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Bioaugmentation and Advanced Oxidation Process for Organic and Inorganic Pollutants Removal and Pathogenic Bacteria Inactivat El-Rahawy Drain, Egypt

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Abstract

River Nile (Rossita Branch) receives massive quantities of untreated or inadequate municipal wastewater, industrial and agricultural wastewater through El-Rahawy drain. Consequently, the objective of the present study was to design a system depending on biological treatment followed by Fenton reaction for treatment of El-Rahawy drain wastewater. The physicochemical and bacteriological parameters were estimated for El Rahawy drain wastewater and treated effluent of the drainage wastewater. The samples were collected after 24h and after the fourth day of the treatment. The bioaugmented bacterial consortium that used to enhance the biological treatment was nonpathogenic and eco-friendly, and the inoculated dose was 10 gm/m^3/day. The removal efficiency of TDS reached 58.9% and 68.6 % by biological treatment after 24 h and 4 days. While the removal % of TDS reached 75.9% after 24 h of Fenton treatment, and this efficiency removal declined to 66.6% on the fourth day of the treatment. The removal efficiency of COD reached 33.3 and 56.8% after 24 h and 4 days of the biological treatment. Whereas the removal % of COD reached to 80% after 24 h, and this removal % declined to 78.8% after 4 days of Fenton treatment. The physicochemical and bacteriological parameters were declined by the treatment time increase. It was found that, the bioaugmented bacterial strains are able to grow well and produce more biodegeding enzymes after 24 h. Moreover, the biological treatment followed by the Fenton reaction is the most suitable solution for El-Rahawy drain treatment

***Keywords**:* bioaugmentation; pollutants; physico-chemical parameters; pathogen; El-Rahawy drain

1. Introduction

After the next 30 years, it is expected that the demand for water will be increased four times for industrial needs and also increase one time for household needs. Moreover, half of the population over the entire world will be facing water shortage even in river basins countries [1]. One of the ultimate keys for overcoming the water shortage is wastewater reuses. In Egypt, the majority of wastewater treatment plants depend on primary treatment, and other plants depend on secondary treatment. Therefore, the majority of these plants produced inadequately treated wastewater that directly discharged and reused in irrigation purposes by discharging into agriculture drains [2]. Significant pollutants in the agriculture drains include salts, nutrients such as phosphate and nitrogen, and toxic organic matters and inorganic pollutants from domestic and industrial sources [3]. El-Rahawy drain is considered one of the most polluted drains in Egypt. El- Rahawy drain receives a considerable

