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The Role of Formed Microorganism in Sludge on Processing of Wastewater Treatment

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Abstract: Several microorganisms such as bacteria, fungi, Protozoa, Rotifera, cystic amoeba and algae diagnosed in activated sludge aerobic (Rustumiya treatment plant) and anaerobic reactor. Results have shown a reduction in the turbidity rates when using activated sludge at Rustumiya plant of 76.3 to 2.653 NTU in pre-treatment and final tank respectively, also COD (chemical oxygen demand) amount reduced from 427.263 to 82 mg/L respectively. In addition, concentrations of phosphates and nitrates decreased from 12.083 to 8.426 mg/L and 3.59 to 2.43 mg/L respectively, by removing 30.2% and 32.3% respectively of the final tank. The ratio of ammonia removing was 89.6% for ammonia, reducing process from 1358 to 140 mg/L. Furthermore, sulfates concentration decreased from 30.883 to 23.337 mg/L. However, the system in the anaerobic reactor depends on non-aerated activated sludge. Results show turbidity reduced from 12.5 to 2 NTU in pre-treatment and final tank respectively, also the COD mount reduced from 191 to 130 mg/L, the percentage removal of 31.9%. In addition phosphates, nitrates and sulfates concentrations were decreased by using activated sludge from 17.15 to 8.15, 1.2 to 0.1 and 28 to 9.2 mg/L respectively. The ammonia concentration has reduced from 1.2 to 0.1 mg/L where at a removal percentage of 90.9%.

Key words: Microorganism, sludge, wastewater, removing, chemical oxygen demand, protozoa, bacteria, algae.

1. Introduction

Different treatment units as primary settling tanks, trickling filters, activated sludge plants ect give out sludge. Sludge from other units as mentioned above contains putrescible organic substances, pathogenic organisms ect which are unsafe to public health. The quantity of produced sludge is huge and its handling and disposal become a big task [1]. In addition, wastewater contains inorganic chemical compounds from waters supplies and many complex organic material which are derived from faces, urine and other organic wastes. Wastewater is normally alkaline when fresh but tends to be acidic as it becomes state [2]. By stabilization it is meant that organic matter has been broken down by bacterial action to simple inorganic substance. Anaerobic bacteria grow and liberate energy in the absence of free oxygen and obtain it from various compounds which are to break down. In anaerobic stabilization, however, reaction is slow and this process gives off unpleasant odors [3]. Aerobic action takes place when free oxygen is available for bacteria, the oxygen may be available from atmosphere or sewage. On the other hand, facultative type of organisms is those which can thrive and continue their life activities both in presence and absence of oxygen. If oxygen is available they become aerobic. All above groups are helpful in the stabilization of sewage and known as Saprophytes [4]. In the liquid layers of the pound, algae begin to grow under favorable conditions, the algae utilize the carbon dioxide in the sewage for photosynthesis during day light hours liberating oxygen which maintains aerobic condition in the upper layers of the pound, algae growth convert solar energy to chemical

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energy in organic form. Empirical studies show that about 6 percent of visible light energy converted to algal energy [5]. The aerobic bacteria grouped upon their role in flock formation. The free-swimming bacteria are floating in the wastewater among activated sludge flocks, if they are present in huge number they may contribute to high effluent BOD5 (biological oxygen demand). The flock forming bacteria are attached to each other and constitute well settling flock-like biomass, which can be separated easily from the liquid phase in the final clarifier. Filamentous bacteria can be useful, because they can work as skeleton for flocks. On the other hand, if lots of filamentous bacteria are present in the sludge, they often cause bulking and foaming problems [6, 7]. Protozoa, which feed on bacteria, are also found active. Higher multi-cellular animal forms, known as metazoan, like nematodes, rotifers, annelid worms feed upon the organic slime and keep the bed porous, thus make the bed fit for re-use after certain interval. The aim of the present study was to screen the role of microorganisms in the activated sludge digestion.

