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RESEARCH ARTICLE

Study of Sludge and comparison for Various Wastewater Treatment

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Abstract

Three stations of sewage wastewater treatment Russtamyia, Zaapharanyia municipal waste water treatment plants and upword – flow anaerobic sludge blanket were selected to study and characterize the activated sludge. Though the experimental period bacteria, fungi, protozoa, rotifera, testate amoebae, ciliates, nematods and algae were identified in aerobic and anaerobic sludge. The species of microfauna were *Streptomyces* sp., *Salmonella* sp., *Shigella* sp., *Staphylococcus aureus*, *Escherichia coli* while *Clostridium perfringens*, *Bacillus* sp. was in anaerobic sludge fungi as *Rhizopus stolonifer*, *Penicillium echinulatum*, *Lecane* sp., *Nematoda* sp., *Philodania* sp., *Arcella discoides*, *Nebela* sp., *Vorticella* sp., *Spirogyra borgeana*, and *Lyngbya digueti* the more frequent. the BOD₅ of Russtamyia, Zaapharanyia municipal waste water treatment plants and upword – flow anaerobic sludge was between 235- 35.009, 164 – 29.162, 103.71 – 76 mg/l respectively, while COD 427 – 263.82, 212.5 – 68, 211 – 130 mg/l and diameter of flocs was between 312.5 – 15, 600 – 32.5 and 562 – 1.25 m.

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INTRODUCTION

Bioremediation of sewage considered as the most important remediation which must applied to the water in the sewage treatment stations, this treatment aimed to oxidize of organic materials in different sewage and Convert them into stable compounds and biomass composed are mostly from bacteria and some of microbiology microorganisms which can be separated from the water and treated separately thus obtaining water practically free from organic pollution, the oxygen and bacteria is the most important two elements of the elements required for the success of bioremediation in addition of another conditions such as temperature and a presence of aid nutrients [1]. Also microorganisms involved to form activated sludge such as Bacteria, Phytoplankton, Zooplankton, Ciliates, Flagellates, and Amoeba, and digesting organic carbons materials which are dissolved or suspended where constitute 60 % of organic carbons materials and almost half of them subject to precipitation. Materials with diameter among 1- 100 mm remain suspended within solution, during remediation are adsorbed inside the bacterial flocs and aggregated bacteria where they were digested adsorbed while the biodegradable parts from organic materials are carbohydrates, protein, amino acids and fats. The aim of biodegradation to reduce organic materials and reduction of concentration of essential nutrients such as phosphorus and nitrogen in the treated water [2]. Using activated sludge considered as classical method in processing of biodegradation to water of wastes because this method depends on degradable capabilities of bacteria to excrete group of shattered enzymes of organic materials and hydrocarbons and must providing a balanced nutritional conditions for the growth of bacteria from pH value of appropriate and sufficient concentration of oxygen, carbon, nitrogen and phosphorus [3]. Were the bacteria oxidizing organic materials to Carbon dioxide CO₂ as a gas directly to the atmosphere and water to the natural resources [4]. Waste waters contains two groups of aerobic bacteria and non-aerobic bacteria, the activity of these

two groups of bacteria in decomposition, oxidizing and remediation of organic materials in waste water depends on the availability of dissolved oxygen. In the case of availability of dissolved oxygen aerobic bacteria will activate and decompose organic material in to in organic material, water, energy, Carbon dioxide and nitrate, this path called "Aerobic decomposition of organic materials", but in the case of unavailable dissolved oxygen in wastewater anaerobic bacteria will become active and decompose organic materials in wastewater and produce inorganic materials, energy, water, gas of Methane, Hydrogen sulfide and Ammonia, this path called anaerobic decomposition of organic materials, therefore aerobic bacteria are considered important and active in bioremediation of wastewater because aerobic bacteria can oxidize organic materials, they also have ability to congregate as form of Floc in viscous which consider an essential factor but rather the main core processes in activated sludge in biological treatment, in addition of bacteria wastewater contains some of the other living organisms more developed and larger named Protozoa, Rotifera, Nematoda and Sarcodena, these organisms very important in processing of bioremediation with activated sludge were activated sludge that is made up of about 90% of the bacterial cells and about 10% from protozoa, the protozoa diameter ranging among (10-20 μm), protozoa work to speed up the activated sludge precipitation as well as feeding and consuming old bacterial cells. Many recent studies are proved the ability to consume organic, hydrocarbon materials, Phosphorous (P) and Nitrogen (N) compounds so as to possess an active enzymatic system [5; 6]. Bacteria in processing of organic materials decomposition depends on the base of energy transfer which represented with Carbon (C) from species to another one [7]. Bacteria are divided in to several classes according to their capabilities and analytical representative to the analyst bacteria which secrete a group of enzymes to fragmentize polysaccharides, fats and protein and change them to organic acids, amino acids, and other simpler compounds, the fermented bacteria perform to fragmentize of polysaccharides, fats and protein to change these compounds to alcohol and fatty acids, then Acetogenic bacteria working to change all of these materials to Acetate and Hydrogen, later degradable bacteria working destroy these compounds and change them to CO_2 and water [8]. In another hand Zooplankton have an fundamental role in bioremediation by activated sludge, when they plays an important role to improve contaminated water via feeding on solids and transferred them to minor nutrients, and decrease solids even to 72 % at waste water treatment were the quantity of zooplankton used as a Bio markers for water contamination compared with their size, fertility and reproduction, also they have ability to indicate that the nature and magnitude of contamination, and playing an important role in removing dispersed bacteria and non-aggregate together especially Rotifera and ciliate [9]. This study aimed to know the components of activated sludge biologically via a acknowledgements of micro living organisms and their role in the sludge treatment processes, with compared sludge to use in different processors.

