A mini review on application of enzymatic bioprocesses in animal wastewater and manure treatment

Dongle Cheng[a1], Yi Liu[b1], Huu Hao Ngo[a,*], Wenshan Guo[a], Soon Woong Chang[c], Dinh Duc Nguyen[c,d], Shicheng Zhang[b], Gang Luo[b], Yiwen Liu[a]

[a]*Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Sydney, NWS 2007, Australia*
[b]*Department of Environmental Science and Engineering, Fudan University, Shanghai 200438, China*
[c]*Department of Environmental Energy Engineering, Kyonggi University, 442-760, Republic of Korea*
[d]*Institution of Research and Development, Duy Tan University, Da Nang, Vietnam*

[1] These authors are contributed equally to this work.

* Corresponding author: *E–mail address:ngohuuhao121@gmail.com*

Abstract

Enzymatic processing has been considered an interesting technology as enzymes play important roles in the process of waste bioconversion, whilst heling to develop valuable products from animal wastes. In this paper, the application of enzymes in animal waste management were critically reviewed in short with respect to utilization in: (i) animal wastewater treatment and (ii) animal manure management. The results indicate that the application of enzymes could increase both chemical oxygen demand (COD) removal efficiency and production of biogas. The enzymatic bioprocesses were found to be affected by the type, source and dosage of enzymes and the operating conditions. Further studies on optimizing the operating conditions and developing cost-effective enzymes for the future large-scale application are therefore necessary.

Keywords: Enzymes, animal wastewater, animal manure, anaerobic treatment, bioenergy

1. Introduction

The world's rapid population growth and improvement in people's living standards increased the demand for animal products and especially for food and non-food purposes. To meet the demand for rising levels of consumption, most of small-scale farms have to be expanded to concentrated animal feeding operations (CAFOs) to increase animal production efficiency (Ramankutty et al., 2018). The global growing trend of cattle, goats, pigs, chickens and aquaculture counts was shown in Fig. 1 (Roser, 2017).

Insert Figure 1

From the year 1951 to 2014, the production of cattle, goats, pigs, chickens and aquaculture increased 1.6, 2.9, 2.4, 5.5 and 54 times, respectively (Roser, 2017). As a result, increasing amounts of animal wastewater and manure were produced during animal production, slaughterhouse, and subsequent meat production processes (Hibbard et al., 1996;

Jayathilakan et al., 2012; Malomo et al., 2018). Continuous production of animal wastewater and manure in large amounts are unavoidable and have become a global environmental issue, especially since they are highly perishable and rich in microorganisms. Many of these carry pathogens that pose serious threats to people's and animals' health (Jayathilakan et al., 2012; Meeker, 2009). Specifically, the direct application of animal wastewaters and manure to soils as irrigation and fertilizer can endanger environment security, due to the accumulation of nutrients in soils resulting in potential surface water and groundwater pollution (Ndambi et al., 2019; Shuval, 1991). Therefore, although animal wastewater and manure play important roles in the global agricultural economy, critical issues including environmental pollution and pathogenic potential related to these wastes need to be resolved urgently.

As reported previously, strategies such as physical, chemical and biological processes can be applied to treat animal wastewater and manure and convert them into valuable bioenergy (biogas) or products (organic fertilizers) (Cantrell et al., 2008; Vanotti et al., 2008). Biological processes are the most popular technologies for animal wastewater and manure treatment due to their benefits of economic attractiveness and simple operation (Aziz et al., 2019). However, animal wastewater produced from dairy, slaughterhouses and meat processing not only contains high levels of organic matters and nutrients but also has amounts of fats and greases, which represent a big challenge for its biological treatment (Bustillo-Lecompte & Mehrvar, 2015). To enhance the biological treatment efficiency, animal wastewater containing fats and greases has to be pretreated. Anaerobic digestion can be a preferred method for animal manure treatment, which convert animal manure to biogas and decrease its volume and toxicity (Banković-Ilić et al., 2014; Nasir et al., 2012). Whereas, the biogas production was limited by the lignocellulose content of animal manure (Triolo et al., 2011).

The application of enzymes in animal wastewater and manure treatment is a promising strategy to overcome difficulties of fats and lignocelluloses biotransformation in the subsequent bioprocesses to enhance their treatment efficiencies and biogas production (Brandelli et al., 2015; Liew et al., 2019). Enzymes are natural and highly efficient catalysts, which can speed up the convention of target compounds without affecting others. In addition, enzyme catalysis has more advantages in degrading the refractory compounds in animal wastewater and manure than physicochemical catalysis because of its mild, highly efficient, eco-friendly reaction and catalytic specificity as well, without affecting other nutrients in the sample (Brandelli et al., 2015; Yao et al., 2020). This review presents an overview and critical discussion on the application of enzymes in the pretreatment of animal wastewater and manure, focusing on the effectiveness, mechanism and impact factor of enzymatic bioprocesses.

2. Enzymatic bioprocesses in animal wastewater treatment

Animal wastewaters from slaughtering, dairy and meat processing generally contain large amounts of fats and greases, which limit their effectiveness in biological treatment. The main reason is that excessive fats and greases can: 1) accumulate on sludge surface, reducing transfer rates of solution substrate to biomass and oxygen to the aerobic microorganisms; 2) inhibit sludge activity and development of filamentous microbial, which in turn influences the sediment of the sludge and results in biomass losses due to the outflow of bioreactors. Furthermore, the appearance of blockages and unpleasant odors problems caused by fats and greases in wastewater are long-term challenges (Cammarota & Freire, 2006). Therefore, the pretreatment process is necessary to hydrolyze fats and greases to improve the further biological treatment efficiency of wastewaters. Traditionally, physicochemical systems such as the grease-trap, adsorption, membrane filtration, flotation and coagulation with different chemical compounds, are used in wastewater treatment plants for the pretreatment of wastewater containing fats and greases. However, these commonly used processes are not only inefficient in removing colloidal and emulsified particle but also costly (Kárpáti et al., 1990; Willey, 2001). The hydrolysis of fats and greases in wastewater by enzymes (mainly lipases) is now an alternative technology. As a biocatalyst, enzymes have proved to be effective for the degradation and transformation of complex triglycerides into simpler free fatty acids (FFAs), enhancing the performance of microorganisms in a later biological treatment process (Jamie et al., 2016; Valladão et al., 2011). Table 1 shows few examples about the enzymatic application in the animal wastewater treatment.

