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# Control of Final Settling Tank Bacterial Content by Image Examination

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**Abstract**: **.** Wastewater commonly includes organic solids, micro-organisms, and toxic compounds. In activated sludge system, aeration process is very costly and it is desirable to reduce microbiological problems.During activated sludge process for wastewater treatment plants (WWTPs), micro-organisms play a vital role as an indicator of wastewater treatment efficiency. Identification of micro-organisms population may be achieved by counting based on image analysis. There are some types of these micro-organisms found in wastewater, such as filamentous bacteria, which cause sedimentation problems in final settling tanks. This paper discusses kinds of microbiological issues that may happen in activated sludge operation and how to control these problems at a pilot plant scale model WWT. In this study the DO set-point was changed every 3 weeks between 0.5 and 4.5 mg/l for a few months.Hydrogen peroxide is one of vital chemical additions in WWTPsto achievegood sedimentation. The aim of this work is also to provethat the addition of optimum $H_2O_2$ /$Fe^{+2}$ = 5 with (DO=1.5 mg/l) was equivalent (DO=3.5 mg/l) without $H_2O_2$/$Fe^{+2}$ for wastewater treatment, this means saving 2 mg/l and providing a high cost of using electricity and mechanical equipment compared to the non-use of hydrogen peroxide in this treatment.

**KEYWORDS**: **:** Wastewater treatment plants (WWTPs); Activated sludge process (ASP); Biological treatment; Image analysis and Hydrogen peroxide ($H_2O_2$).

## 1. INTRODUCTION

### 1.1 RETURN ACTIVATED SLUDGE PROCESS

Wastewater is a combination of solids and water. 0.1% represents pollution and 99.9% represents water. Wastewater must be treated to remove solids which threats to human health [1].Conventional wastewater treatment consists of a consequently stages of physical, chemical, and biological processes for removing organic dissolved and suspended matters [2]. Activated sludge process is an essential type of biological treatment having a main task for removing most of the dissolved solids remaining in the wastewater after primary treatment. Return activated sludge process (ASP) is a common aerobic treatment used to reduce the amount of organic matters from wastewater by using micro-organisms growing in aeration tanks by converting dissolved organic matters into their own biomass.

Wastewater treatment in activated sludge system poses a challenge all over the world [3]. Stabilization of waste activated sludge (WAS) is avitalphase for the reuse or disposal [4].Different patterns of the activated sludge process can be prepared to achieve the wastewater mixing and aerated in an aeration tank where the bacteria form small groups, afterword’s the aeration stops and the combination is moved to a final settling tank [5]. The flocs in wastewater are allowed to separate and settle down from the effluent water. The efficiency of wastewater treatment plants by using activated sludge technique is linked to the return activated sludge technique, which is closely related to micro-organisms population [6]. Microscopic tests of activated sludge may comprehensively control the whole water treatment processes, such tests enable to detect the causes of poor sedimentation by identifying of the filamentous micro-organisms and other micro-organisms [7]. Filamentous bacteria and some of unwanted micro-organisms which lead to bad settlement will appear in case of oxygen reduction [8]. The aim of biological treatment which followed by primary treatment is the additional treatment of wastewater from primary treatment to remove residual organic matter and suspended solids [9]. Biological treatment could be divided into attached growth based and suspended growth based systems [10]. Activated sludge system contains twoimportant units, the aeration basin and the final clarifier. Activated sludge was assumed that adapted micro-organisms in aeration tank increase the treatment efficiency. Many investigations have been done on activated sludge and the effects of factors on this system [11].