Materials and methods

Algae diagnosis

Non diatom algae were diagnosed by light microscope and magnification of 400 X according to international keys [10; 11; 12 & 13].

Diagnosis of Zooplankton

Zooplankton were diagnosed in wastewater after taking a sample from wastewater, a few drops of formalin were added to the wastewater sample for keeping microorganisms safe and alive during lack of oxygen, by a glass dropper amount of 1 ml was taken and placed on the cupped glass slide then examined by light microscope with magnification of 50 X and relying on books classification [14] and [15].

Isolation and diagnosis of bacteria

Samples was diluted three times for each sample, second and third dilution was examined on nutrient agar after appearance of bacterial colonies by variegating these colonies with Gram stain to distinguish between negative and positive bacteria to the stain, then colonies transferred to Manitol salt agar, Pseudomonas agar, McConkie agar, Gauze agar and Shigella Salmonella Agar depends on [16].

Isolation, diagnosis and development of fungi

Four fungal species were isolated and identified from final discharge of Al Russtamyia plant to treat Sewage according [17]. Potato extract agar (PEA) agar with one mg/l of antibiotic (chloramphenicol) was prepared, one ml of wastewater was placed in a sterile dish of glass with diameter of 9 cm. then a sterilized solid media was added and shacked well and putted in an incubator at 25 $^{\circ}\text{C}$ for 48 days with observation the appearance of fungal colonies, several glass slides were made for diagnosis fungal species with light microscope depends on the key of taxonomy [18].

Examination of Biological demand of Oxygen

Biological oxygen demand was examined according to the method [19]. Where attenuator water were prepared via satiating water by feeding water with oxygen through insert air stream by using an air compressor, then nutrient supplement were added to the water which include 22.5 gm / l of Magnesium Sulphate $MgSO_4$ with solution of 5.25 gm / l from Calcium chloride $CaCl_2$. Attenuator water left for 2 hours before using In order to settles the loss of oxygen and the amount is above the saturation. Examination of consumed Dissolved Oxygen by Bacteria and fungi in decomposition organic material in limited period depends on during limited period and temperature of 20 ± 1 °C, processes of decomposition needs 5 or 7 or 20 days for accomplishing according to the nature, compounds of organics materials and mixture of organic materials depending on their ability to decomposition.

Chemical and physical analysis

Demand of Chemical Oxygen.

Chemical oxygen demand COD was measured according to Dichromate method which adopted by American organization of health [20]. Three ml of sample was taken and mixed with 1.5 ml of Potassium Dichromate $K_2Cr_2O_7$. 12.25 gm of $K_2Cr_2O_7$ was taken and solved in 1000 ml of distilled water, then 3.5 ml of H_2SO_4 which prepared from melting 5.5 gm of Silver nitrate $AgNO_3$ in one litter of H_2SO_4 , then left two days for dissolving. Mixture was heated to boiling point in COD- reactor to 120 °C for two hours then titrated after cooling with Ferrous Ammonium Sulfate $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, where dissolved 98 of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ with distilled water, then 20 ml of H_2SO_4 was added to the solution and completed by distilled water to 1000 ml, and then titrated versus K_2CrO_7 , concentration was measured according to following equation:-

$$COD = A - B \times M \times 8000 / \text{Volume of Sample}$$

Where A = Titrated volume of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ with sample

B = Titrated volume of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ for Zeroing solution

M = Molarity of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (0.25)

Results was considered with mg / l

Determination of Nitrate NO_3

For determination of NO_3 , the adopted method of American health association [20], was used by taking 50 ml of water sample and filtered in order to remove suspended materials, then added 1ml of Hydrochloric acid (1 M), and mixed well, then the concentration of NO_3 was measured by Spectrophotometer at wavelength 220 nm. Results was considered with unit mg / l.

Determination of phosphate PO_4

Phosphate was determinate by using adopted method of American health association [20], for PO_4 determination, by adding 8 ml of combined reagent which made up of Ammonium molybdate $(NH_4)_6Mo_7O_{24}$, Sulphuric acid H_2SO_4 , Ascorbic acid $C_6H_8O_6$ and Antimony potassium tartrate $K_2Sb_2(C_4H_2O_6)_2$, to 50 ml of filtered sample water, then the color mixture changed to blue, the intensity of color measured by Spectrophotometer at wavelength 860 nm. Results was considered with unit mg / l.

Total suspended solids (TSS)

Adopted method of American health association [21], was used as follow:

- A- Filter paper size 0.45 μm type Whatman manufactured by Millipore filter was used and dried at oven for 25 mints at 103-105 °C.
- B- Known volume of sample was filtered by vacuum meter through a group of filter paper size 0.45 micron.
- C- Filter paper placed at oven another times after participate suspended micro particles and Impurities
- D- The following equation was used:

$$TSS \text{ (mg/l)} = (A - B) \times 1000 / V$$

where:

A = weight of filter + residue in mg

B = weight of filter in mg

V = ml of filtered sample

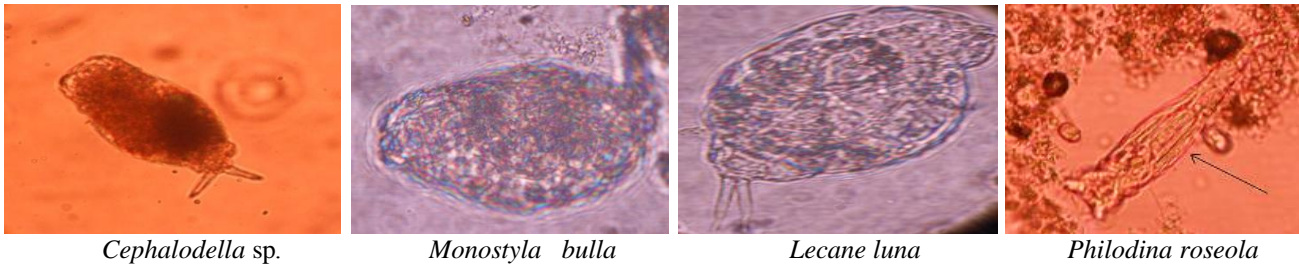
Turbidity

Was measured by turbidity meter HACH, before measuring samples and after celebration equipment by redy standard solutions (0.1, 1.0, 10, 100 Naphelo Turbidity Unit NTU). Samples was diluted when the value of turbidity raised more than range of equipment limited measurements. Results was considered (Naphelo Turbidity Unit) NTU [21].

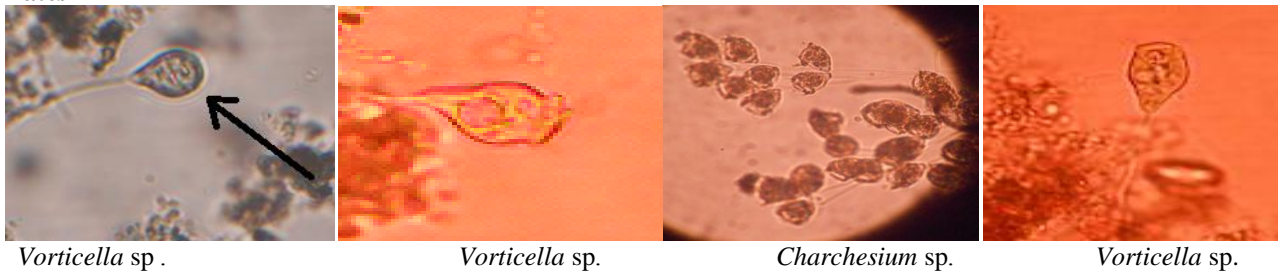
Results

Vital diagnosis for microorganisms in activated sludge (aerobic and anaerobic) Many spices of living organisms was diagnosed in aerobic and anaerobic sludge of Zooplankton in all different groups, Algae, Bacteria and Fungi as shown in figures (1, 2, 3 and 4)

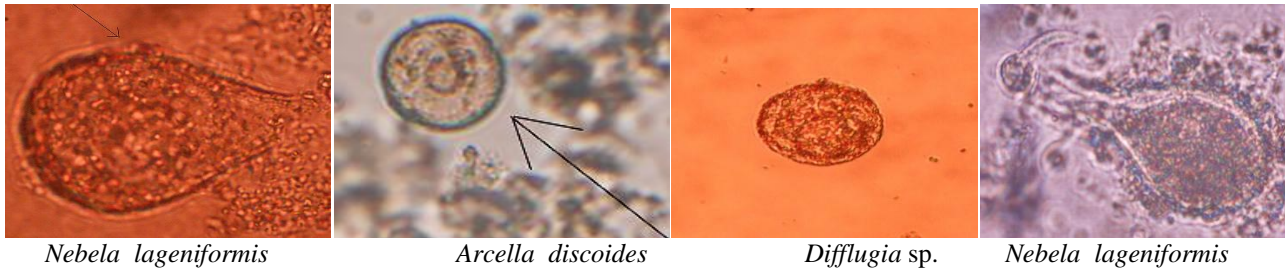
*** Rotifera**



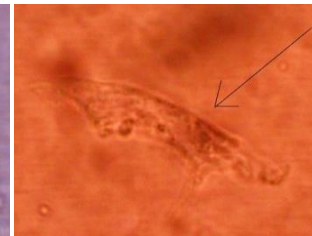
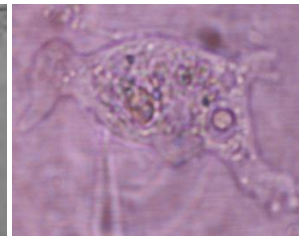
***Ciliates**



***Testate amoebae**



*** Naked amoebae**



***Protozoa**

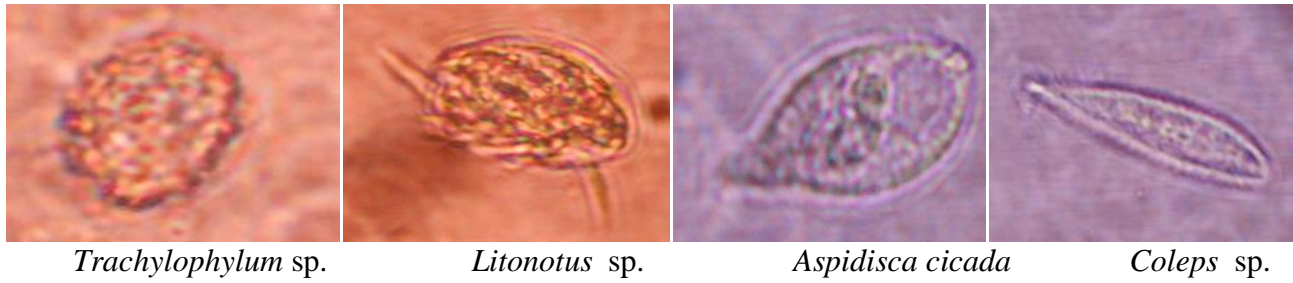


Figure (1). Some of Zooplankton groups in activated sludge (aerobic and anaerobic)