2. Material and Methods

2.1 Isolation and Diagnosis of Bacteria

Depending on Gerardi [8], the samples were diluted to 3rd dilution, 2nd and 3rd dilution were examined by using nutritious agar medium. These plates serve as an incubator at 37 °C for 24 h. At the end of the incubation period, the appearance of bacterial colonies was gram stained for microscopic morphology. Then colonies were transferred to the differential medium follow on Manitol salt agar, Pseudomonas agar, McConkie agar, Gauze agar and Shigella Salmonella.

2.2 Diagnosis of Zooplankton

Zooplankton is found in sewage. 1 mL of sample of zooplankton was examined by light microscope, using depression slide under magnification of 50 × power compound microscope according to Kudo [9].

2.3 Diagnosis of Algae

The non-diatom algae were isolated and diagnosed by microscopically examination, depending on the number of references to classify of non-diatom algae [10-12, 13].

2.4 Isolation and Identification of Yeast

Yeasts were isolated and diagnosed from Al-Rustumiya station by using media consist of (potato dextrose agar and antibiotic chloramphenicol 1 mg/L). 1 mL of sample (every sample) was mixed with media in plate (9 cm diameter) three replicate for every sample, these were incubated at 25-27 °C for 48 h according to Kurtzmar [14]. The isolate yeasts diagnosed by using biochemical testes according to Bennet, Kurtzman, Shrestha and Bennet [15, 16-18].

2.5 Physiochemical Screening

Physiochemical factors such as pH, COD, SO4, NO3, TSS, PO4 and Turbidity measured, according to standard method of analysis [19, 20].

3. Result

3.1 Wastewater Treatment Plants and Anaerobic Reactor

Characterization of activated sludge reveals the ledge of spatiality in sewage wastewater treatment by using anaerobic activated sludge, at a Ministry of Science and Technology, where results showed reduced turbidity by using activated sludge in processing of plant stages entry tank, treatment tank, sludge tank and final tank. Turbidity reduced to 12.5, 9.6, 29.7 and 2 NTU respectively. COD reduced to 191, 181, 230 and 130 mg/L respectively. In addition, phosphate concentrations reduced to 17.15, 19.45, 72.4 and 8.14 mg/L respectively. The nitrate concentrations reduced to 1.2, 0.2, 0.1 and 0.1 mg/L respectively. As to ammonia, concentrations reached to 1.1, 1.6, 2.3 and 0.1 mg/L respectively; whereas absorption reached to 0.218, 0.238, 0.945 and 0.002 nm respectively, the sulfate concentrations reached to 28, 12, 26 and 9.2 mg/L respectively, acidity pH of stages plant 7.2, 7.8, 7.6 and 6.5 respectively (Table 1).

As to TSS (total suspended solids) matter, concentrations reached plant stages to 990, 1020, 1400 and 860 mg/L respectively and Fig. 1.

3. 2 Russtamyia Treatment Plant

Characterization of wastewater treatment and the activated sludge of Russtamyia station sewage wastewater treatment by using activated sludge aerobic processing. Turbidity reduced by using activated sludge during processing stages of plant entry tank, primary tank, secondary tank, sludge tank and final tank 76.3, 16.03, 4993.3, 11333.3 and 2.65 NTU respectively. COD was reduced to 427.263, 8.25, 371, 523.75 and 82 mg/L, respectively.

Phosphate concentrations reduced to 12.083, 6.366, 22.97, 31.455 and 8.426 mg/L, respectively. Nitrate concentrations reduced to 3.59, 1.75, 1.17, 2.86 and 2.43 mg/L, respectively. On the other hand, ammonia concentrations were reduced to 1358, 280, 518, 532 and 140 mg/L respectively; whereas absorption was reduced to 0.018, 0.047, 0.064, 0.407 and 0.027 nm, respectively, sulfate concentrations were reduced to 30.883, 22.809, 18.234, 20.584 and 23.337 mg/L respectively, as acidity pH of plant stages 7.01, 7.3, 7.28, 7.02 and 7.5, respectively Table 2.

