

Analysis of aquatic worms in flocculated digested sludge and extraction of
extracellular polymeric substances in wastewater treatment plants

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To my beloved family and specially my dears father and mother

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ABSTRACT

Activated sludge process in wastewater treatment plant (WWTP) produces large amount of excess sludge. The disposal of sludge in Netherlands is costly (500 EUR/ton wet solids), and therefore reduction techniques of digested sludge are of great interest to improve the costs of wastewater treatment. First part of this study focused on one of the biological approaches to reduce the excess sludge that can be achieved by aquatic worms in short period of time. Worms are efficient degraders to extra mineralization of sludge into biomass, water and CO₂. In optimal condition the total biomass production is low. The investigation on worm predation shows high concentration of ammonia in sludge is toxic and it inhibits the growth of worms, so flocculation was done to separate ammonia from solid. The hypothesis is that flocculated digested sludge (FDS) can be degraded by aquatic worms. In this study, we evaluated the degradation rate, temperature effect, ammonia concentration and worm growth rate. The obtained results from worm experiment showed, in long test (12 days), with the worm biomass of (21.2 g/ww), the degradation of sludge in worm reactor is (15.75 %), while it is (6.65 %) in aerated reactor. Temperature is not conclusive and it could be between (18 to 22° C). On the second part of the experiment, the extraction of extracellular polymeric substances (EPS) method in flocculated digested sludge to compare with non-flocculated digested sludge was evaluated. The results indicated concentration of EPS in digested sludge and flocculated digested sludge is not the same by applying extraction method named cation exchange resin. Although sonication is an applicable technique to destroy the size of flocs in flocculated digested sludge, the result does not show any balance in amount of extracted EPS in both types of sludge.

ABSTRAK

Proses enapcemar teraktif di loji rawatan air kumbahan (WWTP) menghasilkan jumlah besar kumbahan berlebihan. Pelupusan enapcemar di Belanda adalah mahal (500 EUR / tan pepejal basah), dan oleh itu teknik pengurangan enapcemar dicerna mendapat perhatian yang tinggi untuk meningkatkan kos rawatan air sisa. Bahagian pertama kajian ini memberi tumpuan kepada salah satu pendekatan biologi untuk mengurangkan enapcemar lebihan yang boleh dicapai oleh cacing akuatik dalam tempoh singkat. Worms adalah degraders berkesan untuk mineral tambahan enapcemar ke biomass, air dan CO₂. Dalam keadaan optimum jumlah pengeluaran biomass adalah rendah. Siasatan pada cacing pemangsa menunjukkan kepekatan ammonia yang tinggi dalam enapcemar toksik dan ia menghalang pertumbuhan cacing, jadi pemberbukuan dilakukan untuk memisahkan ammonia daripada pepejal. Hipotesis adalah bahawa flocculated enapcemar dicerna (FDS) boleh dihina oleh cacing akuatik. Dalam kajian ini, kami menilai kadar degradasi, kesan suhu, dan kadar pertumbuhan cacing. Keputusan yang diperolehi dari eksperimen cacing menunjukkan, dalam ujian panjang (12 hari), dengan biomas cacing sebanyak 21.2 g (ww), degradasi enapcemar dalam reaktor cacing adalah 15.75 (%), manakala ia adalah 6.65 (%) dalam reaktor berudara . Suhu tidak boleh dipertikaikan dan ia boleh menjadi antara 18 hingga 22° C. Kedua sebahagian daripada eksperimen menilai pengestrakan bahan polimer extracellular (EPS) kaedah dalam enapcemar dicerna flocculated untuk membandingkan dengan bukan flocculated dicerna enapcemar. Hasilnya menunjukkan dengan menggunakan kaedah pengestrakan kationik resin pertukaran (CER) tidak ada kesetaraan dalam jumlah EPS dalam kedua-dua flocculated kumbahan dan enap cemar bukan flocculated. Walaupun sonication adalah teknik yang diguna pakai untuk memusnahkan saiz flocs dalam enapcemar flocculated, hasilnya tidak menunjukkan apa-apa baki dalam jumlah EPS diekstrak dalam kedua-dua jenis enapcemar

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LIST OF SYMBOLS AND ABBREVIATIONS