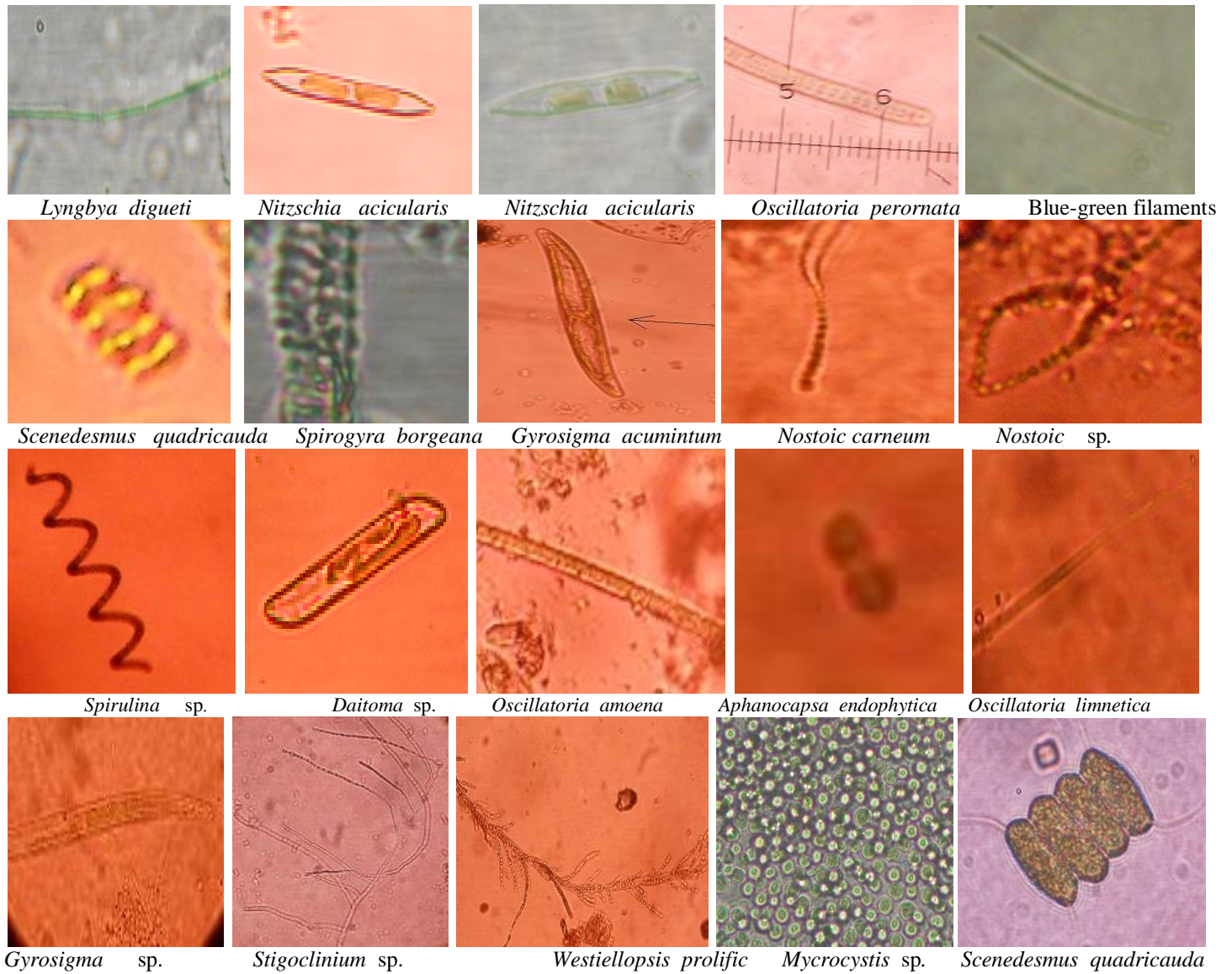
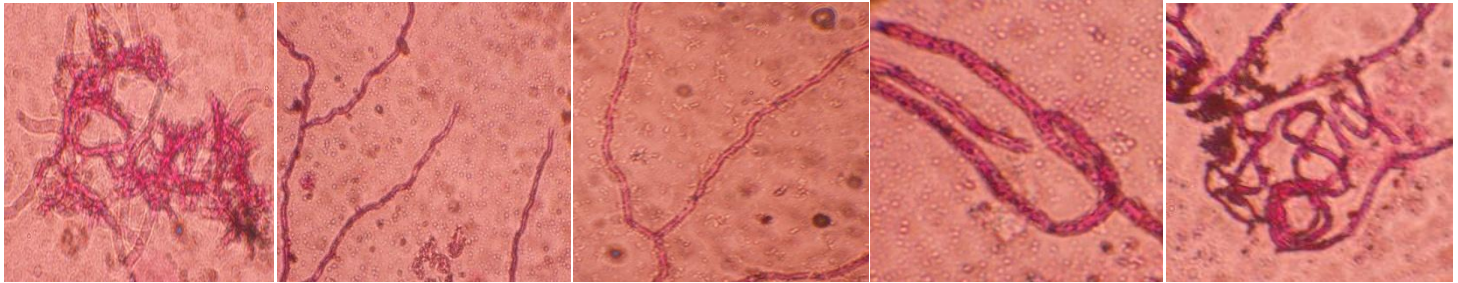


