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# Investigating of wastewater microbial indicators removal in a pilot scale Activated Sludge system

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### ARTICLE INFO

### ABSTRACT

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Activated Sludge (AS) processes are the most widely used biological processes in Wastewater Treatment Plants (WWTPs). Controlling wastewater is important for reducing overall water pollution. Therefore, the main objective of this study is to evaluate the performance of the Activated sludge system in removal microbial indicators from the wastewater sludge. This research was conducted on for 4 months in two seasons. The sludge Samples were taken from the return Activated sludge line. Microbial indicators including Fecal Coliform (FC), Total Coliform (TC), parasitic egg and some parameters such as pH, temperature examined for the comparison between aerobic digester and lime stabilization sludges. The lime stabilization is capable of decreasing large quantities of pathogenic bacteria. The results indicated that Fecal Coliform and parasitic egg decreased to 976MPN/gr.ds and 0MPN/4gr.ds respectively. When limed sludge reached the pH of 12.5. The remaining parasitic egg from lime stabilization of the sludge were 203 MPN/4gr.ds after two months. The average removal efficiency of TSS, TC, FC and parasitic egg were 80.8%, 99% and 100% and 74% respectively. The optimum ratio of lime was identified 245(gr/kg.Ts). The lime stabilization could archived to class B of USEPA category. So at a pH higher than 12 treated sludge with lime meet the guideline for pathogen reduction of class B regarding parasitic egg and has a higher hygienic effect on sludge pathogens and more cost effective than aerobic stabilization when sludge used to inferiority soil improvement, modify acid soils, disposal in the forest but was not used as agricultural fertilizer.

## 1. Introduction

Activated sludge (AS) processes are the most widely used biological processes in wastewater Treatment plants (WWTPs) worldwide, and they have been employed for pollutant removal for more than a century, owing to their high nutrient removal, toxin degradation, and biomass retention capabilities (Yang et al., 2020). The extended aeration activated sludge system is one of the most commonly used wastewater treatment methods that are currently being widely used in Iran (Takdastan et al., 2016 and Lopsik et al., 2013) Microbial community structure and diversity erect the performance and functional stability of WWTPs (Grosser et al., 2017 and Ye et al., 2013). Therefore, knowledge of AS microbial community structure and microbial functions will facilitate sludge population optimization and improve WWTP operation (Grosser et al., 2017 and Fang et al., 2017). Sludge may contain constituents who are potentially harmful to animals and humans who consume the crops. Pathogenic microorganisms such as bacteria, viruses, parasitic worms can be spread by sludge (Xia et al., 2018). There are a lot of processes for treatment and stabilization of sludge, but liming is one of the processes that

can reduce pathogenic organisms significantly .The effect of liming on the microbiological quality is reported to be related to the pH and not to the percentage of added lime (Zeng et al., 2019) It was concluded that the removal of pathogenic microorganisms depended on the pH reached by the sludge, the period of liming activity and the dryness of the sludge.Although parasitic eggs in sludge are more resistant to liming as compared to other microorganisms, sludge treatment with lime reduces their concentration considerably protozoa and eggs. According to the literature, sludge treatment with lime may meet Class A and B requirements in EPA guidelines for control and reduction of pathogens in sewage sludge (USEPA, 1999) The Class A requirement is to reduce the pathogens in sewage sludge to below detectable levels. The Class B requirement is to ensure that pathogens have been reduced to levels that are unlikely to pose a threat to public health and the environment under the specific use conditions. The aim of this study is to evaluate the performance of activated sludge system in removing microbial indicators in the Nowshahr city Wastewater Treatment Plant (NWTP).