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amount of untreated and partially treated municipal and industrial wastewater from primary Zenin and secondary Abou-Rawash wastewater treatment plants from the Giza area, in addition to, the agriculture wastewater from surrounding area [4]. El-Rahawy drain has serious impacts on the water quality of Rossita Branch due to its high organic loads, which affects the suitability of the branch as a source of drinking water supply. This attributed to high load of bacterial pollution and high concentrations of NH_3, total dissolved solids (TDS), electrical conductivity (EC), turbidity, biological oxygen demand (BOD), total alkalinity and recognized depletion in dissolved oxygen (DO) as reported by El Gammal and El Shazely [5]. The major sources of pollution of Rossita Branch are El-Rahawy drain, which located at km 9, Sabal drain located at km 70.4, and Tala drain at km 119.3 (considering Delta Barrage is km 0) [6]. Insufficiently treated wastewater contains vast amounts of pollutants such as solids, organic and inorganic insoluble, and insoluble formulated particles, heavy metals, pathogenic microorganisms, and others. These pollutants may be toxic or mutagenic [7]. The disposal of partially treated and/or untreated wastewater into surface water cause potential or severe pollution and has adverse effects on the water, the health, and the environment [8]. Consequently, the treatment of the El-Rahawy drain before discharging to Nile water is highly needed. Wastewater and drainage water treatment biologically is considered an effective and low cost option [9]. However, biological treatment using indigenous microorganisms couldn't able to degrade all the present pollutants. Similarly, sometimes complex pollutants need different microorganisms as a consortium which might not concurrently be found in the environment in natural way. Accordingly, despite the success of this approach, using indigenous bacteria in the biodegradation process may be inhibited by abiotic factors like pH, temperature, and redox [10,11]. Recently, addition of exogenous microorganisms as a bioaugmentation approach could be beneficially used for accelerates the biological treatment of organic and inorganic pollutants and also, pathogenic bacteria inactivation's [12,13]. Since 20 years ago, advanced oxidation processes (AOPs) has gaining the attention for the efficient removal of organic and inorganic pollutants from aquatic environment [14-16]. AOPs are considered as an eco-friendly chemical, photochemical, photocatalytic, electrochemical and photoelectrochemical methods, which present the common feature of the in situ production of hydroxyl radical (•OH) as the main oxidant. The simplest and most typical chemical AOP is the Fenton method in which a mixture of Fe^{2+} and H_2O_2 (Fenton's reagent) is used to degrade organics [16]. The Fenton reaction or Fenton-like reaction (Fe[III]/excess H_2O_2) have been widely used for treatment of highly concentrated contaminants, although their application under neutral pH conditions is usually limited by the low solubility of iron, low efficiency of reaction, and production of sludge [17]. Therefore, the present study was aimed to design system for biological treatment followed by advanced oxidation for El-Rahawy drain purification.

2. Experimental

2.1 The wastewater source

The drainage wastewater from El-Rahawy drain was collected. The drainage water was collected in a sterile 20 liter bag, according to APHA [18]. Some physicochemical characteristics, moreover bacteriological parameters, including total coliform, fecal coliform, and *E. coli* of El-Rahawy drain water, were also determined according to APHA [18].

2.2 Identification of exogenous bacterial strains

The exogenous bacterial strains were obtained from a commercial substance packet, namely as BIOWISH (BIOWISH Technologies Inc. USA). The powder was rehydrated in a flask containing 10 mL of phosphate-buffered saline (NaCl 8 g/L, 0.2 KCl g/L, Na_2- HPO_4 1.44 g/L, KH_2PO_4 0.24 g/L in distilled water and 7.2 ± 0.2 pH). Then 90 mL of double-strength Tryptic Soy Broth (BD, Germany) was added into the vortexed flask to enrich the bacterial population and enhance growth. The bacterial identifications were carried out using semi-automated BIOLOG GEN III [13]. In brief, the nominated bacterial colonies were injected to inoculated fluid (A and B). The suspension was distributed using multichannel micropipette to 96 wells MicroPlate (BIOLOG, USA). This MicroPlate contains on 94 dissimilar lyophilized carbon and chemical utilizing sources. The inoculated MicroPlate was incubated at 37°C for 18-24 h. The identification of bacterial strain was compared with Biolog's microbial identification systems software in a semi-automated system.

2.3 The use of Fenton reagent

Ferrous sulfateheptahydrate ($FeSO_4 \cdot 7H_2O$) and Hydrogen peroxide (H_2O_2), [Fe^{2+}/ H_2O_2] at pH near (3) as catalytic oxidation was investigated. All experiments were performed at room temperature (25 ± 2) in four Jar Test apparatus used for stirring the reactants. The required dose of catalyst ferrous sulfate was added into the reaction vessel to start the experiments, and the pH was adjusted at the desired value (around 3) by the addition of H_2SO_4 (30%) and kept stable during the reaction.