Insert Table 1

Lipases are the most common enzymes applied to the treatment of wastewater containing fats and greases, which are useful to modify the structure of fats by catalyzing the release of FFAs from long-chain triacylglycerols (C>10) (Hitch & Clavel, 2019). Pascale et al. (2019) attempted to deal with floating fat wastes from dairy and poultry slaughterhouses with a commercial lipase. Results showed that long chain FFAs, especially unsaturated acids released and the amount of it rose when initial pH was adjusted to 7.0. Lipases can be produced either by submerged (SF) or by solid-state fermentation (SSF). The SSF process was preferred to produce lipases due to its advantage of low cost of using raw materials (agro-industrial residues such as babassu oil cake, coconut and soybean meals), simple operation, and energy-saving properties (Cammarota & Freire, 2006). Fungal lipases produced by SSF have been successfully used in treating effluents from dairy, poultry slaughtering, and fish processes (Cammarota & Freire, 2006). As shown in Table 1, most of lipase used in the previous study was produced from *Penicillium sp*. through the SSF process. For instance, Alexandre et al. (2011) investigated the pretreatment of fish-processing wastewater by a lipase-rich solid enzyme pool from the fungus *Penicillium simplicissimum*, and concluded that chemical oxygen demand (COD) removal efficiency increased from 79.9 to 90.9 % when 0.2% enzyme was used to hydrolyze the oil and grease (1500 mg/L) prior to the anaerobic treatment. Rosa et al. (2009) also observed a great enhancement in COD removal efficiencies (from 32 to 90%) in the anaerobic treatment of dairy wastewater (1200 mg/L of oil and grease) when fed with pre-hydrolyzed wastewater by enzymes from SSF of a *Penicillium sp*. fungus. The authors indicated that the difference was linked to the different microbial diversities of the anaerobic sludge with and without the pre-hydrolysis stage.

Furthermore, the production of biogas in the anaerobic treatment of oily wastewater can be improved by enzymatic pretreatment. Valladao et al. (2009) found that biogas production increased by 50% compared to the control (without enzymatic pretreatment) by using lipases (*Penicillium sp*.) pretreatment prior to the anaerobic treatment of wastewater from a poultry slaughterhouse. The study by Valladão et al. (2007) demonstrated that the anaerobic treatment efficiency of poultry slaughterhouse (1200 mg/L oil and grease) was enhanced when a enzymatic pool served in the pretreatment process, with the COD removal efficiency rising from 53% to 85% and biogas production increased from 37 ml to 175 ml in 4 d. Moreover, operating problems, such as accumulation of fat on sludge surface, intense formation of scum, and frequent cleaning requirements, were reduced through the enzyme pretreatment (Alexandre et al., 2011).

The hydrolysis of triglycerides by lipase follows the ping-pong bi-bi mechanism, which is a series of directed progressive reactions to form intermediate diglycerides and monoglycerides (Chew et al., 2008). The binding of lipase and triglyceride can form a lipase–triglyceride complex, which is further converted into an intermediate complex and glycerol by isomerization. Furthermore, the intermediate complex combines with three molecules of water to form a binary complex. The final produced fatty acids are generated and the enzyme is regenerated through unimolecular isomerization at the last step. The attachment of acid or alcohol to the enzyme causes irreversible inhibition (Liew et al., 2019).

Process parameters, including temperature, pH, enzyme concentration and enzymatic hydrolysis time are important for the lipase activity. The lipase activity could be enhanced by increasing the hydrolysis temperature, but declined when the temperature exceeded to a value (Meng et al., 2017). According to previous studies, the optimal temperatures of lipases activities for the hydrolysis of fats and greases in animal wastewater ranged from 30 to 50°C (Boran et al., 2019; Leal et al., 2002; Meng et al., 2017; Rosa et al., 2009). The best pH for the stability of lipase depended on the produced microorganisms, which is usually between the value of 6 and 8 (as shown in Table 1) (Boran et al., 2019). Time for enzymatic hydrolysis is another important parameter that affects the hydrolysis rate (Masse et al., 2001). The hydrolysis rate showed little change by further increasing the hydrolysis time after the reaction reached equilibrium. For example, Meng et al. (2017) observed that lipids were adequately hydrolysed by lipases when the time of hydrolysis reached 24 h, but no obvious difference was found in the hydrolysis rates after the hydrolysis time exceeded 36 h. Moreover, the reaction time for the enzymatic pre-hydrolysis could be shortened under the influence of ultrasound, i.e. from 24 h to 40 min (Adulkar & Rathod, 2014). Hence, it is important to maintain the best conditions to improve lipase activity and enzymatic hydrolysis efficiency. To shorten the reaction time, further research is necessary on the combination of enzymatic hydrolysis with other technologies, for instance ultrasound.