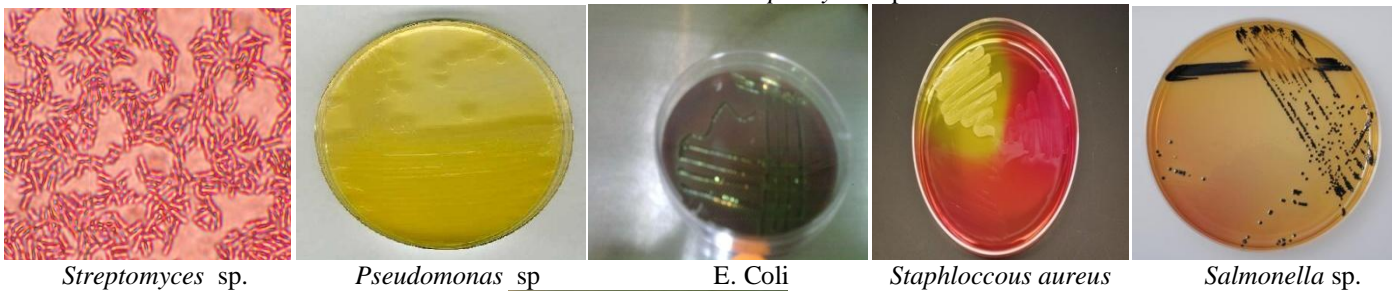
Figure (2). Some of phytoplankton (algae) in activated sludge (aerobic and anaerobic)



Figure (3). Some of fungi in activated sludge (aerobic and anaerobic)



Filaments bacteria *Streptomyces* sp.



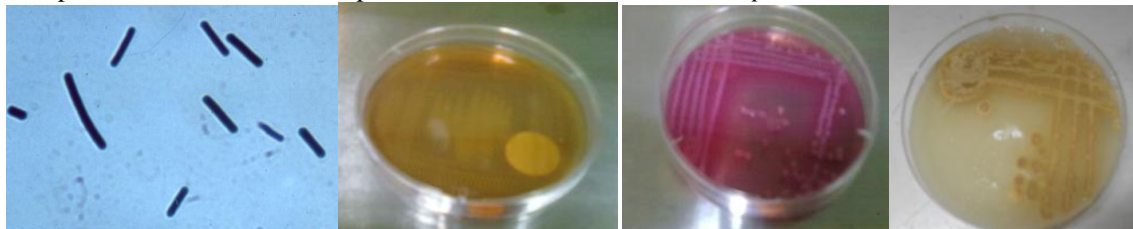
Streptomyces sp.

Pseudomonas sp.

E. Coli

Staphylococcus aureus

Salmonella sp.



Clostridium perfringens

Salmonella sp.

Shigella sp.

Streptomyces sp.

Figure (4). Some of bacteria in activated sludge (aerobic and anaerobic)

Results of chemical tests

Al Russtamyia plant of treatment

Al Russtamyia plant wastewater treatment was selected to test and characterization and study of activated sludge in treatment because of activated sludge importance in wastewater treatment in Al Russafa side, results showed decreasing in a level of turbidity by using activated sludge from 220 to 2.653 NTU at basin before treatment and final basin of treatment respectively for the plant, and Total suspended (TSS) decreased from 602 – 388.66 mg / l respectively, also Total Dissolved Solids (TDS) decreased from 1427 to 1013 mg / l respectively with ratio of removing 70.98 % for final basin, in addition the quantity of COD was reduced from 427.263 to 82 mg / l respectively compared with BOD₅ which reduced respectively from 235 to 35.009 mg/l. in addition to that concentration of botanical nutrients which represented by Phosphate and nitrogen was reduced by using activated sludge where Phosphate concentration of phosphate was reduced from 15.083 to 8.426 mg / l respectively with ratio of removing 55.86 % for final basin, as well as Nitrogen concentrations from 136.43 to 14.75 mg / l respectively for

final basin with ratio of removing 10.81%, the pH was recorded along sampling period which was with range from 7.01 to 7.6 as in table (1).

Table (1). Chemical tests for Al Russtamyia wastewater treatment plant

Phases of Plant	Turbidity NTU	TSS mg/l	TDS mg/l	COD mg/l	PO ₄ mg/l	N mg/l	BOD ₅ mg/l	pH
Before treatment	220	602	1427	427.263	15.083	136.43	235	7.01
Sludge	11333.3	12132.6	1427	523.75	11.455	134.86	218.22	7.02
After treatment	2.653	288.66	1013	82	8.426	14.75	35.009	7.5

Al Zafaranyah plant of treatment

Residential campus of Al Zafaranyah wastewater plant at Baghdad city was focused in order to study and characterize activated sludge and find out their capability in a wastewater of residential campus treatment. Results showed the reduction of turbidity by using levels by activated sludge from 340 to 281 NTU at the basin before treatment and final basin. For the total suspended solids (TSS) the value reduced from 81 to 13 mg/l respectively, also the quantity of Total Dissolved Solids was reduced from 1033 to 1016 mg / l respectively, While the quantity of Chemical oxygen demand (COD) reduced from 212.5 to 68 mg/l respectively compared with Biological Oxygen Demand BOD₅ which reduced from 164 to 29.162 mg/l respectively, in addition to that the concentration of botanical nutrients which represented by Phosphate PO₄ and Nitrogen N was reduced by using activated sludge where the phosphate concentration reduced from 26.23 to 4.23 mg/l respectively with ratio of removing reached to 55.86 %, for final basin concentration of N reduced from 10 to 0.04 mg / l respectively with high removing ratio where reached to 96 % also for final basin. pH value was recorded in range from 7.1 – 7.7 as in table (2).