As to TSS matter concentrations of plant stage, they were reduced to 30, 14100, 1850, 12000 and 11 mg/L respectively and Fig. 2.

Moreover, the Table 3 removal time of all chemical tests between Russtamyia plant treatment and anaerobic plant of final tank.

Biochemical tests of the isolated yeast from anaerobic reactor plant of sludge tank Table 4.

Table 1 Chemical test of anaerobic plant treatment.

Plants	Phosphate mg/L	Nitrate mg/L	Ammonia mg/L	pH	Absorption nm	Turbidity NTU	Sulfate mg/L	COD mg/L
Basin tank	17.15	1.2	1.1	7.2	0.218	12.5	28	191
Treatment tank	19.45	0.2	1.6	7.8	0.238	9.6	12	181
Sludge tank	72.4	0.1	2.3	7.6	0.945	29.7	26	230
Final tank	8.15	0.1	0.1	6.5	0.002	2	9.2	130

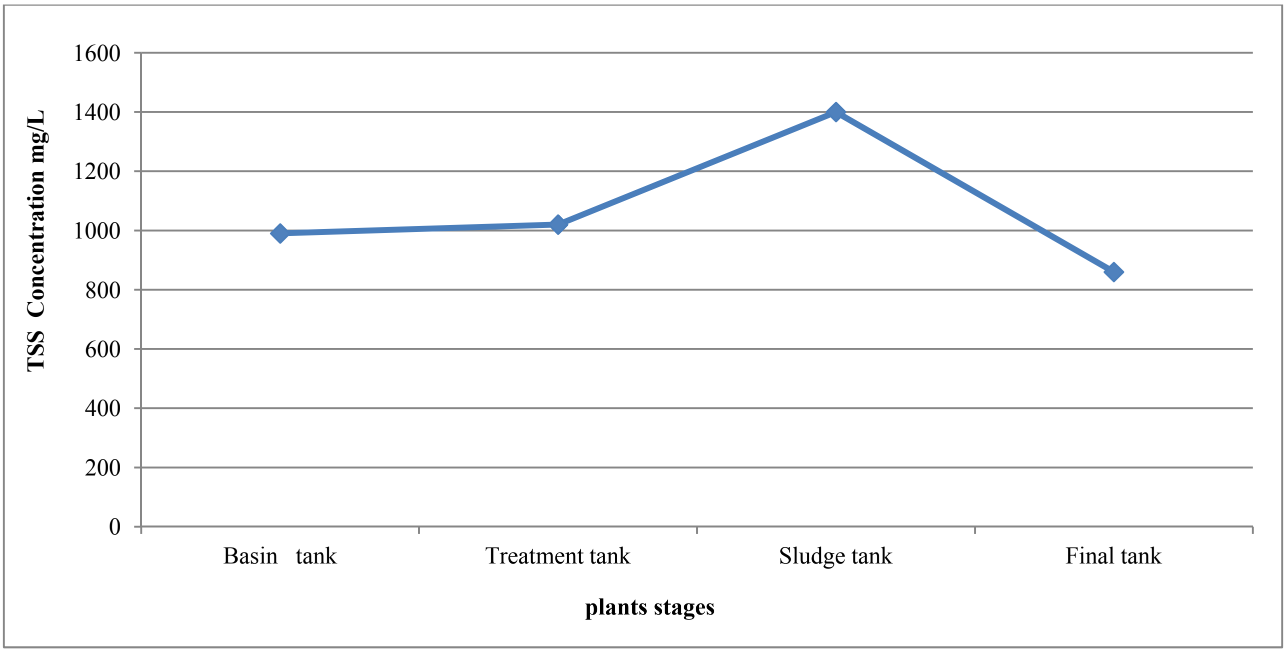


Fig. 1 TSS matter concentrations of anaerobic plant (TSS Concentration mg/L).

Table 2 Chemical test of sample of Russtamyia treatment plant.