For the commercial application of lipase in industrial wastewater treatment, the lipase is required to be stable under harsh conditions, such as high pH and temperature, as well as the presence of organic solvents. Up to now, insufficient research has been done on lipase in degradation of fats in animal wastewater and so more investigations are required to develop thermo- and solvent-stable lipases under industrial conditions for improving catalysis efficiency. A novel lipase with robust activity could be isolated from special spots like thermophilic region and organic solution outlet or employ modern molecular biologic technologies, such as metagenomic sequencing and enzyme evolution (Polaina & MacCabe, 2007; Sahoo et al., 2020). Nehal et al. (2019) isolated a new thermophilic non-induced lipase from oil waste in Algeria with a high organic solvents tolerance and 75% retaining activity the presence of 10 mM Fe^{2+}, K^{+}, and Na^{+} ions. The half -time of this novel lipase could reach 22 h and 90 min at 50℃ and 60℃, respectively. Ktata et al. (2020) discovered a newly thermostable lipase derived from *Aeribacillus pallidus* (GPL) and applied it in oil wastewater treatment. The GPL had maximum activity at 65℃ and pH 10 and exhibited a 96% oil removal efficiency. Nevertheless, more in-depth research is required in further study.

3. Enzymatic bioprocesses in animal manure treatment

Biogas produced from animal manure and slurries through anaerobic digestion can replace fossil fuels in heat, electricity generation and transportation, while the residual digestate can be used as a valuable fertilizer for crops. However, the lignocellulose content of animal manure (30% - 80%) is a major obstacle to biogas generation (Triolo et al., 2011). Møller et al. (2004) indicated that the biodegradability of cattle manure and pig manure is about 32% and 69%, respectively. The biodegradability of cattle manure is lower than pig manure because of the higher lignocellulose content. In anaerobic digestion processes, the conversion of animal manure to biogas occurs mainly through four steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. Products from one stage serve as the substrates of the next, resulting in conversion of organic matter into biogas (Parawira, 2012). The hydrolysis of cellulose, hemicellulose and lignin in animal manure is normally a rate-limiting step, which requires effective methods to enhance the manure biodegradation and biogas/biomethane production (Liew et al., 2020). Therefore, pretreatment of animal manure is required to ensure the cellulose, hemicellulose and lignin are more accessible to microorganisms and enhance hydrolysis and biogas production.

Compared with other mechanical, chemical, physicochemical and microbial processes, enzymatic hydrolysis is preferred due to its properties of high versatility and selectivity, environmental friendliness, low energy input and no chemical requirement (Lovanh et al., 2018; Taherzadeh & Karimi, 2008). Process of enzymatic hydrolysis of lignocellulostic substrates in animal manure during anaerobic digestion is shown in Figure 2.

Insert Figure 2

In addition, enzymes can remain active when coexisting with toxic substrates and in a wide range of conditions, which have more likely to contact substrates compared with microorganisms with smaller size, higher solubility and mobility (Romero-Güiza et al., 2016; Wang et al., 2016). Adding exogenous enzymes have been considered to improve the biodegradation of animal manure. The activity of the enzymes and the efficiency of their hydrolysis could be affected by many factors, including enzyme formulation, enzyme addition methods and system configurations and environmental conditions (Parawira, 2012). Based on previous studies, the contribution of enzyme to biomethane yields during the anaerobic digestion of animal manure is summarized in Table 2.

Insert Table 2

Enzymes could be added into the digester directly or the pretreatment process prior to anaerobic digestion. As shown in Table 2, for using the same enzyme mixture and similar conditions, adding enzymes to the enzymatic pretreatment step will increase methane yield rather than their direct addition to the digester. The study by Sutaryo et al. (2014) stated that no significant effect was found after the addition of enzyme mixture to the dairy cattle manure digester compared with a control digester without the extra enzyme addition. The authors explained that enzymes might be degraded by microorganisms if they were directly added to the digester. Similar results were concluded by Romano et al. (2009), and no difference in biogas and methane yields was observed by adding enzymes in the anaerobic reactor. Weide et al. (2020) indicated that the main effect of the enzyme treatments was to accelerate substrate degradation, since an increase of methane yields (0.3 - 21.1%) were observed between test days 5 and 15, which was almost undetectable after 60 days of testing.

As observed from Table 2, the optimal temperature for the enzymatic treatment is in the 35 - 50°C range, which is best for the growth of microorganisms, and the hydrolysis rate and methane generation might increase as temperature also rises in this range. Quiñones et al. (2012) indicated that the hydrolysis rate decreased by increasing the temperature over 60 °C due to the reduction of enzyme activity caused by the enzyme denaturation.The amount of enzymes used for the digestion should be optimized for the efficiency and economy of the process. Quiñones et al. (2012) demonstrated that higher enzyme concentration was positive with the hydrolysis rate. However, Weide et al. (2020) found that too high an enzyme concentration did not lead to a better outcome. The reason is that the crowding of the enzyme may interfere with its activity, because it is difficult to diffuse through the fibers of the substrate. In reality, the enzyme loadings are related to the types of substrates and enzymes, enzyme activities and other physical and chemical factors (Čater et al., 2014). Therefore, more studies are required to determine the proper concentration of enzymes to maximize the methane generation and minimize the cost of the process.

4. Future perspectives

The proper treatment of animal wastewater and manure has attracted great attention due to the huge amounts of production, hazards to ecological security, and potential resource value of animal wastes. Enzymatic technologies are now interesting alternatives for animal waste treatment based on their various advantages mentioned earlier in this paper. Whereas most current research is on the bench-scale stage, it is necessary to investigate pilot- and full-scale applications of enzymes in animal waste treatment to better evaluate their feasibilities and effectiveness. There is still much work to be done before the large-scale application of enzymes. As discussed above, the reaction rate and efficiency of enzymatic hydrolysis can be influenced by system configurations, operating conditions, as well as enzyme types, dosages, and other methods. Therefore, more investigations are required to optimize these influencing parameters to improve the effectiveness of enzymatic hydrolysis. To maintain the stability and activity of enzymes, the hydrolysis process is required to operate under optimal conditions. Considering the large range of operating conditions in industrial wastewater treatment plants, a novel enzyme that can remain stable under extreme conditions needs to be developed in the near future.