WWTPs	-	Wastewater treatment plants
EPSs	-	Extracellular polymeric substances
CER	-	cation exchange resin
GHGs	-	greenhouse gases
TSS	-	total suspended solids
FSS	-	fixed suspended solids
VSS	-	volatile suspended solids
AD		Anaerobic digestion
CO ₂		carbon dioxide
WAS		waste activated sludge
LB-EPS		loosely bound EPS
g		Gram
HCHO		Formaldehyde
NaOH		Sodium hydroxide
TS		total solid
VS		volatile solid
L		Litter
NO ₃		Nitrate
P		Phosphorus
NaH ₂ PO ₄		Monosodium phosphate
C		Centigrade
UC1		Ultra Check 1
RLU		Relative Light Unit
F-DS		flocculated digested sludge
AD-S		anaerobic digested sludge

EA-FDS	extended aerated flocculated digested sludge
WP-FDS	worm predated flocculated digested sludge
DO	dissolve oxygen
NH ₄	Ammonium
NH ₃	ammonia
g/ww	Gera per wet weight
sCOD	Soluble chemical oxygen demand
ATP	Adenosine triphosphate

CHAPTER 1

INTRODUCTION

1.1 Introduction

1.1.1 Sludge Degradation

Wastewater treatment plants (WWTPs) produce large amount of biological waste sludge that composed of water, bacteria, organic and inorganic components such as phosphorus and nitrogen. There are also several pollutants like heavy metals, organic pollutants and pathogen in waste sludge. Amount of pollution in European Union was almost 11 million tons of dry solid at 2005. As it maybe reaches up to 13 million tons till the time 2020 (Kelessidis and Stasinakis, 2012), Some different methods have applied to dispose the waste sludge such as landfills, incineration and application as fertilizer. These treatments are costly and is almost half of the operational price of wastewater treatment (Mailler, et al., 2014). Therefore it is essential to develop some new methods for sludge minimizing (Wei, et al., 2003a).

A biological method to decrease sludge production is utilization of sludge by aquatic worms. Furthermore the worm biomass is grown up by sludge consumption

that is a source of protein contents (Elissen, et al., 2010). Sludge minimizing by worm is a usual method in small scale performance especially in developing countries. Worm compost is consumed as a fertilizer due to high amount of nitrogen concentration and less amount of heavy metal composition (Ndegwa and Thompson, 2001). The worm biomass is also applied as compost again or as a component for produce detergents and animal feed (de Boer and Sova, 1998).

1.1.2 Extracellular Polymeric Substances

Extracellular polymeric substances (EPSs), that present outside the cell is secreted by microorganisms in biological system like wastewater treatment plants. It consists of polysaccharides, proteins, uronic acids, humic-like substances, lipids, and DNA. The main characteristic of EPS is its function in metabolism of bacteria and survives them in waste sludge. It is consider as a flocculated agent in wastewater treatment (Sheng, et al., 2010).

The construction of EPS also affects settling and bonding properties of sludge and extends the membrane fouling (Ahmed, et al., 2007). The hydrophobic and hydrophilic regions in EPS structure allow it to attach to heavy metals and organic pollutants. Theses binding affinities consider remarkably to eliminate organic and inorganic pollutants (Pan, et al., 2010; Zhang, et al., 2010).

The main methods to extract EPS are sonication, high speed centrifugation, sonication/centrifugation, heating, alkaline, sulfide, EDTA, CER, and formaldehyde/NaOH treatment (Liu and Fang, 2003).

The amount of EPS extraction depends on type of extraction method. The extraction methods also affect the recovery rates and the fluorescence characteristics of EPS. CER and formaldehyde/NaOH are the most popular methods (Sheng, et al., 2005).

This research focused on two aspects: the first one was the consumption of flocculated digested sludge by worm. It explained how compact sludge was eaten by worm at different times. Studies on oligochaete predation showed that large reduction in flocculated digested sludge volume was achieved in a relatively short amount of time. It also indicated predation could increase the overall biodegradability of sludge when paired with anaerobic digestion. Visual characteristics of worm were observed under microscope to understand which species was more strength to digest flocculated sludge.