2.4 Designed system for treatment of El-Rahway drain

The condition and operation of the designed system were adjusted for obtaining efficacy highly in the treatment of target samples. First, the system composed of one tank with capacity approximately 70 L of raw drainage wastewater, the dimension of this tank was 40x60x140 cm with flow rate 0.3L/min, then appropriated dose of bioaugmented substance was inoculated (10 gm/m^3/day) into the tank filled with sample and staying for constant contact time (4 h) to make an excellent opportunities to interact between bioactive substances and organic pollutants, and this process could be called biological augmentation. After that, about half volume (35 L) of sample from tank as mentioned earlier was transferred to another smaller tank with capacity 35 L and dimension (20x30x70 cm with flow rate 0.3L/min) and then filled by biologically treated sample which remained for 2 h a contact time for treatment with advanced Fenton oxidation reaction to enhance the quality of treated drainage samples and the level of elements and pollutants below the Egyptian recommended limits in Law 48/1984, and reusing the final treated effluents in different purposes, especially irrigation and agricultural purposes.

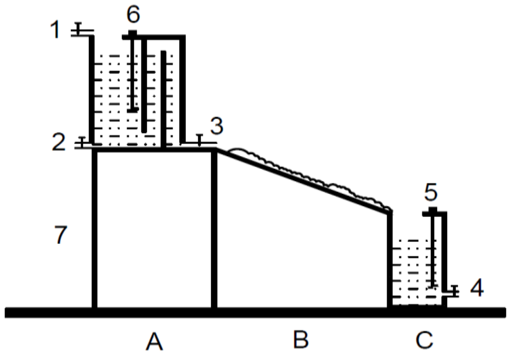

Fig. 1. Schematic diagram of bench-scale engineered system for treatment of drainage wastewater collected from El- Rahway drain. (A) biological treatment, (B) Carrier, (C) Fenton reaction, (1, 2, 3 and 4) faucet, (6 and 5) stirring.

2.5 Samples collection and removal efficiency

The treated drainage water samples were collected after 24 h and also after 4 days of biological and Fenton treatments. Some physicochemical parameters including, pH, TSS, TDS, COD, BOD, TKN, TP, chloride, and sulfate, were determined in the collected, treated samples according to APHA [18]. On the other side, some bacteriological parameters including total coliform, fecal coliform, and *E. coli* using the most probable number (MPN) method were also determined according to APHA [18]. All laboratory experiments in the current study were repeated for three times.

The removal efficiency for each parameter was calculated using the following formula:

$$\% \text{ Removal} = \frac{(Co - C)}{Co} x100$$

Where: Co = parameter concentration before treatment,

C = parameter concentration after treatment.

3 Results and discussion

3.1 Identification of exogenous bacterial strains

The bioaugmented substance contains cooperative bacteria, enzymes, a carbon source, salts, and natural emulsifiers. It was imperative to identify the constituting cooperative bacterial consortium to ensure that these bacteria were nonpathogenic and eco-friendly [13]. In the present study, the exogenous bacterial strains were previously identified by Ibrahim et al. [13]. The bacterial strains were identified as *Bacillus subtilis*, *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Pediococcus pentosaceus*.