## 1.2 TYPES OF MICRO-ORGANISMS

Some types of micro-organisms will cultivate in activated sludge system. So, it is significant that the operator makes an environment that will improve the type of wanted micro-organisms [12]. During activated sludge studying, three different types of micro-organisms will be observed; Bacteria (95%), Protozoa (4%) & Metazoa (1%). Bacteria are single-celled organisms which divided into three main forms; bacillus, round shaped, and spiral-shaped [13]. Aerobic bacteria need dissolved oxygen to be effective in the biological treatment system [14]. Filamentous bulking and foaming phenomenon are big problems in activated sludge system. On the other side, presence of filamentous bacteria may be useful in ASP. Decrease of filamentous bacteria in wastewater can lead to small flocs which settle well [15]. The behavior and the numbers of different types of protozoa will be a good indicator of treatment system performance. Their chief task is to remove non- flocculent bacteria and very small algae that have not settled [15]. For the studying of Protozoa, it classifies in the following five categories according to their behavior in activated sludge; Amoeba, Flagellates, Crawling ciliates, stalked ciliates, and Free-swimming. Amoebae are the most olden single celled protozoa [16]. Amoeba has enormously membranes that allow food to be absorbed through them and they move throughout the MLSS. Generally, Amoeba is found in high numbers in "young" MLSS and is associated with poor settling sludge [1].In case of presence a big amount of amoeba in a collected sample from the aeration tank, this may specify one of the following:

- A sudden load of BOD which indicate that extra food available in wastewater.
- Amount of oxygen is low; Amoebae transfer very slowly.
- A large amount of solid particles.

A lot of flagellates and bacteria absorb many nutrients in wastewater so they compete heavily with bacteria to get as much nutrients and dissolved nutrients [16]. Flagellates are found in high numbers where high organic load is found (BOD) [1].The presence of ciliates in the wastewater is a strong indicator of the quality of treatment[1]. They are crucial to remove excess bacteria and small algae from the wastewater and clarifying the effluent. Ciliates can be classified into three categories; Crawling ciliates, Stalked ciliates & Free-swimming ciliates. Free-swimming ciliates are usually covered with a range of cilia that are used for movement and for food collection. They appear when flagellates begin to disappear. As the bacteria count rises, a lot of the organic nutrients have been removed [17]. When bacteria decrease, Crawling ciliates compete to swim and feed on bacteria [17]. Stalked ciliates have contained cilia surrounding the mouth and usedit to bring food. These Stalked ciliates appear in established sludge and dominate when removing most of the dissolved nutrients. In Mature sludge, crawling ciliates and stalked ciliates compete for dominance [18]. Metazoa organisms can have a simple physical structure and are usually found in treatment systems for sanitation. Following are metazoa such as; Rotifers and Nematodes [18]. Rotifers are commonly found in wastewater treatment systems and play an important role in the activated sludge treatment but are not major in the system; they are a strong indicator of the presence of toxic substances in wastewater [19]. As shown in Fig. (1), the bacteria Nematodes may be seen in important amounts in older sludge. Nematodes eat bacteria, protozoa, and fungus. Some nematodes have teeth and others can stick to them as prey [18].