Plants	Phosphate mg/L	Nitrate mg/L	Ammonia mg/L	pH	Absorption nm	Sulfate mg/L	COD mg/L
Entry tank	12.083	3.59	1358	7.01	0.018	30.883	427.263
Primary tank	6.366	1.75	280	7.3	0.047	22.809	8.25
Secondary tank	22.97	1.178	518	7.28	0.064	18.234	371
Sludge tank	31.455	2.86	532	7.02	0.407	20.584	523.75
Final tank	8.426	2.43	140	7.5	0.027	23.337	82

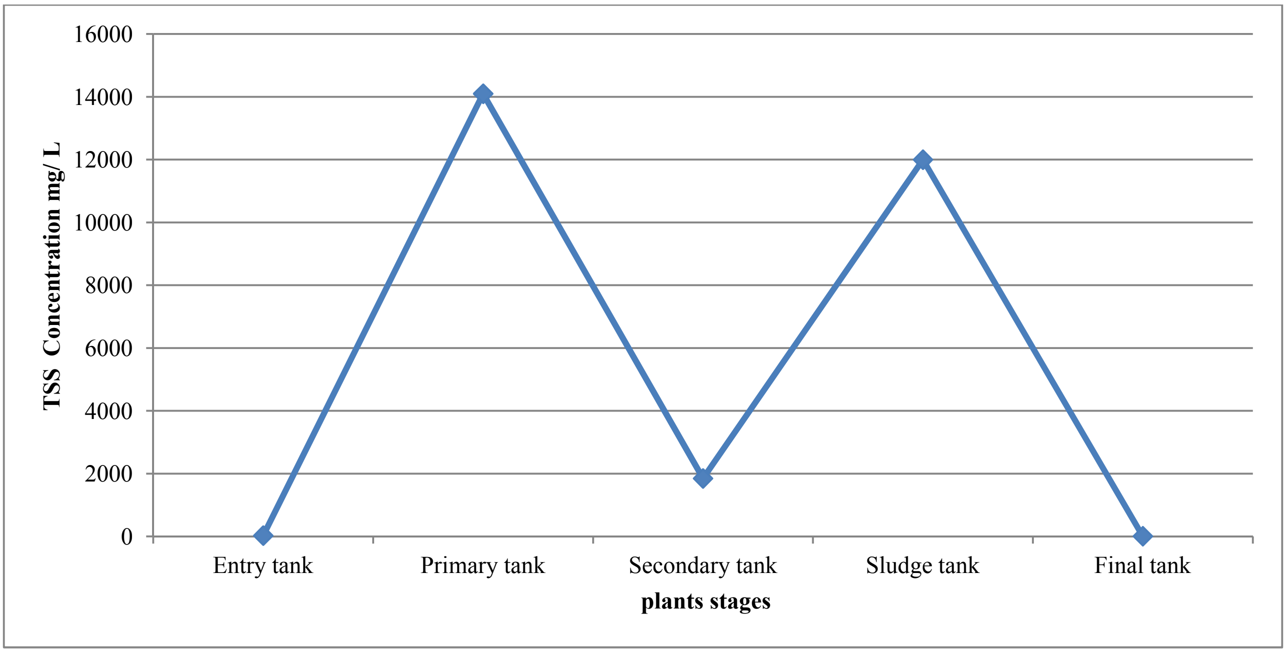


Fig. 2 TSS matter concentrations of Russtamyia treatment plant stages.