Furthermore, the high cost of current commercial enzymes remains a big challenge for their large-scale application in animal wastes management. Although several promising technologies such as immobilized enzymes, microwave assistance and ultrasound assistance, have been developed to try to shorten the enzymatic hydrolysis time and reduce the cost of enzymatic application, further development is necessary. The production of enzymes from cheaper substrates is also important to reduce the application cost. Animal wastes could be used as potential substrates for cultivating the enzyme-producing microorganisms. Hence, a combined enzymatic process of enzyme production and further application should be an environmentally friendly and cost-effective strategy for animal wastes management, which requires much more in-depth research.

5. Conclusion

Anaerobic processes are commonly used to treat animal wastewater and manure and produce biogas. Whereas, fats and greases in animal wastewater and lignocellulose in animal manure limit the treatment efficiency and quantity of biogas production. The application of enzymatic bioprocesses in animal wastewater and manure treatment could improve their treatment efficiency with high COD removal and biogas production by hydrolyzing the fats/greases and lignocellulose to easily degradable compounds. Furthermore, more investigations are required for the production of low-cost enzymes and their large-scale applications.

Acknowledgement

This research was supported by the Centre for Technology in Water and Wastewater, University of Technology, Sydney, Australia (UTS, RIA NGO) and the Korea Institute of Energy Technology Evaluation and Planning (KETEP) and the Ministry of Trade, Industry & Energy (MOTIE) of the Republic of Korea (Grant No. 20173010092470).

References

1. Adulkar, T.V., Rathod, V.K., 2014. Ultrasound assisted enzymatic pre-treatment of high fat content dairy wastewater. Ultrason. Sonochem. 21 (3), 1083-1089.

2. Alberton, D., Alexander Mitchell, D., Cordova, J., Peralta Zamora, P., Krieger, N. 2010. Production of a fermented solid containing lipases of Rhizopus microsporus and its application in the pre-hydrolysis of a high-fat dairy wastewater. Food Technol. Biotechnol., 48 (1), 28-35.

3. Alexandre, V., Valente, A., Cammarota, M.C., Freire, D.M., 2011. Performance of anaerobic bioreactor treating fish-processing plant wastewater pre-hydrolyzed with a solid enzyme pool. Renew. Energy 36 (12), 3439-3444.

4. Aziz, A., Basheer, F., Sengar, A., Khan, S.U., Farooqi, I.H., 2019. Biological wastewater treatment (anaerobic-aerobic) technologies for safe discharge of treated slaughterhouse and meat processing wastewater. Sci. Total Environ. 686, 681-708.

5. Banković-Ilić, I.B., Stojković, I.J., Stamenković, O.S., Veljkovic, V.B., Hung, Y.-T., 2014. Waste animal fats as feedstocks for biodiesel production. Renew. Sust. Energ. Rev. 32, 238-254.

6. Bhange, V.P., Suke, S.G. 2018. Effect of lipase from different source on high fat content wastewater of dairy industry. Indian J. Biotechnol. 17, 244-250

7. Boran, R., Ugur, A., Sarac, N., Ceylan, O., 2019. Characterisation of Streptomyces violascens OC125-8 lipase for oily wastewater treatment. 3 Biotech 9 (1), Article number: 5.

8. Brandelli, A., Sala, L., Kalil, S.J., 2015. Microbial enzymes for bioconversion of poultry waste into added-value products. Food Res. Int. 73, 3-12.

9. Bustillo-Lecompte, C.F., Mehrvar, M. 2015. Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: A review on trends and advances. J. Environ. Manage., 161 287-302.

10. Cammarota, M., Freire, D., 2006. A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. Bioresour. Technol. 97 (17), 2195-2210.

11. Cantrell, K.B., Ducey, T., Ro, K.S., Hunt, P.G. 2008. Livestock waste-to-bioenergy generation opportunities. Bioresour. Technol., 99 (17), 7941-7953.

12. Čater, M., Zorec, M., Logar, R.M., 2014. Methods for improving anaerobic lignocellulosic substrates degradation for enhanced biogas production. Springer Sci. Rev. 2 (1-2), 51-61.

13. Chew, Y.H., Chua, L.S., Cheng, K.K., Sarmidi, M.R., Aziz, R.A., Lee, C.T., 2008. Kinetic study on the hydrolysis of palm olein using immobilized lipase. Biochem. Eng. J. 39 (3), 516-520.

14. Dors, G., Mendes, A.A., Pereira, E.B., de Castro, H.F., Furigo, A. 2013. Simultaneous enzymatic hydrolysis and anaerobic biodegradation of lipid-rich wastewater from poultry industry. Applied Water Science, 3 (1), 343-349.

15. dos Santos Ferreira, J., de Oliveira, D., Maldonado, R.R., Kamimura, E.S., Furigo, A. 2020. Enzymatic pretreatment and anaerobic co-digestion as a new technology to high-methane production. Appl. Microbiol. Biotechnol., 1-12.

16. Hibbard, D.C., Raghunath, B., Guyse, C.S. 1996. Food processing wastewater treatment and recovery process, Google Patents.

17. Hitch, T.C., Clavel, T., 2019. A proposed update for the classification and description of bacterial lipolytic enzymes. Peer J. 7, e7249.

18. Jamie, A., Alshami, A.S., Maliabari, Z.O., Ali Ateih, M., Al Hamouz, O.C.S., 2016. Immobilization and enhanced catalytic activity of lipase on modified MWCNT for oily wastewater treatment. Environ. Prog. Sustain. Energy 35 (5), 1441-1449.

19. Jayathilakan, K., Sultana, K., Radhakrishna, K., Bawa, A., 2012. Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. J. Food Sci. Technol. 49 (3), 278-293.

20. Jung, F., Cammarota, M., Freire, D. 2002. Impact of enzymatic pre-hydrolysis on batch activated sludge systems dealing with oily wastewaters. Biotechnol. Lett., 24 (21), 1797-1802.