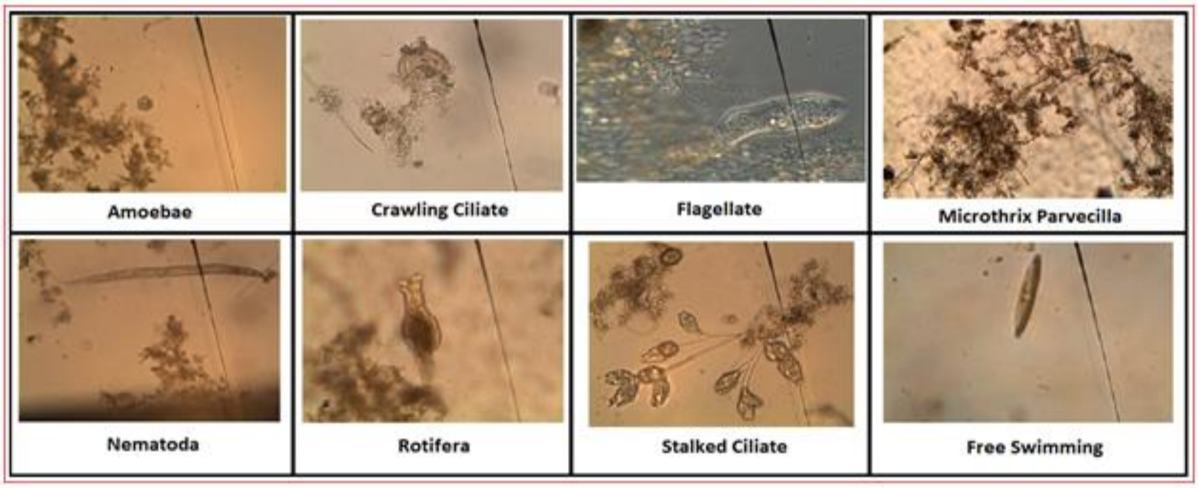


**FIG 1.**Types of micro-organisms in mixed liquor suspended solids as microscopic images

## 1.3 FINAL CLARIFIER

The aim of biological treatment is the additional treatment of wastewater from primary treatment to remove residual organic matter and suspended solids. In addition, biological treatment is followed by primary treatment and involves the removal of biodegradable organic matter using aerobic biological treatment processes [9]. Biological treatment could be divided into attached growth based and suspended growth based systems [10]. Activated sludge system contains twoimportant units, the aeration basin and the final clarifier. The activated sludge principles have been developed, since 1914 [2]. Activated sludge consists of micro-organisms, colloidal matters and mineral particles; thus, determination of floc structures between them is very complex task. It was assumed that adapted micro-organisms in aeration tank increase the treatment efficiency. Many investigations have been done on activated sludge and the effects of factors on this system [11]. Final clarifier allows settling to the bottom, allowing clear wastewater to leave the tank. The MLSS may be removed from the bottom of the tank [1].

## 1.4 ADDITION OF HYDROGEN PEROXIDE

Two types of effective chemicals, such as; chlorine and hydrogen peroxide were successfully used to control filamentous organisms and control micro-organisms. Despite biological purification in wastewater treatment plants, wastewater must also be subjected to additional purification processes such as hydrogen peroxide additions with fixed doses of iron sulphate [20]. Hydrogen peroxide releases a lot of oxygen atoms to increase treatment efficiency. Once control of micro-organisms is obtained, the dose may be ranged between (25-50) mg/l $H_2O_2$ tolimit filamentous bulking of bacteria [21]. Current approaches for controlling problems of wastewater treatment include operational changes, chlorination, chemical or polymer additions, and additional structures. Hydrogen peroxide ($H_2O_2$) is one of vital chemical additions in WWTPs to high efficiency which can be properly evaluated by microscopic analyses of activated sludge. Previous studies proved that hydrogen peroxide can be used with sodium carbonate as ratio 2/3 to improve COD efficiency, the COD index in the range reduced from 9 to 29% [22]. $H_2O_2$ can be used alone or with catalysts such as iron ($Fe^{+2}$ or $Fe^{+3}$) to release oxygen atoms for improving treatment efficiency. By using Fenton reaction, $H_2O_2$ is decomposed and $Fe^{+2}$ ions are necessary for the release of hydroxyl radicals from hydrogen peroxide. In the common Fenton process (Reaction 1), the $Fe^{+3}$ ions are formed. Under the effect of UV light, photo reduction from $Fe^{+3}$ ions to $Fe^{+2}$ is run; moreover, the additional $OH^{\bullet}$ radicals are formed (Reaction 2) [22]. The cost of using hydrogen peroxide can save coagulant use. Experiments were conducted to obtain best conditions for Fenton reaction with different $H_2O_2$ and salts of iron and the best ratio between $H_2O_2$/ $Fe^{+2}$ was 5, respectively [23].Conversion $Fe^{+2}$ to $Fe^{+3}$ which help in the process of oxidation, remove color in the effluent wastewater [24].