Second aspect was the extraction of extracellular polymeric substances (EPSs) by cation exchange resin (CER) method in anaerobic digested sludge and flocculated digested sludge. The emphasis was on improvement of EPS extraction method in flocculated digested sludge to achieve the same amount of EPS in both types of sludge. In this case sonication was applied to reduce the size of flocs in point of more extraction.

1.2 Problem Statement

The high growth of industrialization and urbanization are the main causes of large amount of pollution from wastewater treatment plants (WWTPs). Destroying of sludge is always the main concern in wastewater treatment because it contains almost more than half of price in total plant and the rules regarding sludge removal are being significantly stringent (Pilli, et al., 2011).

Principal techniques for sludge removal are landfill, land application and incineration. Land application is limited to stop health dangers to human being and animals because of probably poisonous components in the sewage sludge, such as heavy metals, pathogens, and strong organic contaminants. Decrease of available land area, along with growing strict rules governing the pattern of innovative landfills, construction, and operating new landfills have increased greatly. Commonly, incineration is usually the last alternative for sewage sludge disposal. The method produces ash, which often runs into landfill since it is unable to dispose somewhere else because of large amount of heavy metals and overall toxicity. Therefore, the existing legal limitations, the growing costs and public awareness of sewage sludge disposal have supplied significant fame to develop technologies for minimization of sludge generation (Wei, et al., 2003b).

To solve these problems some new techniques were utilized specifically in the activated sludge process. Several suggested strategies depend on decreasing sludge generation by growing the total sludge age. Some other methods are generally based on hydrolysis enhancement rate and also increase of biodegradability. Applied methods consist of mechanical, chemical, thermal and biological processes and might be located as pre-treatment step. The disadvantage of many of these types of methods is which they are actually energy and cost demanding. Due to environmental and economical limitations, there is a desire for cost-effective and ecological technologies for sludge treatment. The majority of suggested strategies are not relying on cycling of organic carbon in environment. Complicated organic carbon in environment is usually converted by higher organisms such as benthic earthworms and it seems a reasonable way to integrate worms into a process of sludge digestion in wastewater treatment plant (Tamis, et al., 2011).

As large amount of activated sludge are produced in wastewater treatment plant, dewatering is great interest to decrease the volume of sludge and manage the properties improvement. Organic polymer is broadly used more than other chemicals in wastewater industry.

Therefore the specific study of EPS is certainly an interesting subject not only in case of improving our information about biological wastewater treatment, but also enhancing the effectiveness of treatment by optimization of functional parameters. Due to the necessity of EPS in flocs configuration and stability, a wide range of researches have tried to extract and evaluate EPS from activated sludge ,so a number of extraction methods have been applied for this reason.

1.3 Objectives

The aims of the study are:

- To analyse the aquatic worms in flocculated digested sludge
- To analyse extraction of extracellular polymeric substances in flocculated digested sludge and anaerobic digested sludge

1.4 Significance of the Study

The use of biological methods in wastewater treatment has received a lot of attention nowadays due to low cost and high efficiency. This study will aid to reduce the amount of flocculated digested sludge in wastewater by using worms as predators. Furthermore, the worms were applied in this study have previously been screened to degrade activated sludge and that result is promising to pursue the experiment for flocculated sludge. The worm predation process has been shown to be affected by ammonia concentration, so it has to remove during experiment. The

worms are able to survive easily in lab environment. Consequently it can be an economical treatment.

Cation exchange resin is a chemical method to extract extracellular polymeric substances. This study will assist to get the same amount of EPS in both flocculated digested sludge and anaerobic digested sludge by CER method.

1.5 Scope of Study

The potential of aquatic worms to degrade flocculated digested sludge in wastewater treatment plant by measuring total solid and volatile solid. The amount of ammonia, total nitrogen, phosphorus, COD, dissolved oxygen, pH, and temperature were examined during experiments. Also the physical features of worms were observed under microscope to find which types of worm can survive during the experiment.

Beside that the EPS was extracted by CER method in flocculated digested sludge and anaerobic digested sludge. Sonication was applied for flocculated digested sludge to get the same output for both types of sludge.