21. Kárpáti, A., Bencze, L., Borszéki, J., 1990. New process for physico-chemical pretreatment of dairy effluents with agricultural use of sludge produced. Water Sci. Technol. 22 (9), 93-100.

22. Ktata, A., Krayem, N., Aloulou, A., Bezzine, S., Sayari, A., Chamkha, M., Karray, A., 2020. Purification, biochemical and molecular study of lipase producing from a newly thermoalkaliphilic Aeribacillus pallidus for oily wastewater treatment. J. Biochem. 167 (1), 89-99.

23. Leal, M., Cammarota, M., Freire, D., Anna Jr, S., 2002. Hydrolytic enzymes as coadjuvants in the anaerobic treatment of dairy wastewaters. Braz. J. Chem. Eng. 19 (2), 175-180.

24. Liew, Y.X., Chan, Y.J., Sivakumar, M., Chong, M.F., Chong, S., Tiong, T.J., Lim, J.-W., Pan, G.-T., 2019. Enzymatic pretreatment to enhance anaerobic bioconversion of high strength wastewater to biogas: A review. Sci. Total Environ. 713, p. 136373.

25. Lovanh, N., Ruiz-Aguilar, G., Loughrin, J. 2018. Enzymatic pre-treatment of high content cellulosic feedstock improves biogas production. In 2018 ASABE Annual International Meeting. American Society of Agricultural and Biological Engineers. p. 1.

26. Malomo, G.A., Madugu, A.S., Bolu, S.A., 2018. Sustainable animal manure management strategies and practices. Agricultural Waste and Residues, 119-137.

27. Masse, L., Kennedy, K., Chou, S., 2001. Testing of alkaline and enzymatic hydrolysis pretreatments for fat particles in slaughterhouse wastewater. Bioresour. Technol. 77 (2), 145-155.

28. Meeker, D.L., 2009. North American Rendering: processing high quality protein and fats for feed. Rev. Bras. Zootec 38 (SPE), 432-440.

29. Mendes, A.A., Pereira, E.B., Furigo Jr, A., Castro, H.F.d. 2010. Anaerobic biodegradability of dairy wastewater pretreated with porcine pancreas lipase. Braz Arch Biol Technol, 53 (6), 1279-1284.

30. Meng, Y., Luan, F., Yuan, H., Chen, X., Li, X., 2017. Enhancing anaerobic digestion performance of crude lipid in food waste by enzymatic pretreatment. Bioresour. Technol. 224, 48-55.

31. Mobarak, Q.E., Kasra, K.R., Nosrati, M., Amani, T. 2012. Enzymatic pre-hydrolysis of high fat content dairy wastewater as a pretreatment for anaerobic digestion. Int. J. Environ. Res.6 (2), 475-480.

32. Møller, H.B., Sommer, S.G., Ahring, B.K., 2004. Methane productivity of manure, straw and solid fractions of manure. Biomass Bioenergy 26 (5), 485-495.

33. 85. Nasir, I.M., Mohd Ghazi, T.I., Omar, R., 2012. Anaerobic digestion technology in livestock manure treatment for biogas production: a review. Eng. Life Sci. 12 (3), 258-269.

34. Ndambi, O.A., Pelster, D.E., Owino, J.O., De Buisonje, F., Vellinga, T.V., 2019. Manure management practices and policies in sub-Saharan Africa: implications on manure quality as a fertilizer. Front. Sustain. Food Syst. 3, 29.

35. Nehal, F., Sahnoun, M., Dab, A., Sebaihia, M., Bejar, S., Jaouadi, B., 2019. Production optimization, characterization, and covalent immobilization of a thermophilic Serratia rubidaea lipase isolated from an Algerian oil waste. Mol. Biol. Rep. 46 (3), 3167-3181.

36. Parawira, W., 2012. Enzyme research and applications in biotechnological intensification of biogas production. Crit. Rev. Biotechnol. 32 (2), 172-186.

37. Pascale, N., Chastinet, J., Bila, D., Sant̀Anna, G., Quitério, S., Vendramel, S., 2019. Enzymatic hydrolysis of floatable fatty wastes from dairy and meat food-processing industries and further anaerobic digestion. Water Sci. Technol. 79 (5), 985-992.

38. Polaina, J., MacCabe, A.P. 2007. Industrial enzymes. Netherlands: Springer, 531-547.

39. Polizelli, P.P., Facchini, F.D.A., Bonilla-Rodriguez, G.O. 2013. Stability of a Lipase extracted from seeds of Pachira aquatica in commercial detergents and application tests in poultry wastewater pretreatment and fat particle hydrolysis. Enzyme Res., Article ID 324061.

40. Quiñones, T.S., Plöchl, M., Budde, J., Heiermann, M., 2012. Results of batch anaerobic digestion test–effect of enzyme addition. Agricultural Engineering International: CIGR Journal 14 (1), 38-50.

41. Ramankutty, N., Mehrabi, Z., Waha, K., Jarvis, L., Kremen, C., Herrero, M., Rieseberg, L.H., 2018. Trends in global agricultural land use: Implications for environmental health and food security. Annu. Rev. Plant Biol. 69, 789-815.

42. Rigo, E., Rigoni, R.E., Lodea, P., De Oliveira, D., Freire, D.M., Treichel, H., Di Luccio, M. 2008. Comparison of two lipases in the hydrolysis of oil and grease in wastewater of the swine meat industry. Ind. Eng. Chem. Res., 47 (6), 1760-1765.

43. Romano, R.T., Zhang, R., Teter, S., McGarvey, J.A., 2009. The effect of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass. Bioresour. Technol. 100 (20), 4564-4571.

44. Romero-Güiza, M., Vila, J., Mata-Alvarez, J., Chimenos, J., Astals, S., 2016. The role of additives on anaerobic digestion: a review. Renew. Sust. Energ. Rev. 58, 1486-1499.