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^{-} + OH \quad (1)$$

$$Fe^{+3} + H_2O \rightarrow Fe^{+2} + OH^{\bullet} + H^{+} \quad (2)$$

## 2. MATERIALS AND METHODS

### 2.1 PILOT PLANT MODEL

Physicochemical studies in this paper by using microscopic analyses of activated sludge are essential to evaluate the effect of DO doses and effect of $H_2O_2$additions on activated sludge system [25]. To apply this study, a pilot plant model for the addition of chemicals must be made with the necessary calibration of this model.As shown in Fig. (2) pilot plant model was started from November 2nd, 2017 by the following steps;

1- Pilot plant model was determined to select design flow rate (Q) equals 840 liters per day as 210 liters each six hours (One Cycle) and the pilot plant was controlled as a continuous flow system.
2- The model was located beside the primary sedimentation tank of Zeinen WWTP for easy wastewater transfer to the system.
3- The model consists of three separate tanks: feeding tank, aeration tank and final sedimentation tank.
4- Feeding tank is located a head of the system's biological treatment units and contains 0.5m3 of water coming from primary settling tank.
5- This tank is a rectangular shape and dimensions of the aeration tank are as follows (0.55 x 0.74 x 0.52) m.
6- The active depth of the tank is 0.52 m and it is where the influent and the returned activated sludge are mixed.
7- The raw wastewater flow rate (from feeding tank) was set at 0.58 L/min., the excess sludge supposed to be taken out from the tank intermittently based on the sludge retention time calculations.
8- Sludge is returned to the aeration tank for 1 minute each 10 minutes with rate 7 liters each minute, this design criterion was carried out to calibrate this model with second plant of Zeinen WWTP.
9- Samples were collected from the pilot plant which located at Zeinen WWTP in Giza, Egypt in the first step.
10- The treatment system which used in this plant is an extended aeration process and all samples which used for this study were collected from primary tank where located in at Zeinen WWTP.
11- Different doses of dissolved oxygen were performed in aeration tank of pilot plant and DO doses were varied between (0.5-4.5) mg/l.

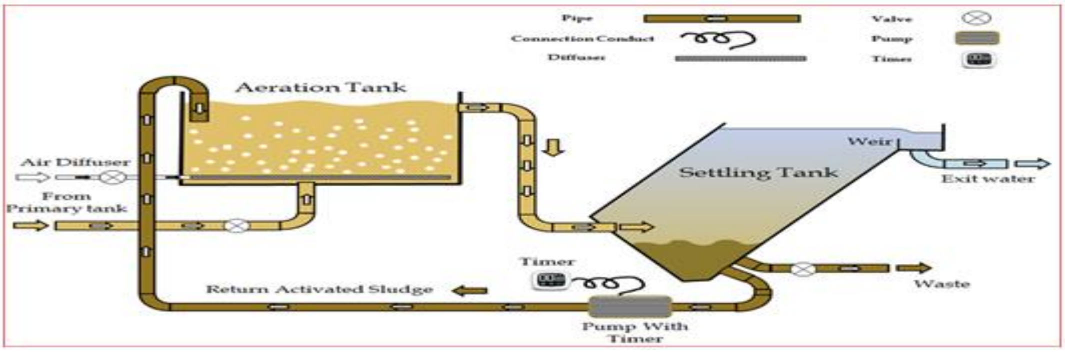


**FIG 2.**Schematic diagram of the pilot plant

## 2.2 SAMPLES AND MICROSCOPIC WORK

Experiments were performed to conclude the effective of the best ratio of $H_2O_2/Fe^{+2}$ ions on improvement of treatment efficiency in final clarifier. The ratio of $H_2O_2/Fe^{+2}$ was 5 as 30/6, respectively [23]. After samples collection from model, use microscope for preparing slides and making required analysis as the following steps;

- Clean slide and use pipette to take a sample of MLSS solution.
- Place one drop of MLSS solution from the pipette to the middle of the glass slide.
- As soon as cover slip touches drop of MLSS allow cover slip to fall onto glass slide.
- Place slide on microscope stage.
- Move stage on 10 X and identify organisms in the MLSS.