REFERENCE

- Ahmed, Z., Cho, J., Lim, B.-R., Song, K.-G., & Ahn, K.-H. (2007). Effects of sludge retention time on membrane fouling and microbial community structure in a membrane bioreactor. *Journal of Membrane Science*, 287(2), 211-218.
- Albalasmeh, A. A., Berhe, A. A., & Ghezzehei, T. A. (2013). A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydrate polymers*, 97(2), 253-261.
- Appels, L., Baeyens, J., Degrève, J., & Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science*, 34(6), 755-781.
- Bland, J. M., & Altman, D. G. (1996). Statistics notes: measurement error. *Bmj*, 313(7059), 744.
- Bolto, B., & Gregory, J. (2007). Organic polyelectrolytes in water treatment. *Water research*, 41(11), 2301-2324.
- Buyers-Basso, M. (2014). *Measuring and Modelling the Impact of Oligochaete Predation Based Activated Sludge Reduction on Wastewater Treatment Plant Operation*. Delft University of Technology.
- Buys, B. R., Klapwijk, A., Elissen, H., & Rulkens, W. (2008). Development of a test method to assess the sludge reduction potential of aquatic organisms in activated sludge. *Bioresource technology*, 99(17), 8360-8366.
- Chohey, N. P., & Hicks, T. G. (2004). *Handbook of chemical engineering calculations* (Vol. 2): McGraw-Hill New York.
- Comte, S., Guibaud, G., & Baudu, M. (2006). Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties: Part I. Comparison of the efficiency of eight EPS extraction methods. *Enzyme and Microbial Technology*, 38(1), 237-245.

- D'Abzac, P., Bordas, F., Van Hullebusch, E., Lens, P. N., & Guibaud, G. (2010). Extraction of extracellular polymeric substances (EPS) from anaerobic granular sludges: comparison of chemical and physical extraction protocols. *Applied microbiology and biotechnology*, 85(5), 1589-1599.
- De Boer, G., & Sova, O. (1998). *Vermicomposting as a Resource for Biodegradable Detergents*. Paper presented at the 4th ZERI World Congress, Windhoek, Namibia.
- Drewes, C. D. (1997). Sublethal effects of environmental toxicants on oligochaete escape reflexes. *American zoologist*, 37(4), 346-353.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, 28(3), 350-356.
- Eastman, J. A., & Ferguson, J. F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *Journal (Water Pollution Control Federation)*, 352-366.
- Eddy, M. (2004). *Wastewater Engineering: Treatment and Reuse*: Singapore: McGraw-Hill.
- Elissen, H., Mulder, W., Hendrickx, T., Elbersen, H., Beelen, B., Temmink, H., et al. (2010). Aquatic worms grown on biosolids: Biomass composition and potential applications. *Bioresource technology*, 101(2), 804-811.
- Elissen, H. J. (2007). *Sludge reduction by aquatic worms in wastewater treatment: with emphasis on the potential application of Lumbriculus variegatus*: Wageningen University.
- Elissen, H. J., Hendrickx, T. L., Temmink, H., & Buisman, C. J. (2006). A new reactor concept for sludge reduction using aquatic worms. *Water research*, 40(20), 3713-3718.
- Feng, X., Lei, H., Deng, J., Yu, Q., & Li, H. (2009). Physical and chemical characteristics of waste activated sludge treated ultrasonically. *Chemical Engineering and Processing: Process Intensification*, 48(1), 187-194.
- Flemming, H.-C., & Wingender, J. (2010). The biofilm matrix. *Nature Reviews Microbiology*, 8(9), 623-633.
- Frølund, B., Palmgren, R., Keiding, K., & Nielsen, P. H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water research*, 30(8), 1749-1758.