# 2. Materials and Methods

## 2.1. Materials and system description

Two pilots (sludge dryer and aerobic digester) are made to determine the efficiency of sludge stabilization process in the removal of studied parameters A method for the lime stabilization of wastewater sludge, includes the steps of dewatering sludge so as to produce a sludge cake containing from about 10 to 60% by weight of dry solids and rapidly and intimately mixing and reacting the sludge cake with calcium oxide (kor et al., 2009). Thus a single glass reactor with 15 liters capacity .A cylindrical vessel with a height of 100 and diameter of 50 cm was built to sludge drving bed. In the bottom of the tank, 20 holes with a diameter of 1 cm were created. The thickness of the sand layer inside the bed was 30 cm sludge. The sand layer thickness and the particle diameter were 46 cm and 3 - 25 mm respectively. The raw sludge of the secondary settling pond was transferred to sludge drying bed. For finding the optimum ratio of lime addition to sludge, this action was done in seven stages. After 15 days of sludge dewatering, we

transferred 2 kg of sludge to a glass reactor and added a certain proportion of lime. Then the mixing operation performed to keep the pH above 12.5. During the stabilization period, Total coliform, fecal coliform, parasite eggs were detected according to EPA 1992 guidelines (U.S.EPA, 1999). Enumeration of total coliform and fecal coliform were carried out using multitubes methods and the results expressed as MPN per 100 ml. The number of TC, FC and parasitic egg in the reactor sludge was monitored and compared with USEPA standards. In order to survey the process of sludge stabilization in aeration reactor, a single glass reactor with a volume of 10 liters Was made. Aeration action was done by 4 aquarium aeration pumps continuously and a high mixer. A wooden box with dimensions 40 cm x 40 cm was placed on the bioreactor and On top of that, Two number of 100 volt bulbs were installed to maintain the temperature inside the bioreactor from 37 - 45 °C (Mousavian et al., 2016). We transferred 7 lit of return actives sludge to bioreactor and then mixing was done and aeration action start. During the stabilization period, the number of TC, FC, parasitic egg in the reactor sludge was monitored and compared with USEPA standards. Design of aerobic bioreactor and sludge dryer are presented fig 2.

# 2.2. process descriptions 2.2.1. Stabilization

Stabilization as a process of reducing sewage sludge hazards. Stabilization is a process where sludge is treated to make it disposable and reusable. Three main processes are used: digestion, lime stabilization, and heat/thermal treatment. In the aerobic digestion treatment, the organic matter is converted into a CO2 and H2o by aerobic bacteria. Digestion process is mainly used in big wastewater treatment plants. The sewage sludge digestion process is one of the stabilization method. This process is carried out mainly in mesophilic conditions (30-42°C). Mesophilic digestion ensures stabilization, but The without sufficient hygienization. thermophile fermentation (50-60°C) gives better results in lowering the level of pathogens. Conducting stabilization in thermophile conditions also shortens the digestion time. However, the thermophile digestion process compared to the mesophilic digestion is more sensitive to small changes in the process parameters and requires more energy to heat the sewage sludge to the required temperature. Increasing the degree of sewage sludge digestion (Grosser et al 2017, Vera et al, 2013)

#### 2.3. Sampling and Analytical Techniques

This descriptive-analytical study was carried out in the Wastewater Treatment Plant of Nowshahr for 2 seasons from winter 2016 to autumn 2016. Briefly, the structure of the Nowshahr treatment plant includes a screen, a pumping station, a grit chamber, two aeration ponds, and four sedimentation ponds. The sludge dewatering and thickening system are strain filtration and gravity thickening, respectively. Disinfection of the output effluent is carried out with sodium hypochlorite. In the wastewater treatment plant, solid waste and large materials in the wastewater are separated by two mechanical and a manual solid waste collectors. (Fig 1) Seven samples of raw sludge were taken and transferred to the Environmental Health Laboratory, Nowshahr wastewater plant .During this study, grab samples were taken per month from return activated sludge .The Total Suspended Solids (TSS), the fecal Coliforms (FC) and the Total coliform (TC), pH, Temperature, and the number of parasitic egg were detected according to the Standard Methods (APHA, 2005).