Finally, the results of this experimental work are compared with the recorded values of observation, then the pilot plantmodel is ready for simulation.Figure (3)illuminates the flowchart of the steps of experimental work andsimulation model.

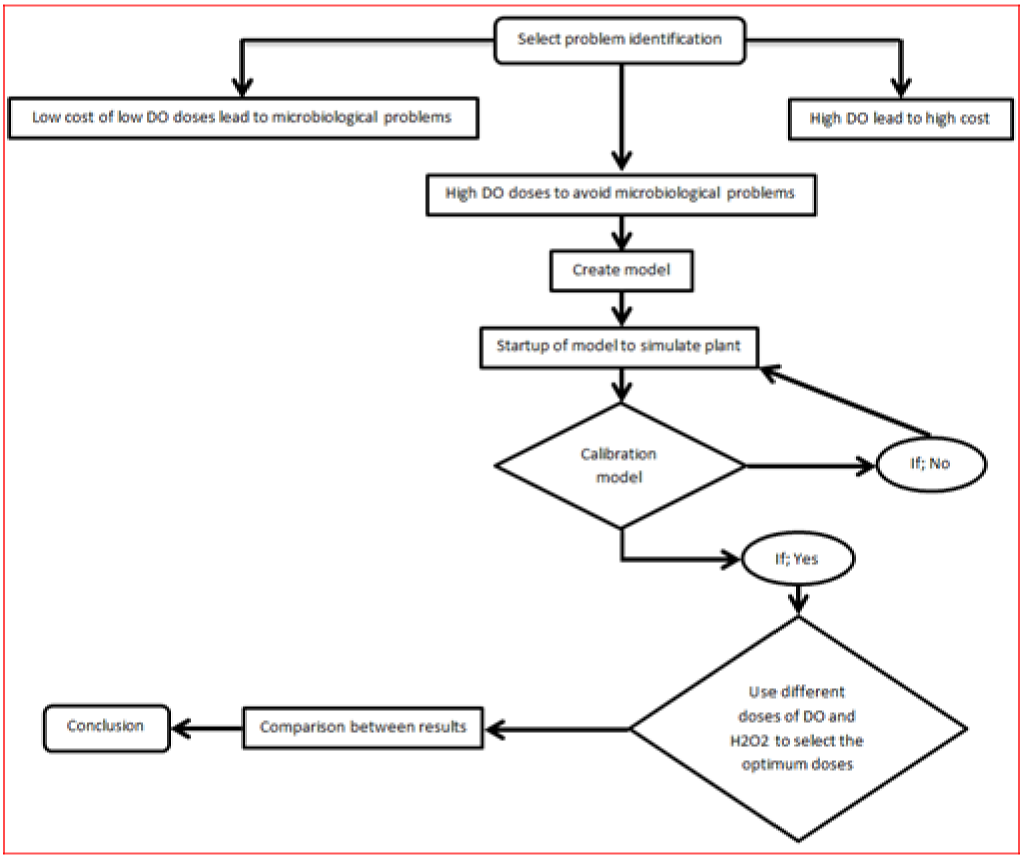


**FIG 3.**The Work Program Flow Chart

## 3. RESULTS AND DISCUSSION

### 3.1 MICROBIOLOGY OF A.S.P. IN PILOT PLANT BY USING MICROSCOPE

ASP Identification of micro-organisms activity and population may be successfully achieved by counting based on image analysis as shown in Figures (4, 5); this technique will indicate the overall bacteriological health which indicates the ASP overall capability for treatment using the activated system through monitoring micro-organic balance versus sludge volume. In this experiment, the lowest percentages of dissolved oxygen (0.5, 1.0 & 1.5 mg/l) were selected which representing the Shock organic load of the wastewater treatment plant.The best efficiency of organics removal was appeared, and knows the optimum DO dose was guide to the best efficiency of final clarifier and growth of microbial bacteria. During this step, DO concentration was changed from 0.5 mg/l to 4.5 mg/l approximately every 3 weeks to calculate which of these dosages will have any good impact on the activated sludge system and notes different types of micro-organisms in table like the following Table(1). Experiments were performed to conclude the effective of the best ratio of $H_2O_2/Fe^{+2}$ ions on improvement of treatment efficiency in final clarifier. The ratio of $H_2O_2/Fe^{+2}$ was 5 as 30/6, respectively.

TABLE 1.Example of count worksheet for (10 samples) with different doses of DO

| Micro-Organisms | DO (mg/l) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 |
| | Count of micro-organisms for 10 samples | | | | | | | | |
| Microthrix Parvicella | 18 | 11 | 7 | 2 | 0 | 0 | 0 | 0 | 0 |
| Amoebae | 17 | 12 | 11 | 8 | 7 | 4 | 2 | 3 | 2 |
| Flagellate | 14 | 12 | 13 | 9 | 8 | 5 | 4 | 2 | 3 |
| Free-swimming Ciliates | 2 | 8 | 31 | 38 | 53 | 66 | 72 | 68 | 61 |
| Stalked ciliates | 17 | 61 | 108 | 154 | 247 | 302 | 283 | 257 | 222 |
| Paramecium | 0 | 0 | 4 | 12 | 11 | 14 | 15 | 17 | 22 |
| Rotifers | 2 | 3 | 11 | 18 | 27 | 32 | 45 | 38 | 44 |
| nematodes | 4 | 7 | 21 | 43 | 65 | 72 | 69 | 62 | 59 |