- Gardner, W. S., Briones, E. E., Kaegi, E. C., & Rowe, G. T. (1993). Ammonium excretion by benthic invertebrates and sediment-water nitrogen flux in the Gulf of Mexico near the Mississippi River outflow. *Estuaries*, *16*(4), 799-808.
- Hendrickx, T., Temmink, H., Elissen, H., & Buisman, C. (2009a). Aquatic worms eating waste sludge in a continuous system. *Bioresource technology*, *100*(20), 4642-4648.
- Hendrickx, T., Temmink, H., Elissen, H., & Buisman, C. (2009b). The effect of operating conditions on aquatic worms eating waste sludge. *Water research*, *43*(4), 943-950.
- Hendrickx, T., Temmink, H., Elissen, H., & Buisman, C. (2010a). Aquatic worms eat sludge: Mass balances and processing of worm faeces. *Journal of hazardous materials*, *177*(1), 633-638.
- Hendrickx, T., Temmink, H., Elissen, H., & Buisman, C. (2010b). Design parameters for sludge reduction in an aquatic worm reactor. *Water research*, *44*(3), 1017-1023.
- Hendrickx, T. L., Elissen, H. H., Temmink, H., & Buisman, C. J. (2011). Operation of an aquatic worm reactor suitable for sludge reduction at large scale. *Water research*, *45*(16), 4923-4929.
- Huang, W. F., Shu, Y. G., Cai, L., & Jin, J. H. (2012). Utilization of energy and growth of aquatic worms during the process of sludge reduction by aquatic worms. *Advanced Materials Research*, *573*, 1073-1078.
- Huang, X., Liang, P., & Qian, Y. (2007). Excess sludge reduction induced by *Tubifex tubifex* in a recycled sludge reactor. *Journal of biotechnology*, *127*(3), 443-451.
- Jiang, T., Du, S., Sun, P., & Zhu, M. (2012). Effect of oligochaete worm body fluids on biological phosphorus removal in a bench-scale EBPR system. *Environmental technology*, *33*(15), 1755-1762.
- Kelessidis, A., & Stasinakis, A. S. (2012). Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. *Waste management*, *32*(6), 1186-1195.
- Kruger, N. J. (2009). The Bradford method for protein quantitation *The protein protocols handbook* (pp. 17-24): Springer.

- Lagerkvist, A., & Morgan-Sagastume, F. (2012). The effects of substrate pre-treatment on anaerobic digestion systems: a review. *Waste management*, 32(9), 1634-1650.
- Lapinski, J., & Tunnacliffe, A. (2003). Anhydrobiosis without trehalose in bdelloid rotifers. *FEBS letters*, 553(3), 387-390.
- Lee, B.-M., Shin, H.-S., & Hur, J. (2013). Comparison of the characteristics of extracellular polymeric substances for two different extraction methods and sludge formation conditions. *Chemosphere*, 90(2), 237-244.
- Li, X., Chen, H., Hu, L., Yu, L., Chen, Y., & Gu, G. (2011). Pilot-scale waste activated sludge alkaline fermentation, fermentation liquid separation, and application of fermentation liquid to improve biological nutrient removal. *Environmental science & technology*, 45(5), 1834-1839.
- Liang, P., Huang, X., Qian, Y., Wei, Y., & Ding, G. (2006). Determination and comparison of sludge reduction rates caused by microfaunas' predation. *Bioresource technology*, 97(6), 854-861.
- Liu, H., & Fang, H. H. (2002). Extraction of extracellular polymeric substances (EPS) of sludges. *Journal of biotechnology*, 95(3), 249-256.
- Liu, Y., & Fang, H. H. (2003). Influences of extracellular polymeric substances (EPS) on flocculation, settling, and dewatering of activated sludge.
- Lou, J., Cao, Y., Sun, P., & Zheng, P. (2013). The Effects of Operational Conditions on the Respiration Rate of Tubificidae. *PloS one*, 8(12), e81219.
- Mailler, R., Gasperi, J., Rocher, V., & Chebbo, G. (2014). Priority and emerging substances in sludge and fate during sludge treatment. *Waste Management*, 34(7), 1217-1226.
- Min, B., & Logan, B. E. (2004). Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. *Environmental science & technology*, 38(21), 5809-5814.
- Ndegwa, P., & Thompson, S. (2001). Integrating composting and vermicomposting in the treatment and bioconversion of biosolids. *Bioresource technology*, 76(2), 107-112.
- Nielsen, P. H., & Jahn, A. (1999). Extraction of EPS *Microbial extracellular polymeric substances* (pp. 49-72): Springer.

