bacillus megaterium SP1 and its application in aquaculture wastewater treatment. BioMed Research International, 2758168.
38. Arafa, R.A., El-Rouby, M.N., Abass, H.A. and El-Khier, Z.A., 2014. Bioflocculants produced by bacterial isolates from Egyptian soil 1-

characterization and application of extracellular bioflocculants and nanoparticles for treatment of river Nile water. Journal of Pharmacy and Biological Sciences, 9(5), 103-114.

39. Bewley, J.D. and Black, M., 1994. Seeds: Physiology of Development and Germination. 2 ed. Plenum Press, New York.
40. Vwioko, D.E. and Fashemi, D.S., 2005. Growth response of Ricinus communis L (Castor Oil) in spent lubricating oil polluted soil. Journal of Applied Sciences and Environmental Management, 9(2), 73-79.
41. Njoku, K.L., Akinola, M.O. and Taiwo, B.G., 2009. Effect of gasoline diesel fuel mixture on the germination and the growth of Vigna unguiculata (Cowpea). African Journal of Environmental Science and Technology, 3(12), 466-471.
42. Vauhkonen, V., Lauhanen, R., Ventelä, S., Suojaranta, J., Pasila, A., Kuokkanen, T., Prokkola, H. and Syväjärvi, S., 2011. The phytotoxic effects and biodegradability of stored rapeseed oil and rapeseed oil methyl ester. Agricultural and Food Science, 20(2), 131-142.
43. Silva, J.A., and Uchida, R.S., 2000. Plant nutrient management in Hawaii's soils: Approaches for tropical and subtropical agriculture. College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa, 1–6.
44. Peralta-Videa, J.R., De la Rosa, G., Gonzalez, J.H. and Gardea-Torresdey, J.L., 2004. Effects of the growth stage on the heavy metal tolerance of alfalfa plants. Advances in Environmental Research, 8(3-4), 679-685.
45. Regar, D.L., Dadhich, P. and Jaiswal, P., 2023. Assay on the Impact of Vegetable Oil Mill Effluent on Seed Germination and Seedling Growth of Brassica compestris L. and Oryza sativa L. Journal of Experimental Agriculture International, 45(2), 1-9.
46. Dohare, D. and Meshram, R., 2014. Biological treatment of edible oil refinery wastewater using activated sludge process and sequencing batch reactors-a review. International Journal of Engineering Research and Science & Technology, 3, 251-260.