Aeration process in activated sludge system is very high cost in terms of electrical and mechanical. Aeration process is desirable to control the good treatment in WWTP and reduction of DO concentration as possible leads to risking poor quality of treatment without any chemical oxidants which release oxygen atoms. The good condition of activated sludge that is described by: high content of ciliates, high content of Bacillus species, amount of rotifers and nematodes, low count of flagellates and amoebae, absence of fungi and filamentous bacteria. The poor condition of activated sludge that is cha described by: low content of ciliates, presence of filamentous bacteria, flagellates, fungi and amoebae. DO was encountered using at first and by the same technique with the aid of chemical admixture $H_2O_2$. The final results may be summed up in the following points;

- The bacteriological count of (Microthrix Parvicella, Amoebae and Flagellate) decreases significantly as DO levels increased.
- On the other hand the bacteriological count of (ciliates, rotifers and nematodes) which indicates a good condition of activated sludge increases significantly as DO levels increased.
- Because of aeration process in activated sludge system is very costly, so using chemical addition ($H_2O_2$) which has two advantages; release oxygen instead of high costly of aeration process and cheap in cost.

### 3.2 Addition of Hydrogen Peroxide (H2O2)

In this experiment, the lowest percentages of dissolved oxygen (0.5, 1.0 & 1.5 mg/l) were selected which representing the Shock organic load of the wastewater treatment plant. Hydrogen peroxide was added with ratio of $H_2O_2$ /$Fe^{+2}$ were 5 as 30/6 mg/l and the results were compared as shown in Table (2) and Fig. (5).

TABLE 2.Example of Count Worksheet for (10 samples) with low doses of DO with ratio of $H_2O_2/Fe^{+2}$ was 5 as 30/6 mg/l

| Micro-Organisms | Without any additions of $H_2O_2$ | | | With ratio of $H_2O_2/Fe^{2+}$ was 5 as 30/6 mg/l | | |
|---|---|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 0.5 | 1.0 | 1.5 |
| | Count of micro-organisms for 10 samples | | | | | |
| Microthrix Parvicella | 18 | 11 | 7 | 10 | 3 | 1 |
| Amoebae | 17 | 12 | 11 | 9 | 6 | 2 |
| Flagellate | 14 | 12 | 13 | 11 | 6 | 4 |
| Free-swimming Ciliates | 2 | 8 | 31 | 9 | 26 | 58 |
| Stalked ciliates | 17 | 61 | 108 | 61 | 86 | 197 |
| Paramecium | 0 | 0 | 4 | 1 | 3 | 8 |
| Rotifers | 2 | 3 | 11 | 4 | 7 | 18 |
| Nematodes | 4 | 7 | 21 | 12 | 19 | 35 |