- Nozaic, D., Freese, S., & Thompson, P. (2001). Longterm experience in the use of polymeric coagulants at Umgeni Water. *Water Science & Technology: Water Supply*, 1(1), 43-50.
- Pan, X., Liu, J., Zhang, D., Chen, X., Song, W., & Wu, F. (2010). Binding of dicamba to soluble and bound extracellular polymeric substances (EPS) from aerobic activated sludge: a fluorescence quenching study. *Journal of colloid and interface science*, 345(2), 442-447.
- Park, C., & Novak, J. T. (2007). Characterization of activated sludge exocellular polymers using several cation-associated extraction methods. *Water research*, 41(8), 1679-1688.
- Pilli, S., Bhunia, P., Yan, S., LeBlanc, R., Tyagi, R., & Surampalli, R. (2011). Ultrasonic pretreatment of sludge: A review. *Ultrasonics sonochemistry*, 18(1), 1-18.
- Rao, S. S. (2001). *Applied numerical methods for engineers and scientists*: Prentice Hall Professional Technical Reference.
- Ratsak, C. H., & Verkuijlen, J. (2006). Sludge reduction by predatory activity of aquatic oligochaetes in wastewater treatment plants: science or fiction? A review *Aquatic Oligochaete Biology IX* (pp. 197-211): Springer.
- Rensink, J., & Rulkens, W. (1997). Using metazoa to reduce sludge production. *Water Science and Technology*, 36(11), 171-179.
- Sesay, M. L., Özcengiz, G., & Dilek Sanin, F. (2006). Enzymatic extraction of activated sludge extracellular polymers and implications on bioflocculation. *Water research*, 40(7), 1359-1366.
- Sheng, G.-P., Yu, H.-Q., & Li, X.-Y. (2010). Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. *Biotechnology Advances*, 28(6), 882-894.
- Sheng, G.-P., Yu, H.-Q., & Yu, Z. (2005). Extraction of extracellular polymeric substances from the photosynthetic bacterium *Rhodospseudomonas acidophila*. *Applied microbiology and biotechnology*, 67(1), 125-130.
- Sinha, R. K., Herat, S., Bharambe, G., & Brahmabhatt, A. (2009). Vermistabilization of sewage sludge (biosolids) by earthworms: converting a potential biohazard destined for landfill disposal into a pathogen free, nutritive & safe biofertilizer for farms. *Waste Management & Research*.

- Tamis, J., Van Schouwenburg, G., Kleerebezem, R., & van Loosdrecht, M. (2011). A full scale worm reactor for efficient sludge reduction by predation in a wastewater treatment plant. *Water research*, 45(18), 5916-5924.
- Thapa, K., Qi, Y., & Hoadley, A. (2009). Interaction of polyelectrolyte with digested sewage sludge and lignite in sludge dewatering. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 334(1), 66-73.
- Wang, C., Chang, C., Chu, C., Lee, D., Chang, B.-V., & Liao, C. (2003). Producing hydrogen from wastewater sludge by *Clostridium bifermentans*. *Journal of biotechnology*, 102(1), 83-92.
- Wei, Y., & Liu, J. (2006). Sludge reduction with a novel combined worm-reactor *Aquatic Oligochaete Biology IX* (pp. 213-222): Springer.
- Wei, Y., van Houten, R. T., Borger, A. R., Eikelboom, D. H., & Fan, Y. (2003a). Comparison performances of membrane bioreactor and conventional activated sludge processes on sludge reduction induced by Oligochaete. *Environmental science & technology*, 37(14), 3171-3180.
- Wei, Y., Van Houten, R. T., Borger, A. R., Eikelboom, D. H., & Fan, Y. (2003b). Minimization of excess sludge production for biological wastewater treatment. *Water research*, 37(18), 4453-4467.
- Wei, Y., Zhu, H., Wang, Y., Li, J., Zhang, P., Hu, J., et al. (2009). Nutrients release and phosphorus distribution during oligochaetes predation on activated sludge. *Biochemical Engineering Journal*, 43(3), 239-245.
- Zhang, D., Pan, X., Mostofa, K. M., Chen, X., Mu, G., Wu, F., et al. (2010). Complexation between Hg (II) and biofilm extracellular polymeric substances: an application of fluorescence spectroscopy. *Journal of hazardous materials*, 175(1), 359-365.
- Zhang, X., Tian, Y., Wang, Q., & Lin, H. (2013). Waste sludge reduction using *Limnodrilus hoffmeisteri*: Growth, development and sludge predation potential of aquatic worm correlate with process conditions. *Eco Engineering*, 58, 406-413.