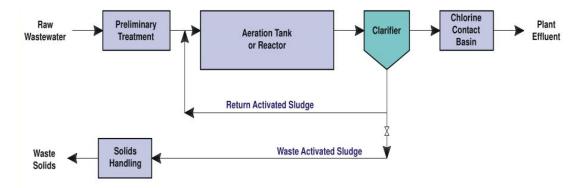


Figure 1. Activated sludge system Schematic of NWTP



a) Aerobic bioreactor



b)Sludge Dryer Pilot Figure2: a) Aerobic bioreactor; b) Sludge Dryer Pilots

## 3. Results

The results obtained from this study are summarized in Tables 1 and 2. The average pH was 7/4 in the raw sludge of NWTP. The average initial microbial concentration in the raw sludge for Total and Fecal Coliforms were 235x10\*4 and 22x10\*5 per gram of dried sludge respectively and the viable parasitic egg was 796 per gram of dry sludge solids.

The amount of lime measured to increase the pH to more than 12 was 245 gram. Per liter. The summary results shown in tables 1 and 2. The average removal efficiency of TSS, TC, FC and parasitic egg were 80.8%, 99% and 100% and 74% respectively (fig 3). The optimum ratio of lime was choose the amount, which could be raised the PH of mixture over 12 after 4 steps was 245 (gr/Kg.Ts).(fig 4)

Parameters	No. of samples	рН	Raw sludge	After 5 days	After 10 days	After 15 days	After 25 days	After 40 days	After 50 days	After 60 days
Total coliform (MPN/g)	7	12/75	235x10 <sup>4</sup>	223x10 <sup>4</sup>	2235x10 <sup>3</sup>	2135x10 <sup>3</sup>	21x10 <sup>5</sup>	18x10 <sup>5</sup>	223x10 <sup>4</sup>	$2205 \text{ x}10^3$
Fecal Coliform (MPN/g)	7	12/65	22x10 <sup>5</sup>	235x103	976	0	0	0	0	0
Total solids (%)	7	11/97	1,015	0/689	0/543	0/434	0/334	0/284	0/242	0/212
Temperatur e©	7	12/62	18/2	19/2	17/45	18/45	20/12	21/2	19/45	21/35
Parasite eggs (MPN/4gr.d s)	67	12/59	796	702	624	540	460	375	298	203

**Table 1.** The results obtained with sludge liming at different pH

Table 2. Lime dosage and variation of pH in different times of storage

Parameters	Raw - sludge pH	Average Lime Dosage (gr/Kg.Ts)	pH after 5 days	pH after 10 days	pH after 15 days	pH after 25 days	pH after 40 days	pH after 50 days	pH after 60 days
pH	12/75	245	12/65	11/97	12/15	12/85	12/65	12/62	12/59

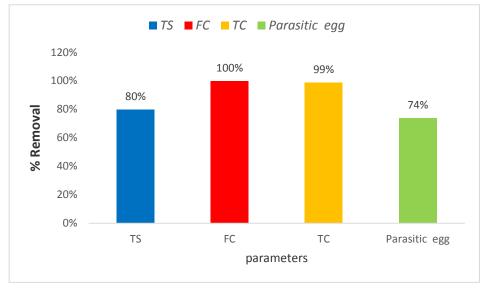


Figure 3: Removal efficiency of the studied parameters in NWTP

Parameters	Step 1	Step 2	Step 3	Step 4
Activation		<b>1</b>	<b>1</b>	
degree of	77/88	77/88	77/88	77/88
Ca(OH) <sub>2</sub> %				
Ratio of g				
Ca(OH) <sub>2</sub> to g dry	210	245	245	245
solids				
Parasitic	812	763	640	620
egg(MPN/4gr.ds)	012	705	040	020

Table 3: Results of Lime stabilization sludge of Nowshahr wastewater plant

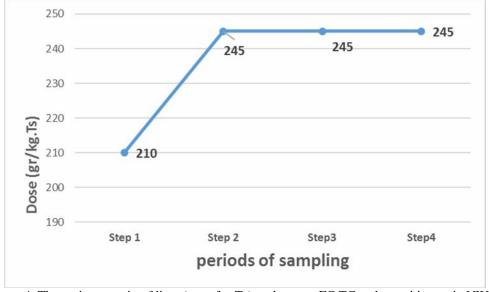


Figure 4. The optimum ratio of lime (gram/kg.Ts) to decrease FC,TC and parasitic egg in NWTP