Table (2). Chemical tests of Al Zafaranyah camp plant of treatment plant

Phases of Plant	Turbidity NTU	TSS mg/l	TDS mg/l	COD mg/l	PO ₄ mg/l	N mg/l	BOD ₅ mg/l	pH
Before treatment	340	81	1033	212.5	15.083	10	164	7.2
Sludge	329.3	2651	1020	198	11.455	5.58	-	7.7
After treatment	281	13	1016	68	8.426	0.4	29.162	7.1

Anaerobic treatment system (Anaerobic reactor with an ascending layer)

Anaerobic reactor with an ascending layer at Ministry of Science and Technology was selected for Characterization of sludge and find out ability of non-ventilated activated sludge for wastewater treatment. Results showed decreasing of turbidity by using activated sludge from 222.5 to 171 NTU at the basin before treatment and final basin at the plant respectively, and for Total Suspended Solids (TSS) was decreased from 50 to 32 mg / l respectively, also the quantity of Total Dissolved Solids was decreased from 1314 to 1308 mg / l respectively, as well as the Chemical Oxygen Demand (COD) was decreased from 211 to 130 mg / l respectively compared with quantity of Biological Oxygen Demand BOD₅ were decreased from 103.71 to 76 mg / l respectively, in addition to that results showed decreasing of botanical nutrients which represented by Phosphate PO₄ and Nitrogen N was reduced by using activated sludge where the concentration of PO₄ was decreased from 14.65 to 11.65 mg / l and the concentration of N was decreased from 43.60 to 39.76 mg/l respectively also the pH value for plant phases ranged from 6.2 to 7.3 as shown in table (3).

Table (2). Chemical tests of Anaerobic treatment system (Anaerobic reactor with an ascending layer)

Phases of Plant	Turbidity NTU	TSS mg/l	TDS mg/l	COD mg/l	PO ₄ mg/l	N mg/l	BOD ₅ mg/l	pH
Before treatment	222.5	50	1314	211	14.65	43.60	103.71	7.3
Sludge	105	149	1427	230	52.3	87.28	-	6.2
After treatment	171	32	1308	130	11.65	39.76	76	7.1

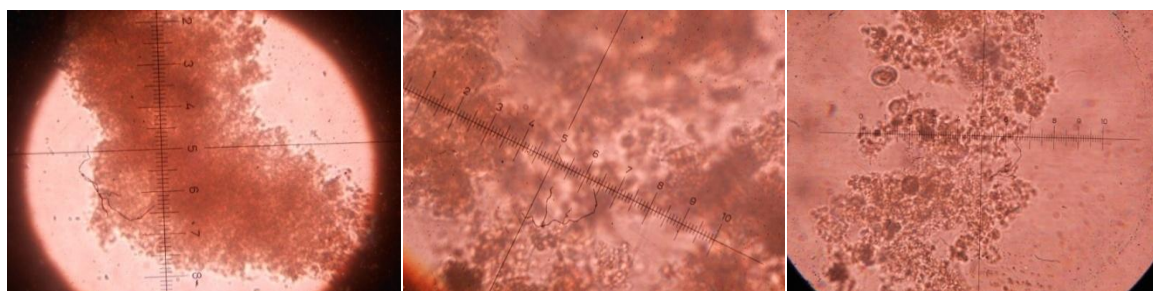
Results of Flocs**Al Russtamyia plant of treatment**

Diameters of Bio-flocs was measured in all phases of plant to know the range of ability to Precipitate bio-flocs 20 times for each plant when the minimum, intermediate and the maximum measuring of flocs for all phases, the minimal value of flocs diameters of plant phases which started with (before treatment phase, primary basin, secondary basin, sludge and final basin) with diameters 0.5, 2.25, 1.5, 15, and 0.7 μm respectively, but the intermediate volume of flocs diameters for same phases was 11.01, 23.09, 18.48, 137.85 and 3.22 μm respectively, in another hand the maximum limits for flocs diameters was 243, 125, 130, 312.5 and 9 μm respectively as in table (4).

Table (4). Diameters values of bio flocs for phases of Al Russtamyia plant

Plants	Diameters of Flocs (μm)		
	Minimum value	Intermediate	Maximum value
Before treatment	0.5	11.01	243
Primary	2.25	23.09	125
Secondary	1.5	18.48	130
Sludge	15	137.85	312.5
Final	0.7	3.22	9

Figures (5) showed the forms of bio-flocs which found in sludge basin at Russtamyia plant, which diagnosed by light microscope with magnification 40 X, bio-flocs diameters was measured by using Ocular ruler which compared with microscope Lenses.

**Figure (5). Forms of represented bio-flocs in activated sludge at Al Russtamyia plant**

Al Zafaraniyah residential campus plant of treatment

The values of measured diameter of bioflocs for Al Zafaraniyah campus plant of treatment which represented by phases (before treatment, limited aeration basin, sludge basin and final basin) was 42.5, 50, 32.5 and 1 μm respectively as minimum value of bioflocs diameters. But the recorded intermediate value of bioflocs diameters was 22.5, 100, 245 and 3 μm respectively, while diameters of maximum value reached to 312.5, 140, 600 and 6.25 μm respectively as shown in table (5).

Table (5). Values of bioflocs diameters for treatment phases at Al Zafaraniyah residential campus plant of treatment

Plants	Diameters of Flocs (μm)		
	Minimum value	Intermediate	Maximum value
Before treatment	42.5	225	312.5
Limited aeration	50	100	140
Sludge	32.5	245	600
Final	1	3	6.25

Figure (6). Show the forms of bioflocs that found at Al Zafaraniyah residential campus plant of treatment at sludge basin and diagnosed by light microscope under magnification 40 X, bio-flocs diameters was measured by using Ocular ruler which compared with microscope Lenses.