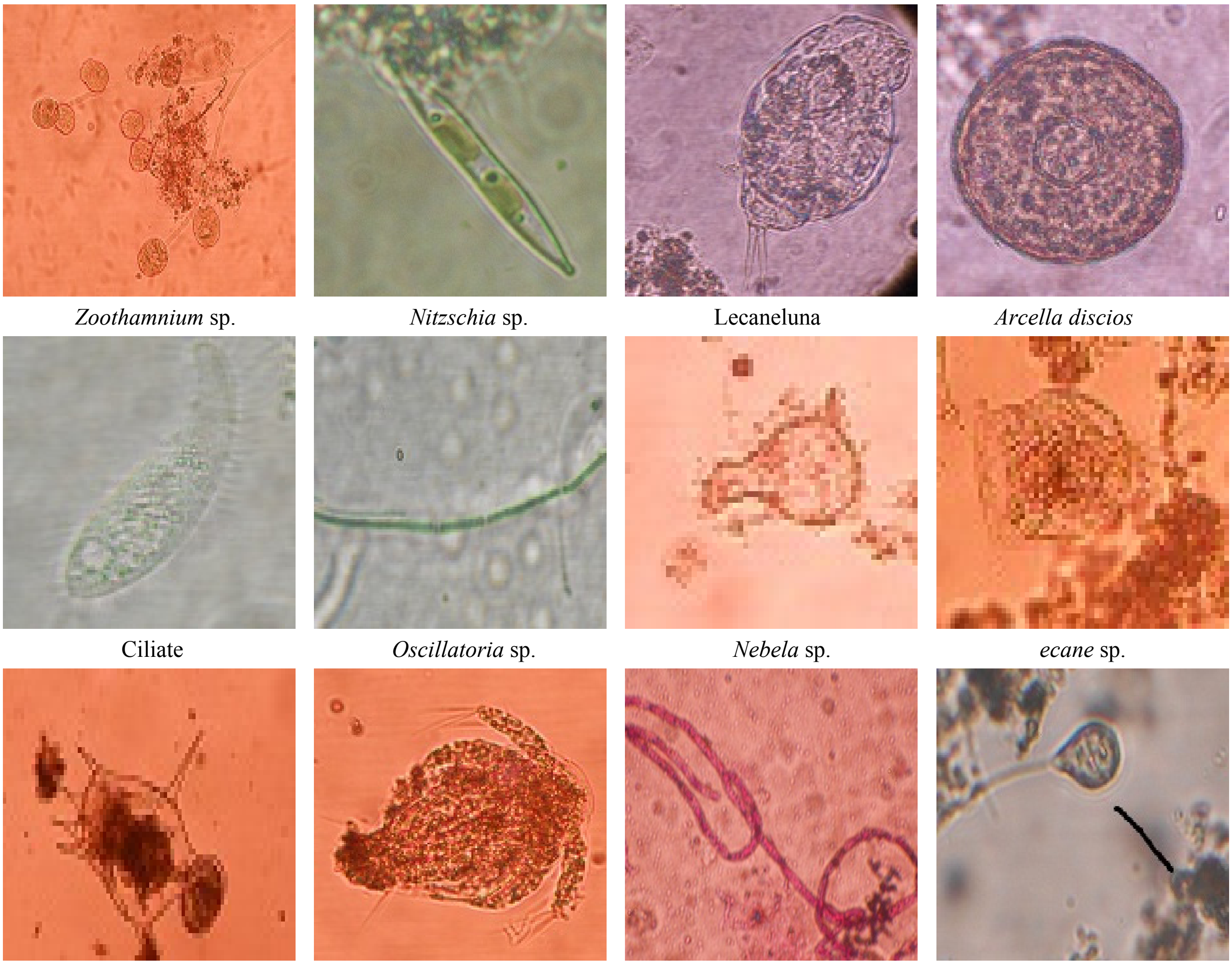
Table 3 Chemicals removal % of final tank for Russtamyia and anaerobic reactor plants.

Test mg/L	plants	
	Russtamyia (%)	Reactor anaerobic (%)
COD	69.2	31.9
Sulfate	24.4	67.1
Phosphate	30.2	52.4
Nitrate	32.3	91.6
Ammonia	89.6	90.9

Table 4 Biochemical tests of isolated yeast.

Yeast species	1	Sugar Assimilation											13
		2	3	4	5	6	7	8	9	10	11	12	
Lipomyce sarxii	+	+	-	-	-	-	+	-	+	+	-	-	-
Rhodotorula mucilaginosa	+	+	-	+	+	-	+	+	+	+	+	Weak	+

1-Urealysis. Sugar Assimilation: 2-Glucose 3-Inulin 4-Sucrose 5-Raffinose 6-Lactose 7-Trehalose 8-Maltose 9-D-Xylose 10-D-Arabinose 11- L-Arabinose 12-Manitol 13-Simon citrate.