45. Rosa, D.R., Duarte, I.C., Saavedra, N.K., Varesche, M.B., Zaiat, M., Cammarota, M.C., 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. Bioresour. Technol. 100 (24), 6170-6176.

46. Roser, H.R.a.M. (2017). "Meat and Seafood Production & Consumption", Vol. 2019, Our World in Data. https://ourworldindata.org/meat-and-seafood-production-consumption.

47. Sahoo, R.K., Das, A., Gaur, M., Sahu, A., Sahoo, S., Dey, S., Rahman, P.K., Subudhi, E., 2020. Parameter optimization for thermostable lipase production and performance evaluation as prospective detergent additive. Prep. Biochem. Biotechnol., 1-7.

48. Shuval, H. 1991. Effects of wastewater irrigation of pastures on the health of farm animals and humans. Rev. Sci. Tech., 10 (3), 847-66.

49. Sutaryo, S., Ward, A.J., Møller, H.B., 2014. The effect of mixed-enzyme addition in anaerobic digestion on methane yield of dairy cattle manure. Environ. Technol. 35 (19), 2476-2482.

50. Taherzadeh, M.J., Karimi, K., 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int. J. Mol. Sci. 9 (9), 1621-1651.

51. Triolo, J.M., Sommer, S.G., Møller, H.B., Weisbjerg, M.R., Jiang, X.Y., 2011. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: Influence of lignin concentration on methane production potential. Bioresour. Technol. 102 (20), 9395-9402.

52. Valladao, A., Sartore, P., Freire, D., Cammarota, M., 2009. Evaluation of different pre-hydrolysis times and enzyme pool concentrations on the biodegradability of poultry slaughterhouse wastewater with a high fat content. Water Sci. Technol. 60 (1), 243-249.

53. Valladão, A., Torres, A., Freire, D., Cammarota, M., 2011. Profiles of fatty acids and triacylglycerols and their influence on the anaerobic biodegradability of effluents from poultry slaughterhouse. Bioresour. Technol. 102 (14), 7043-7050.

54. Valladão, A.B.G., Freire, D.M.G., Cammarota, M.C., 2007. Enzymatic pre-hydrolysis applied to the anaerobic treatment of effluents from poultry slaughterhouses. Int. Biodeterior. Biodegrad. 60 (4), 219-225.

55. Vanotti, M., Szogi, A., Vives, C. 2008. Greenhouse gas emission reduction and environmental quality improvement from implementation of aerobic waste treatment systems in swine farms. Waste Manage., 28 (4), 759-766.

56. Wang, X., Li, Z., Zhou, X., Wang, Q., Wu, Y., Saino, M., Bai, X., 2016. Study on the bio-methane yield and microbial community structure in enzyme enhanced anaerobic co-digestion of cow manure and corn straw. Bioresour. Technol. 219, 150-157.

57. Weide, T., Baquero, C.D., Schomaker, M., Brügging, E., Wetter, C., 2020. Effects of enzyme addition on biogas and methane yields in the batch anaerobic digestion of agricultural waste (silage, straw, and animal manure). Biomass Bioenergy 132, p. 105442.

58. Willey, R., 2001. Fats, oils, and greases: the minimization and treatment of wastewaters generated from oil refining and margarine production. Ecotoxicol. Environ. Saf. 50 (2), 127-133.

59. Yao, Y., Wang, M., Liu, Y., Han, L., Liu, X., 2020. Insights into the improvement of the enzymatic hydrolysis of bovine bone protein using lipase pretreatment. Food Chem. 302, p. 125199.

Figure captions

Fig. 1 Total number of livestock animals worldwide, with exception to aquaculture figures, which are reported in weight (Roser, 2017).

Fig. 2 Process of enzymatic hydrolysis of lignocellulostic substrates in animal manure during anaerobic digestion.

Table Captions

Table 1 Examples on enzymatic application in the treatment of animal wastewater

Table 2 Examples for biomethane production in the anaerobic digestion of animal manure with enzymes application.