The microscopic analysis included activated sludge working in pilot scale plant and estimated numbers of micro-organisms are presented in Figures (4, 5). During the analysis carried out, different groups of micro-organisms were identified. Some of these micro-organisms are desirable and others are undesirable. The first type of these micro-organisms which desirable micro-organisms are stalked ciliated, Paramecium, Rotifers, Nematodes and Free swimming ciliates. An increase in the number of these micro-organisms was observed after increasing DO concentrations. At DO = 3 mg/l, Stalked ciliated was achieved maximum numbers in pilot plant. After DO = 3 mg/l, numbers of Stalked ciliated were decreased. Another desirable of micro-organisms were increased up to 3 mg/l, the amount of increasing became unnoticeable after DO = 3 mg/l. The number of undesirable groups was showed in figure (5). The second type of these micro-organisms which undesirable micro-organisms are Microthrix Parvicella, Amoebae, and Flagellate was showed in Fig. (5).

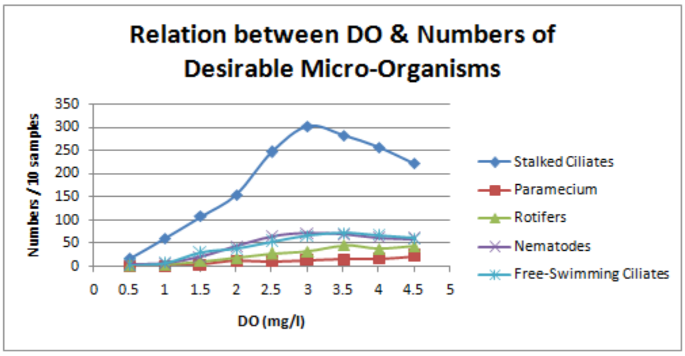


**FIG 4.** Numbers of desirable micro-organisms (Stalked Ciliates, Paramecium, Rotifers, Nematodes and Free Swimming Ciliates) in the pilot plant and relation between micro-organisms and dissolved oxygen (DO)

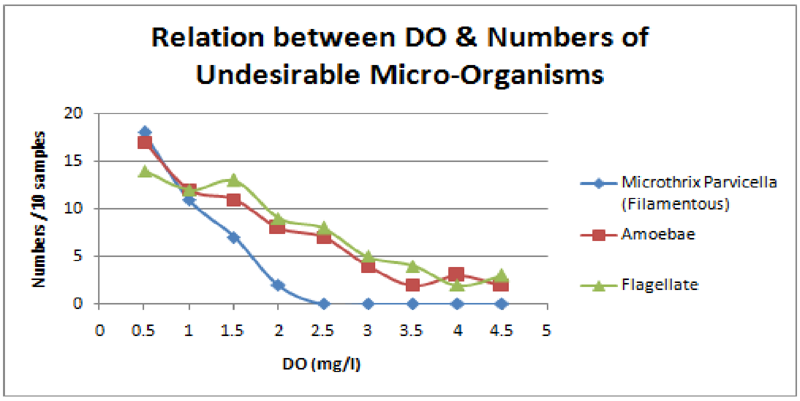


**FIG 5.**Numbers of undesirable micro-organisms (Microthrix Parvicella, Amoeba and Flagellate) in the pilot plant and relation between micro-organisms and dissolved oxygen (DO)

The good condition of activated sludge that is described by: high content of ciliates, high content of Bacillus species, amount of rotifers and nematodes, low count of flagellates and amoebae, absence of fungi and filamentous bacteria. The poor condition of activated sludge that is cha described by: low content of ciliates, presence of filamentous bacteria, flagellates, fungi and amoebae. DO was encountered using at first and by the same technique with the aid of chemical admixture $H_2O_2$. The final results of this experimental work by using addition of hydrogen peroxide were showed in Figure (6).