Zoothamnium sp. | *Nitzschia* sp. | Lecaneluna | *Arcella discios*

Ciliate | *Oscillatoria* sp. | *Nebela* sp. | *ecane* sp.

Brachionus calyciflorui | Cyclop | Streptomyces | *vorticella* sp.

Fig. 3 More important organisms were founded in sludge tank of reactor plant anaerobic.

A permanent species of bacteria was founded in the aerobic activated sludge like Filamentous bacteria such as *Streptomyces* sp. On the other hand, the non-filamentous bacteria such as *Escherichia* sp., *Salmonella* sp., *Shigella*, *staphloccous* aureus, the bacteria were founded in the anaerobic sludge such as *Clostridium perfringens* and *Bacillus* sp. While fungus such as *Rhizopus stolonifer* and *Penicillium echinulatu*, and the large numbers of zooplankton such as *Arcella discoides*, *Lecane* sp., *Vorticella* sp., Ciliata, Philodania, *Nebela* sp. and *Nematoda* sp., and the important algae were founded such as *Oscillatoria limnetica*, *Spirogyra borgeana*, *Lyngbya digueti*, *Microcystes flos-aquae*, *Navicula cryptocephala* and *Nitzschia acicularis* (Fig. 3). All these organisms were identified depending on which played on important role in the treatment process [8-12, 15, 21].

4. Discussion

Activated sludge consists of approximately 95% bacteria and 5% higher organisms such as protozoa, metazoan, fungi, rotifers, etc. These microorganisms have a role in the activated sludge process to treat various types of wastes [22]. Bacteria are responsible for the biological oxidation of organic substrates, nitrification of ammonia, denitrification of nitrate and accumulation of phosphorous and they constitute the major component of activated sludge [23]. Furthermore, bacteria, may be divided into groups according to their response to free molecular oxygen.

These groups are strict aerobes, facultative anaerobes, and anaerobes. In the case of availability of oxygen, activate aerobic bacteria and decomposition of organic materials to CO_2, water, nitrate, and energy. But if it is not available oxygen activate anaerobic bacteria and decomposition of organic materials to energy, water, H_2S, and ammonia [8]. More than 80% of biological waste water plants are based on the principle of activated sludge process, in which suspended bacteria oxidize the carbonaceous and nitrogen compounds to produce an effluent that is in accordance with legal standards, and that corresponds to a minimal environmental impact [5]. The activated sludge flocks contain mostly bacterial cells as well as other microorganisms (Fungi, Rhizopoda, Flagellates, Ciliates, Rotifers) and inorganic and organic particles. That has a role in biological process [24], this result came consistent with Ghazy [25] has showed COD, BOD, TSS and ammonia decreased through stages of treatment process. The removal of these parameters during studied period ranged 86-95, 91-96, 90-94 and 71-85% respectively. Micro fauna, amoebae and flagellates appeared at low DO (dissolved oxygen) and high organic load, dominance of crawling and stalked ciliates affected efficiency of treatment. Rotifers count ranged from 1.3 × 103 to 9 × 104, indicating good sludge quality. Showed by Usharant [25] the efficiency of *Pseudomonas* sp. to phosphate removal to be maximum of 68% in synthetic phosphate wastewater with glucose carbon source followed by starch (66%), sucrose (65%) and lactose (62%) after 72 h at neutral pH (7.0 ± 2).