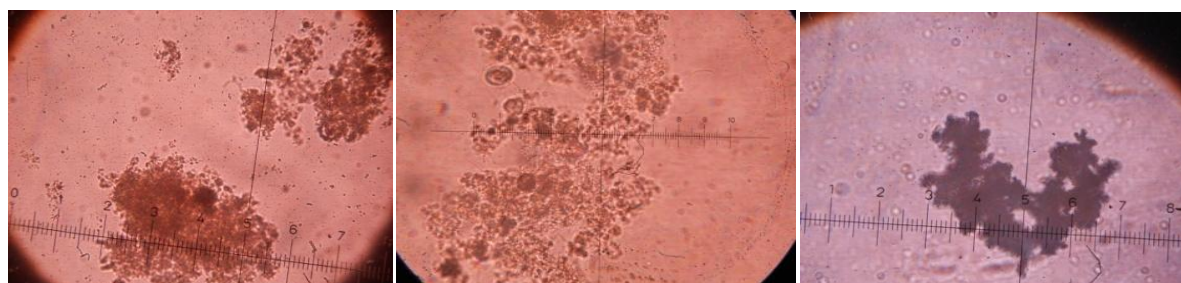


Figure (6). Forms of represented bioflocs at activated sludge at Al Zafaraniyah residential campus plant of treatment

Anaerobic treatment system (Anaerobic reactor with an ascending layer)

Bioflocs diameters of Anaerobic treatment system of four phases (before treatment basin, precipitation basin, sludge basin and final basin) reached to 1.75, 1.25, and 1.25 μm respectively as minimum value of bioflocs, when was intermediate value reached to 47.5, 6.5, 112.5 and 35 μm respectively, while maximum value reached to 130, 115, 562.5 and 80 μm respectively, as shown in table (6) and Figure (7).

Table (6). Values of bioflocs diameters for treatment phases at anaerobic treatment system anaerobic reactor with an ascending layer.

Plants	Diameters of Flocs (μm)		
	Minimum value	Intermediate	Maximum value
Before treatment	1.75	47.5	130
Precipitation	1.25	6.5	115
Sludge	1.25	112.5	562.5
Final	1.25	35	80

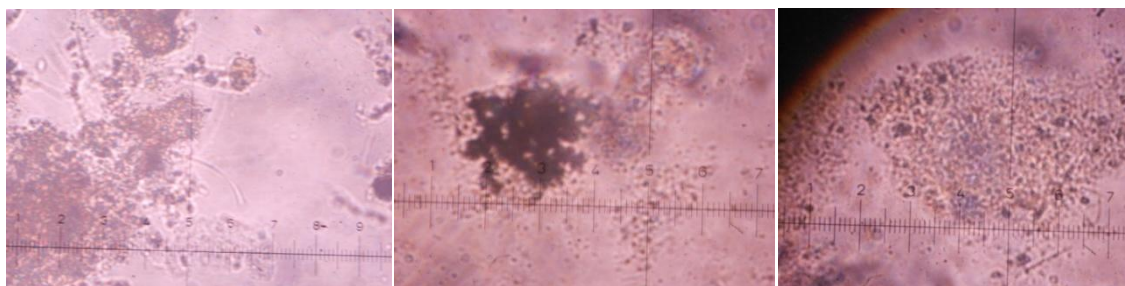


Figure (7). Show some represented bioflocs at anaerobic treatment system anaerobic reactor with an ascending layer which diameters were measured by light microscope by using Ocular ruler.

Discussion

Bacteria are considered as one of the most important microorganisms which are used in bioremediation of wastewater to decompose and oxidize suspended, colloid and dissolved material in water to change it to inorganic fixed materials with the presence of Oxygen. Wastewater contains groups of bacteria, aerobic and anaerobic, the activity of these two groups of bacteria for decomposing and oxidizing inorganic materials, depends on dissolved oxygen availability or not, in the case of oxygen availability effect and activate aerobic bacteria such as *Salmonella* sp., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* sp., *Citrobacter* sp., *Shigella* sp. and *Streptomyces* sp. while the bacteria *Streptomyces* sp. which is a filamentous type can decompose organic materials to inorganic materials, water, energy, Carbon dioxide and Nitrate NO_3 , this path called aerobic decomposition for organic materials, but anaerobic bacteria are active in the case of unavailability of dissolved Oxygen O_2 in wastewater *Clostridium perfringens*. which also can grow in aerobic conditions, because of *Bacillus* sp. behavior as a facultative in living in both aerobic and anaerobic conditions as in an anaerobic reactor with an ascending layer for this study where decompose organic materials in wastewater to produce inorganic materials, energy, water, methane CH_4 , Hydrogen sulfide H_2S and Ammonia NH_3 , this path called anaerobic decomposition (Fermentation) of organic materials. Therefore aerobic bacteria are considered as important and active bacteria in biodegradation for wastewater [22]. Results of this project to the ability of Al Russtamyia treatment plant to reduce COD from 427.263 to 82 mg/l with removing ratio 80%, while Al Zafaranyah residential campus plant of treatment, recorded a reduced value from 212.5 to 68 mg/l with removing ratio 68%, while anaerobic reactor system value of COD was reached ranged from 211 to 130 mg / l with removing ratio 38%, the disparity in values of reducing depends on ability of bacteria for excretion a wide range of lysozymes which work to break down organic and hydrocarbon materials and convert them to simpler materials and adsorb a lot of heavy materials by extracellular in addition to spreading and of bacteria in all body water and rapid growth. Several studies refer to the efficacy of *Bacillus*, *Pseudomonas* and some species of *Micrococcus* species highly efficient for breaking down dissolved organic materials due to secreted lysozymes [23]. For the quantity of BOD_5 , the ratio of decreasing for both aerobic plants was 82.2% and 85.1% for Al Zafaranyah campus plant of treatment and Al Russtamyia plant of treatment respectively, but for Anaerobic treatment system reached to 26.7%, also results showed an efficiency of activated sludge to reduce PO_4 and NO_3 from wastewater because of ability to consume and reducing concentrations of PO_4 and NO_3 significantly from contaminated water, the study of [24] ability of bacteria to reduce PO_4 , NO_3 , NO_2 in contaminated waters and change them to nutrients granules stored in bacterial cells. For aerobic activated sludge a significant role to reduce the amount of total suspended solids (TSS), it was observed that at Al Russtamyia plant of treatment when TSS reduced from 602 – 288.66 mg/ l. with reducing ratio value 52%, also to Al Zafaranyah residential campus plant of treatment results showed reducing value of TSS from 81 to 13 mg / l. with reducing ratio 83%, but the value of TSS reducing of Anaerobic system was gave results less than aerobic plants (Al Russtamyia and Al Zafaranyah) where TSS reduced from 50-32 mg / l. with ratio 36%.