# 4. Discussion

The results obtained from this study were analyzed with regard to EPA regulations for control of pathogens in sewage sludge. The U.S. Environmental Protection Agency (EPA) has established a regulation for the use and disposal of sewage sludge (U.S.EPA, 1999). This regulation protects public health and the environment through requirements designed to reduce the potential for contact with the diseases-bearing microorganisms in sewage sludge applied to the land or placed on a surface disposal site (Jimenez et al., 2000). These requirements are divided into two categories: The requirements designed to control and reduced pathogens in sewage sludge and those to reduce the ability of the sludge to attract vectors. A. The effect of lime on pathogen reduction. The pathogen reduction requirements for sewage sludge are divided into two categories: Class A and Class B. (U.S.EPA, 1992). In Class A the density of fecal coliform in the sewage sludge must be less than 1000 MPN per gram total solids (dry weight basis). Class B requirements can be met only by fecal coliform density in the treated sludge of 2 million MPN or CFU per gram total solids sewage (dry weight basis) (U.S.EPA, 1992, Jimenez, 2000). The results of microbial analyzes showed that lime stabilized were classified on class B of USEPA category. As can be seen from Table 1 at pH higher than 12 after two months the sludge met the requirements for Class B. The results also showed that lime treatment increase the removal efficiency of parasitic eggs at pH above 12. This study showed liming improved that the microbiological quality of sludge. The efficiency of sludge liming in terms of the elimination of pathogenic microorganisms depends on the pH achieved in the sludge and duration of liming activity. Liming improved the microbiological quality of sludge provided that the pH was maintained at higher than 12 for two months (met Class B) based on the studies conducted (by Farzadkia et al., 2005 and 2008; Bina et al., 2004, Mignotte 2001; Jimenez et al., 2000 and Fang et al., 2018) the lime stabilization could be beneficially reused as landfill cover material ,poor soil reconditioner and co-composting material (Djukic et al., 2016). If fertilizer using of disposal would be noted to microbial quality

must be improved up to Class A of USEPA category and parasitic egg decrease the pH to higher than 12 after 2 months. The optimum ratio of lime was identified 245 (gr/kg.Ts).

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### Refereces

- APHA, A. (2005). WEF: Standard Methods for the Examination of Water and Wastewater: Centennial Edition. Washington, DC.
- Ahmed, A.U., Sorensen, D.L. (1999). Kinetics of pathogen destruction during storage of dewatered biosolids. Wat Environ Res, 67: 143-50
- Bina, B., Movahedian, A., Kord, I. (2004).The Effect of Lime Stabilization on the Microbiological Quality of Sewage SludgeIranian J Env Health Sci Eng, 1(1): 34-38
- Djukic, M., Jovanoski, I., Ivanovic, O. M., Lazic, M., & Bodroza, D. (2016). Costbenefit analysis of an infrastructure project and a cost-reflective tariff: A case study for investment in wastewater treatment plant in Serbia. Renewable and Sustainable Energy Reviews. 59:1419-1425.
- Fang, H., Zhang, H., Han, L., Mei, J., Ge, Q., Long, Z., & Yu, Y. (2018). Exploring bacterial communities and biodegradation genes in activated sludge from pesticide wastewater treatment plants via metagenomic analysis. Environmental Pollution. 243: 1206-1216.
- Farzadkia M, Jafarzadeh, N., Loveimi asl, L., and Ghalambor, A. (2008). Wastewater sludge stabilization using lime a case study of west Ahwaz wastewater treatment plant. Journal of Water and Wastewater. 19(4):67-71 (in Persian).
- Farzadkia, M., Taher Khani, H. (2005). Evaluation of sludge management in sewage treatment plant in Hamadan Province. Journal of Mazandaran

University of Medical Sciences. 15(47):19-25.