Awad and Karume [26] reported that occurrence of fungi in aerobic and anoxic activated sludge from MBRs (membrane bioreactors). Under aerobic condition, the *Geotrichum* was found at (8.8%) followed by *Penicillium* (75.0%), Yeasts (65.7%) and Trichoderma (55.5%), while Yeasts (77.1%) *Geotrichum candidum* and *Penicillium* (61.1%) species were the most prevalent in anoxic activated sludge. Showed by Kacprzak [27] study comparative analysis of fungal communities occurring in wastewater and sewage sludge. Additionally some physiochemical factors such as pH, BOD and COD were determined. The pH values were generally neutral at the range from 6-7.85. The COD and BOD values of untreated waste water amounted from 202 to 618 mg/dm^3 and 100 to 440 mg/dm^3 respectively. The COD and BOD values of treated wastewater oscillated from 42 to 66 mg/dm^3 and 10 to 40 mg/dm^3, respectively. The comparative qualitative analysis showed that, most quantitative occurred genus *Penicillium* and usually occupied about 50% in all studied communities, occupied yeast 30% in untreated wastewater, 13% in treated wastewater and 19% in sewage sludge [21] observed reduction in COD rates oscillated from 86-92% in 21 days when treatment olive black water by using *Aspergillus oryzae*. It was also found that the *Penicillium corylophilum* was capable of removing 94.40% of COD and 98.95% of turbidity of filtrate with minimum dose of inoculum of 10% v/v in domestic waste water treatment plant sludge (1% w/w) [28]. The percent removal of sulfate, nitrate, ammonia and phosphate in anaerobic reactor was more significant than aerobic reactor. For the presence of fungi that contain a group of extracellular enzymes that facilitate the biodegradation of phenolic compounds, dyes, and PAH (poly aromatic hydrocarbons), among others, through non-specific oxidation reactions [29]. A number of genera of protozoa have been identified in activated sludge such as Papadimitriou [17] reported that *Aspidisca cicada* and *Vorticella microstsoma* were correlated to BOD5 removal capacity, while *Podophrya fixa* could be used as an indicator of low effluent SS content. Further, Liang [30] stated that 78% of NH_4 could be removed in the combined system of *Chlorella vulgaris* and *Bacillus licheniformis*, while 29% in single algae system and only 1% in single bacteria system. Approximately 92% of total phosphate was removed in the combined system, compared with 55% and 78% in single algae and bacteria system respectively.

5. Conclusions

(1) Activity of aerobic treatment plants is more than that of anaerobic plants, where COD reduction by aerobic attained 69.2%, while anaerobic plants reduced to 31.9%.

(2) Reduction TSS by aerobic plants is greater than that of the anaerobic plants because of the abundant presence of zooplankton.

References

[1] Luz, E. B., and Yoav, B. 2008. "Recent Advances in Removing Phosphorus from Wastewater and Its Future Use with Activated Sludge." *Water Research* 38: 4222-46.

[2] Mino, T. 2007. "Microbial Selection of Pollutants Accumulating Bacteria in Activated Sludge Wastewater Treatment Processes for Enhanced Biological Removal." *Biochemistry (Moscow)* 65 (3): 341-8.

[3] Mary, B. L., Petra, P., Martina, M., Toma, M., David, P., and John, S. 2006. "Polychlorinated Biphenyl (PCB)-Degrading Bacteria Associated with Trees in a PCB-Contaminated Site." *Appl. Environmental Microbiology* 72: 2331-42.

[4] Mauro, T., Sandro, P., Dittmar, H., and Peduzzi, R. 2003. "Spatio-Temporal Distribution of Phototrophic Sulfur Bacteria in the Chemocline of Meromictic Lake Cadagno (Switzerland)." *Microbiology Ecology* 43: 89-98.

[5] Tiwari, K. B., Shrestha, L. M., and Agrawal, V. P. 2007. "Abundance of Lactose Assimilating Yeasts from Nepalese Murcha (Yeastcake)." *Int. J. Life Sci.* 1: 1-5.

[6] Awad, M. F., and Kraume, M. 2010. "The Occurrence of Fungi in Activated Sludge from MBRs." *International Journal of Chemical and Biological Engineering* 3 (4): 180-3.

[7] Chatterjee, A. K. 2010. *Water Supply Waste Disposal and Environmental Engineering*. New Delhi: Khanna Publishers.

[8] Gerardi, M. 2003. *The Microbiology of Anaerobic Digesters*. New York: Wiley-Inter science.

[9] Kudo, R. 1977. *Protozoology*. New Delhi. 1172 pp.