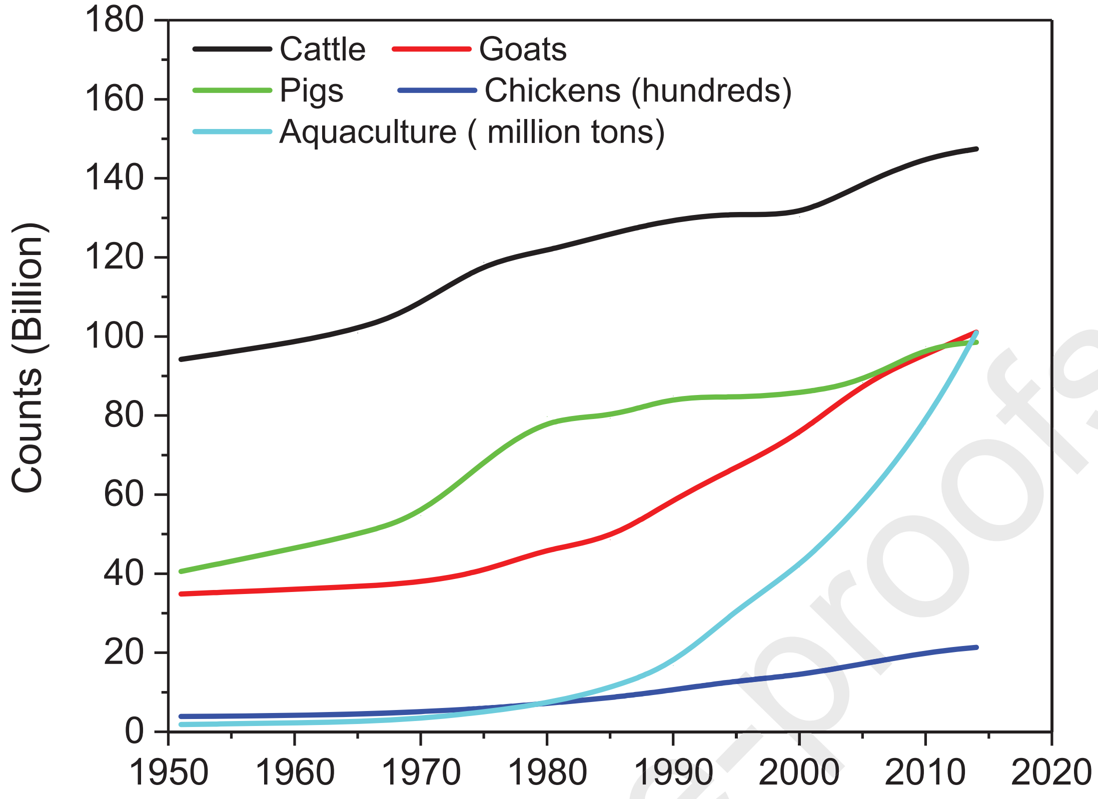


Fig. 1 Total number of livestock animals worldwide, with exception to aquaculture figures, which are reported in weight (Roser, 2017).

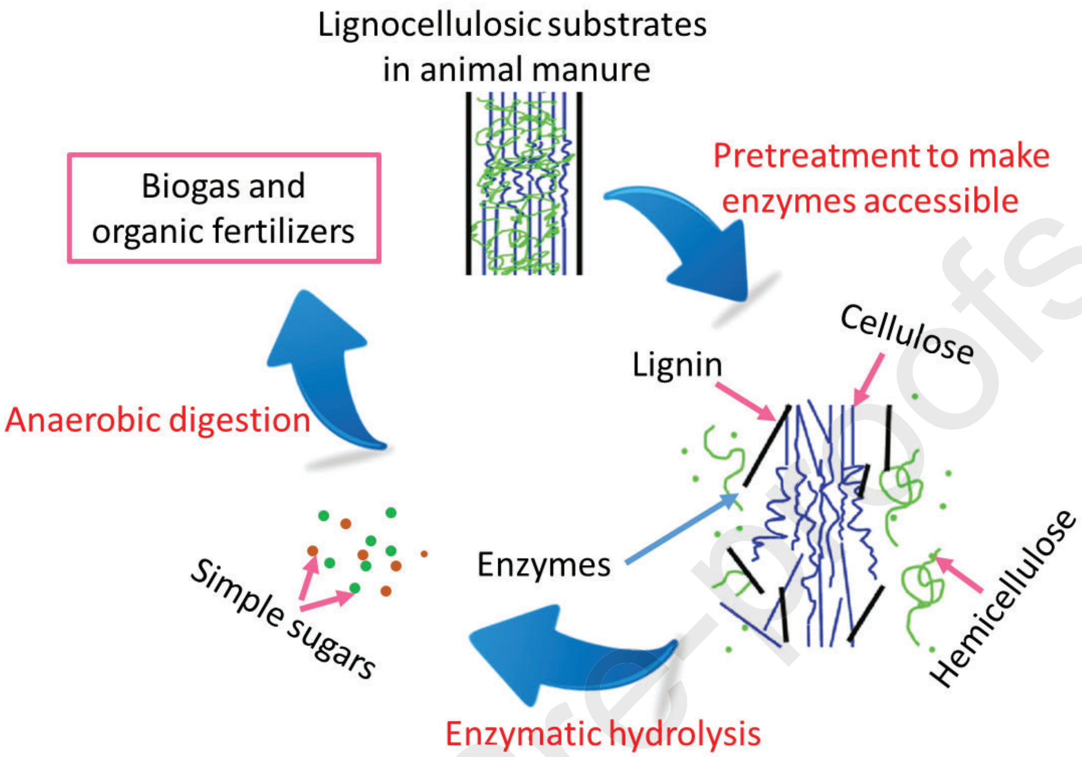


Fig. 2 Process of enzymatic hydrolysis of lignocellulostic substrates in animal manure during anaerobic digestion.