- Farzadkia, M., Jafarzadeh, H.N.E., & Loveymi, A.L. (2009). Optimization of Bacteriological Quality of Biosolids By Lime Addition. 1: 29-34
- Jimenez, B., Barrios, J.A., Maya, C. (2000). Class B Biosolids Production from Wastewater Sludge with High Pathogenic Content Generated in an Advanced Primary Treatment. Wat Sci Tech. 42(9):103-110.
- Grosser, A., Wrowag, M., Rorat, A. (2017). Sewage sludge disposal strategies for sustainable development, Envirmenal Research. 156: 39-46.
- Kor, Y., Zazoli, M., Keramat, S., et al. (2009).
  Survey of performance and optimizing methods of aerated lagoons of bandargaz wastewater treatment plants. The Journal of Tolooe-behdasht. (1-2): 1-2.
- Lopsik, K. (2013). Life cycle assessment of small-scale constructed wetland and extended aeration activated sludge wastewater treatment system. Int J Environ Sci Technol. 10(6):1295-308.
- Mousavian, S., Takdastan, A., Seyedsalehi, M., & Akhavani, S. (2016). Determining the kinetics' coefficients in treatment of sugarcane industry using aerobic activated sludge by complete-mix regime. J Chem Pharm Res. 8(4): 1342-1349.
- Mignotte-Cadiergues, B., Maul, A., Huyard, A., Capizzi, S., & Schwartzbrod, L. (2001). The effect of liming on the microbiological quality of urban sludge. Water Science and Technology. 43(12): 195-200.
- Metcalf, L., Eddy, H. P., & Tchobanoglous, G. (2003). Wastewater engineering: treatment, disposal, and reuse (Vol. 4). New York: McGraw-Hill.
- Shahi, D.H., Ebrahimi, A., Esalmi, H., Ayatollahi, S. and Dashty, N. (2012). Efficiency of straw plants in removal of indicator pathogens from sub surface flow constructed wetlands of municipal wastewater in Yazd, Iran. Health and Development Journal. 1(2): 147-154.
- Shahi, D.H., Eslami, H., Ehrampoosh, M.H., et al. (2013). Comparing the efficiency of

Cyprus alternifolius and Phragmites australis in municipal wastewater treatment by subsurface constructed wetland. Pakistan journal of biological sciences: PJBS. 16(8): 379-384.

- Takdastan, A., Kordestani, B., Nisi, A. et al. (2016). Study of Operational and Maintenance Problems and Parameters of Extended Aeration Activated Sludge Process in Golestan Hospital Wastewater Treatment Plant, Ahvaz, and Their Solutions. Journal of Environmental Health Enginering. 3(4): 270-279.
- US Environmental Protection Agency.(1992). Standard for use or disposal of sewage sludge, final rules, Federal Reg, 58:9248.
- US .EPA, E. (1999). Control of pathogens and vector attraction in sewage sludge. Environmental Regulations and Technology, USEPA, Cincinnati.
- Vera, I., Sáez, K. and Vidal, G. (2013). Performance of 14 full-scale sewage treatment plants: Comparison between four aerobic technologies regarding effluent quality, sludge production and energy consumption. Environmental technology. 34(15): 2267-2275.
- Xia, Y., Wen, X., Zhang, B. and Yang, Y. (2018). Diversity and assembly patterns of activated sludge microbial communities: a review. Biotechnology advances, 36(4), pp.1038-1047.
- Ye, C., Yang, X., Zhao, F.J. and Ren, L. (2016). The shift of the microbial community in activated sludge with calcium treatment and its implication to sludge settleability. Bio resource technology, 207, pp.11-18.
- Yang, Y., Wang, L., et al (2020). Activated Sludge Microbial Community and Treatment Performance of Wastewater Treatment Plants in Industrial and Municipal Zones. International Journal of Environmental Research and Public Health. 17(2): 436-439.
- Zeng, J., Li, J., Gou, M., Xia, Z.Y., and Tang, Y.Q. (2019). Effective strategy for improving sludge treatment rate and microbial mechanisms during chromium bioleaching of tannery sludge. Process Biochemistry. 83: 159-167.