[10] Desikachary, T. V. 1959. *Cyanophyta Indian Council of Agricultural Research*. New Delhi 686 pp.

[11] Edward, G. B., and David C. S. 2010. *Freshwater Algae: Identification and Use as Bioindicators*. Chippenham: Wilts.

[12] Felisberto, S. A., and Rodrigues, L. 2004. "Periphytic Desmids in Corumba', Goiás, Brazil: Genus *Cosmarium Corda*." *Braz. J. Biol*. 64 (1): 1-2.

[13] Prescott, G. W. 1964. *The Fresh-Water Algae*. Iowa: William, C. Brown Co.

[14] Kurtzman, C. P., and Fell, J. W. 1998. *The Yeast : a Taxonomic Study*. Elsevier Science.

[15] Bennet, J. W., Wunch, K. G., and Fasion, B. D. 2008. *Use of Fungi Biodegradation. Manual of Environmental Microbiology*. Washington, DC.: ASM Press.

[16] Kurtzman, C. P., Fell, J. W., and Boekhout, T. 2011. *The Yeasts: A Taxonomic Study*. Elsevier Science.

[17] Papadimitriou, S., Kennedy, H., Kattner, G., Dieckmann, G. S., and Thomas, D. N. 2004. "Experimental Evidence for Carbonate Precipitation and CO2 Degassing During Sea Ice Formation, Geochim." *Cosmochim. Acta* 68: 1749-61

[18] Tabak, H., and Cooke, W. B. 1968. "The Effects of Gaseous Environments on the Growth And Metabolism of Fungi." *The Botanical Review* 34: 126-252.

[19] APHA. 1989. *Standard Methods for the Examination of Water and Wastewater*. New York: American Public Health Association.

[20] APHA. 1999. *Standard Methods for the Examination of Water and Wastewater, Water Environment Federation*. Washington, DC.

[21] Öztepe, C., and Yurt, M. 2011. "Biological Degradation of Olive Black Water by *Aspergillus oryzae*: A Pilot Plant Investigation Canadian." *J. on Environmental, Construction and Civil Engineering* 2 (3): 24-31.

[22] Irvine, R. L., Wilderer, P. A., and Flemming, H. C. 1997. "Controlled Unsteady State Processes and Technologies—An Overview." *Water Science and Technology* 35 (1): 1-10.

[23] Bitton, G. 1994. *Wastewater Microbiology.* New York: Wiley-Liss Publication pp. 150-155.

[24] Bitton, G. 2005. *Wastewater Microbiology.* New York: Wiley-Liss Publication pp. 230-237.

[25] Tizghadam, M., Dagot, C., and Baudu, C. 2008. "Wastewater Treatment in a Hybrid Activated Sludge Baffled Reactor." *Journal of Hazardous Materials* 154: 550-7.

[26] Jaouani, A., Guillén, F., Penninckx, M. J., Martínez, A. T., and Martínez, M. J. 2005. "Role of *Pycnoporus coccineus* Laccase in the Degradation of Aromatic Compounds in Olive Oil Mill Wastewater." *Enzyme and Microbial Technology* 36: 478-486.

[27] Jeon, C. O., and Park, J. M. 2000. "Enhanced Phosphorus Removal in a Sequencing Batch Reactor Fed with Glucose as a Sole Carbon Source." *Water Res.* 34: 2160-70.

[28] Shrestha, B., and Sharma, A. P. 1995. *Manual on Practical Pharmaceutical Microbiology*.

[29] Usharani, K., and Lakshmana perumalsamy, P. 2010. "Bio-treatment of Phosphate from Synthetic Wastewater Using *Pseudomonas* sp YLW-7." *J. Appl. Sci. Environ* 14 (2): 75-80

[30] Liang, S., Zhao, X., Yuan, W., Liu, S., Cheng, X., and Xiao, Z. 2013. "A Long-Term Global Land Surface Satellite, (GLASS) Dataset for Environment Studies." *Int. J. Digital Earth*.