Table 1 Examples on enzymatic application in the treatment of animal wastewater

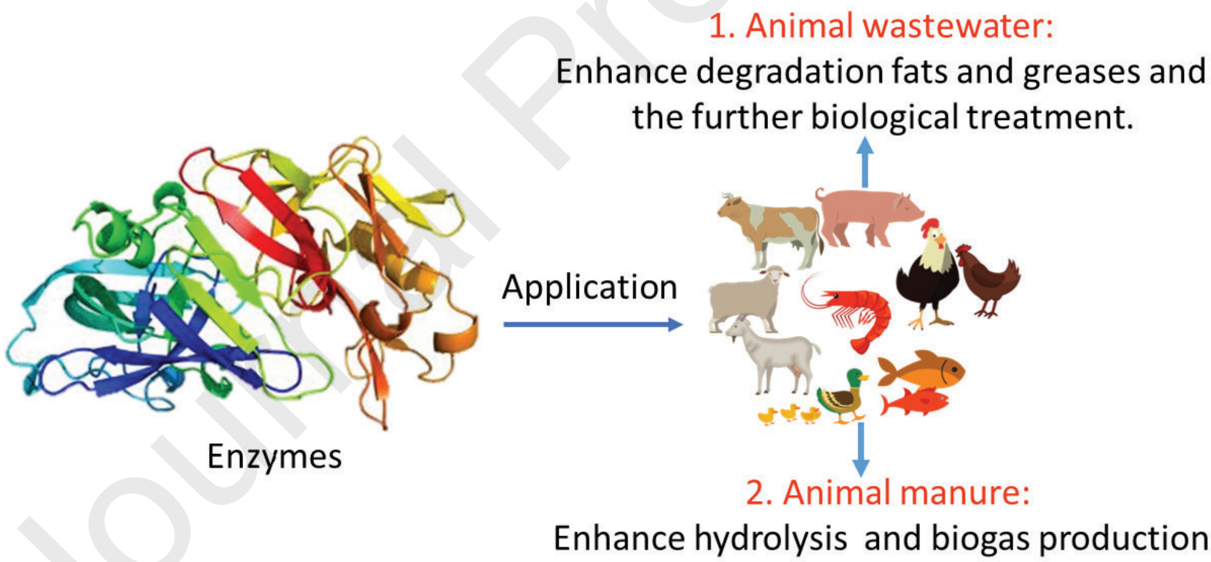
Animal wastewater	Fat and grease concentration (g/L)	Enzymes sources	Enzyme dosage	Operating parameters	Contribution to the treatment	References
Dairy wastewater	-	Lipase from raw milk and crude enzyme extract,	4% w/v	37 °C for 72 h	Enhance COD and TS removal; Balance the pH	(Bhange & Suke, 2018)
	0.8	Lipases produced by *Penicillium restrictum*	0.2% w/v	30 °C for 8 h, pH 7.0	COD removal enhaced from 0 to 82%; Volatile suspended solids reduction of 10 times	(Jung et al., 2002)
	1.2	Lipase produced by *Penicillium restrictum*	10% v/v	35°C for 12h	Fat removal enhanced from 19 to 80%; COD removal increased 10 times	(Leal et al., 2002)
	1.2	lipase producred by *Penicillium sp.*	0.1% w/v	30 °C for 24 h, pH 7.0	Free acid release increased 8 times; COD removal increased from 32 to 90%	(Rosa et al., 2009)
	1.3	Lipase produced by *Rhizopus microsporus*	0.3 % m/v	35 °C for 72 h	76.9% reduction of oil and grease	(Alberton et al., 2010)
	-	Porcine pancreas lipase	0.05% w/v	37 °C for no longer than 4 h, pH 8.0	High COD and color removal independent of the lipase concentration; Lower content of suspended solids and less turbidity	(Mendes et al., 2010)
	1	Lipase produced by *Pseudomonas aeruginosa* KM110	10% v/v	30°C for 24 h, pH 7.0	COD removal enhaced from 66 to 99%; Biogas production increased from 2330 ml to 4710 ml	(Mobarak et al., 2012)

Slaughterhouse wastewater	-	Fusarium verticillioides lipase	-	30°C for 96 h	3-fold reduction of fat	(Valladao et al., 2009)
	0.8	Lipase produced by *Penicillium sp.*	0.1%, 0.5% and 1.0% w/v	30°C for 24 h, pH 7.0	COD removal enhanced from 53.4 - 69.8% to 93.8 - 98.4%; Biogas production increased by 50%; Fat adhered to the sludge reduced from 19.9% to 8.6%	(dos Santos Ferreira et al., 2020)
Poultry industry wastewater	1.2	Lipase produced by *Penicillium restrictum*	0.1% w/v	35 °C for 22 h, pH 7.0	COD removal enhanced from 53% to 85%; Biogas production increased from 37 ml to 175 ml in 4 days	(Valladão et al., 2007)
	3	Porcine pancreatic lipase	-	35°C for 30 d, pH 8	Enhance COD removal (3 times) and formation of methane	(Dors et al., 2013)
	-	Lipase from oleaginous seeds of Pachira aquatica	-	40°C for 90 minutes, pH 8.0-9.0.	Free fatty acids release increased by 7.4 times	(Polizelli et al., 2013)
Dairy and poultry slaughterhouse wastewater	-	Commercial enzym	0.10% w/w	30 °C for 12 or 24 h, pH 7.0	Enhance the release of long chain free fatty acids (LCFAs)	(Pascale et al., 2019)
Swine meat processing wastewater	10	Commercial lipase (Lipolase 100T) and lipase produced by *Penicillium restrictum,*	5.0% w/v	45 °C for 9 - 15 h, pH 6	Enhance the release of free acids yield	(Rigo et al., 2008)
Fish-processing plant wastewater	1.5	Lipase produced by *Penicillium simplicissimum*	0.5% or 0.2% w/v	30 °C for 8 h	COD removal increased from 79.9% to 85.3% (0.5% lipase) and 90.9% (0.2% lipase); Reduced operational problems	(Alexandre et al., 2011)

Table 2 Examples for biomethane production in the anaerobic digestion of animal manure with enzymes application.

Animal manure	Enzymes	Enzymatic addition strategies	Temperature (°C)	Methane yields increase (%)	References
Dairy cattle manure	Enzyme mixture: cellulase, protease, pectate lyase	Direct addition to AD	50	No significant effect	(Sutaryo et al., 2014)
		Pre-treatment prior to AD		4.44	
Solid cattle manure	Enzyme mixture: cellulase, hemi-cellulase, xylanase, pectinase, xylan esterase, pectin esterase, lipase, amylase glucosidase and protease	Pre-treatment prior to AD	40	106.06	(Quiñones et al., 2012)
Cow manure and corn straw	Amylase	Direct addition to AD	37	110.79	(Wang et al., 2016)
	Cellulase	Pret-reatment prior to AD	55	103.2	
	Protease	Direct addition to AD	37	1.47	
Chicken manure	Cellulase	Direct addition to AD	40	Max: 11.2; after 60 d: 9.4	(Weide et al., 2020)

Horse manure	Cellulase	Max: 4.6; after 60 d: -2.3	(Weide et al., 2020)
Cattle manure and maize silage mixture	Enzyme mixture: cellulase, xylanase2, and beta-glucanase3	Max: 11.0; after 60 d: 10.3	(Weide et al., 2020)
	Enzyme mixture: xylanase, glucosidase, and endo-pectinase	Max: 6.8; after 60 d: 2.2	



Highlights