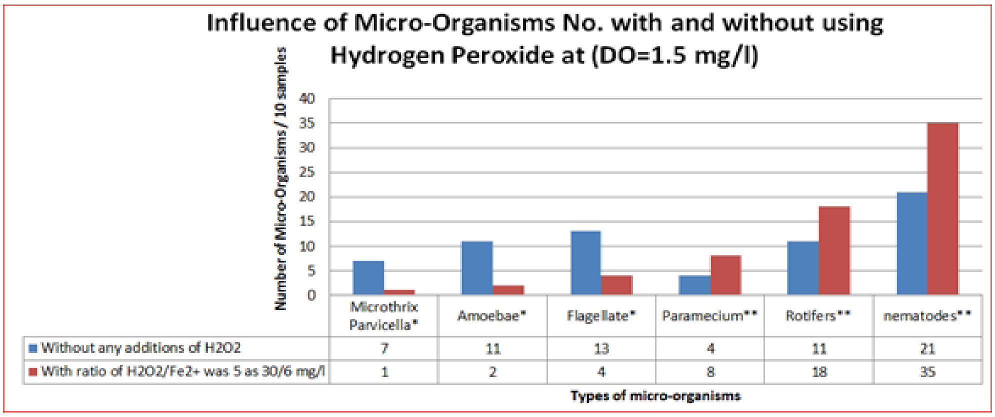


**FIG 6.**Influence of micro-organisms No. with and without using Hydrogen Peroxide at (DO=1.5 mg/l)
Type*: Undesirable Bacteria; Type**: Desirable Bacteria

## 4. Conclusion

Based on the experimental program executed in this research, and incremental biodegradability was evaluated by monitoring population of micro-organisms changes during $H_2O_2$additions. The microscopic examinationinvolved activated sludge functioning in the biological part of the pilot plant model. For improving the performance of final clarifier, several notes were observed. The experiments in this paper were performed to find the optimumdosageof using different $H_2O_2$concentrations in ASP system; the following conclusions had been reached:

- The optimum dose of hydrogen peroxide in ASP systems with ratio of $H_2O_2$ /$Fe^{+2}$ was 5 as 30/6 mg/l respectively.
- After addition of $H_2O_2$ in pilot plant, undesirable bacteria (Microthrix Parvicella, Amoebae and Flagellate) were decreased by 85.7%, 81.8% and 69.2% respectively.
- On the other side, desirable bacteria (Paramecium, Rotifers and Nematodes) were increased by 100.0%, 63.6% and 66.7% respectively.
- Obvious decreasing of undesirable bacteria (Microthrix Parvicella, Amoebae and Flagellate) after addition of hydrogen peroxide with ratio of $H_2O_2$/$Fe^{+2}$ was 5, were (85%, 82%, & 69%) respectively at (DO=1.5 mg/l)compared with DO=2.5 mg/l [26].
- Furthermore the increase was more positive when increasing the chemical $H_2O_2$catalyst at (DO=1.5 mg/l) by (82%, 87%, 64% & 67%) for stalked ciliated, free swimming ciliates, Rotifers and Nematodes respectively.
- The addition of optimum $H_2O_2$ /$Fe^{+2}$ = 5 with (DO=1.5 mg/l) was equivalent using aeration system with (DO=3.5 mg/l) without $H_2O_2$/$Fe^{+2}$ for wastewater treatment, this means saving 2 mg/l and providing a high cost of using electricity and mechanical equipment compared to the non-use of H2O2 in this treatment.

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