The high rate of reducing of TSS at aerobic plants belongs to the presence of many species of Zooplankton such as *Arcella discoides*, *Trachelophyllum* sp., *Aspidisca* sp., *Litonotas* sp., *Bodo candatus*, *Lecane* sp., *Vorticella* sp., *Monostyla bulla*, *Charchesium* sp., *Paramecium* sp., *Nematoda* sp., *Cephalodella* sp., *Keratella vulgy.*, *K. himinalus* and *Philodania* sp., which they represent as a biological filter which refer to good treatment, but the anaerobic system has a few species of zooplankton, but some species like *Coleps* sp. and *Nebela lageniformis* can live at anaerobic conditions, there are very important role of Zooplankton at activated sludge which include Rotifera, Ciliate and Crustacea (*Daphnia* and Copepoda) where constitute about 5% of the biomass of the Activated Sludge, and play a major and important role in improving contaminated

waters by feeding on solid materials and change them to nutrients where decrease about 72% of TSS at wastewater treatment plant [25]. Zooplankton used as biomarkers for water contamination and compared with size and fertilization and reproduction of Zooplankton which have ability to indicates the nature and magnitude of pollution. But ciliate feeding on bacteria but not decomposed organic materials, with highly ability to reduced BOD₅, bacteria and flagellates compete to fed on decompose nutrients, but ciliate compete with another ciliate and rotifera to ingest bacteria, zooplankton refer to the quality of the sludge because they exist after forming flocs and after removing most of organic nutrients. For rotifera rarely found in a large numbers in wastewater treatment processing. The main role of Zooplankton is removing the bacteria and developing flocs also they contribute to remove turbidity of water emerging from the plant by consuming non flocced bacteria.

Mucoid materials which produced by the mouths or feet of rotifera helping in forming flocs, rotifer needs more time for the presence at processing of treatment which refers to increasing fixing of organic wastes [26]. There was high reduction of bio-flocs sizes in all stations of this study, because of the filamentous bacteria and fungi presence such as *Penicillium brevicompactum*, *Penicillium echinulatum*, *Penicillium glabrum*, *Rhizopus stolonifer*, which have the ability for surviving at low pH and low concentration of N, this capability associated with the ability to decomposing and dismantling of cellulose Making it relevant to the treatment of wastewater, especially industrial wastewater [27]. Also the algae have a role in wastewater treatment, when algae sharing anaerobic bacteria for treatment wastewater with ratio of 10% from non-treated water representing a removal of color ratio 52%. For algae also an important bioremediation for two reasons, first at oxidizing lakes by producing oxygen O₂ by photosynthesis and consuming Carbon dioxide CO₂ at day [28]. Several studies refers to using filamentous algae like *Oscillatoria perornata* with ratio 5% to reduce or decompose organic materials during photosynthesis path where living organisms produce Ions and Molecular oxygen with presence of H₂O₂ also Cyanobacteria can use Melanoidin as a Nitrogen N better than carbon recourse. Also bio flocs represents in wastewater performed several roles in those states where lead a different roles in the processes of digestion and decomposition of organic materials through its presence in wastewater containing high concentrations of organic wastes and work to decompose these materials to simpler elements which can used as a nutrients from Bacteria, Fungi, Algae, protozoa and others [28]. Micro flocs precipitation by used micro-organisms belong to existing charges among suspended materials and cells walls of microorganisms which make them flocculate and then precipitate, or to the active groups which Contained therein these organisms which make them gravitated to the fitted groups of suspended materials [29]. Fungi growing in low pH at no aerated sludge with pH from 2-9 which helps to reduce the quantity of organic materials and density of fungal hyphae, and organic materials like saccharides can changed to ethanol CH₃CH₂OH in anaerobic conditions [30]. It's also corresponds to the results of the current study that arrived pH of sludge of bioreactor to 6.2 where the fungi are working to reduce the organic compounds in wastewater.

Conclusions and perspective

- 1- Method of remediation by activated sludge in small and medium plants, but its uneconomical in the case of large plants, because the sludge needs aeration for several days so that need several days and large volume aeration basins with large equipments.
- 2- Fermented non aerated sludge do not produce foul odor
- 3- Produced fermentation from non-activated sludge leads to reduction of numbers of bacteria and pathogenic fungi.
- 4- Using of non-activated sludge for remediation is difficult, because of anaerobic bacteria which do the processing for decomposition of organic materials do not exist adequately in raw wastewater, in addition to that their growth rate is very slow when comparing to aerobic bacteria
- 5- Average of development for anaerobic bacteria is too slow compared aerobic bacteria

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