3.2 Some physicochemical and bacteriological characteristics of the used El-Rahawy drain wastewater

The El-Rahawy drain receives about 1,450,000 m^3/day of primary treated wastewater from the Abu-Rawash wastewater treatment plant (WWTP) and about 450,000 m^3/day of secondary treated wastewater from the Zenen WWTP. The average TOC, DO, TDS, TSS, BOD, and COD concentrations in the Zenen WWTP effluents were recorded to be 1.50, 4.50, 412.0, 30.0, 34.50, and 70.0 mg/L, respectively. As well, the average pH value was recorded to be 7.4. After applying 2.0 mg/L of aluminum chloride at pH 6.14, the average concentrations of TOC, DO, TDS, TSS, BOD, and COD of Abu-Rawash WWTP effluent were recorded to be 5.60, 0.92, 355, 41.50, 57.60, and 106 mg/L, respectively [3]. Table (1) illustrates the minimum, maximum, and average of El-Rahawy drain water. The average pH value was 6.9. Moreover, the average values of TSS, TDS, COD, BOD, TKN, TP, chloride and sulfate were 12.7, 3691.2, 174, 66.4, 24.8, 11.5, 787.6 and 972.8 mg/L, respectively. In separate study, the average concentrations of total suspended solids (TSS), total organic carbon (TOC), dissolved oxygen (DO), biological oxygen demand (BOD), total dissolved solids (TDS), and chemical oxygen demand (COD) in the El- Rahawy drain were 159.25, 9.20, 1.45, 146.70, 720, and 270 mg/L, respectively [19]. Ezzat et al. [20] found low concentration of DO and high concentrations of turbidity, TDS, COD, BOD, ammonia (NH_3), bicarbonate (HCO_3), and total alkalinity at the discharge point of the El-Rahawy drain. Regarding the bacteriological parameter, the average of total coliform, fecal coliform, and *E. coli* were $1.9x10^9$, $6.7x10^7$, and $6.5x10^7$ MPN-Index/100mL, respectively (Table 1). The microbial loads in El-Salam Canal that receives Nile water mixed with El-Serw and Hadous drainage water were determined by Yehia and Sabae [21]. The indicator bacteria values were ranged as 9×10^2 – 1.1×10^5, 9×10^2 – 4.6×10^4 and 350- 1.5×10^4 MPN/ 100mL for total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS), respectively. In another research, Abdel-Satar and Elewa [22] observed high levels of physicochemical parameters in the Rosetta branch at the discharge point of the El-Rahawy drain. In the present study, it can be concluded that a considerable amount of pollutants and microorganisms are discharged directly to the El-Rahawy drain without any treatment, which results increase in pollution levels at the El-Rahawy drain and the Rossita Branch [23].

3.3 Designed system for treatment of El-Rahway drain

In the present study, El-Rahawy drain is suffering from the pollution that comes from Zenin and Abou-Rawash wastewater treatment plants and direct discharges of domestic and agricultural wastewater. From all of these, the biological treatment followed by Fenton reaction could be the simple and cost-effective methods for El-Rahawy drain treatment. Biological augmentation (also means adding selected mixed bacterial strains to wastewater) is a promising technique for degrading the inorganic and organic pollutants and enhancing removal efficiency [24]. Bioaugmentation is a convenient and affordable approach, which could be a useful tool in alleviating this challenge [13]. In this study, although the high ratio of BOD/COD, the AOPs, including the Fenton reaction was proposed to avoid the problems of the broad areas needed when utilizing the biological treatment technologies, which are the cheapest.

In the present study, the pH values were 6.9 in El-Rahawy drain water. Moreover, the pH fluctuated between 6.9 and 7.03 after biological and Fenton treatment processes. According to El-Bestawy [25] who mentioned that there TSS, COD, and BOD parameters in wastewater are the most parameters expressed to contamination level and considered as efficiency indicators for any treatment process. Regarding the total suspended solids (TSS) of the effluent water of El-Rahawy drain was 12.7 mg/L, which declined to 3.2 and 1.1 mg/L using biological treatments after 24 h and 4 days, respectively. TSS was fully removed (100%) after Fenton reaction treatment after 24 h and 4 days (Table 1). In the current experiment, the removal efficiency of TDS reached 58.9% and 68.6 % by biological treatment after 24 h and 4 days. While the removal % of the same parameter reached 75.9% after 24 h of Fenton treatment, and this efficiency removal declined to 66.6% on the fourth day of the treatment (Fig. 2). Concerning to the COD, it is removal efficiency reached 33.3 and 56.8% after 24 h and 4 days of the biological treatment, respectively. Whereas, the removal % reached to 80% after 24 h and this removal % declined to 78.8% after 4 days of Fenton treatment. In the present study, the removal efficiency of BOD reached 30.2 and 52.8% by biological treatment after 24 h and 4 days. While the removal % of the same parameter was reached to 89.4% after 24 h of Fenton treatment also this efficiency removal decreased to 89.1% on the fourth day of the treatment (Table 2). From the obtained results, it can be found that the concentrations of TDS, COD, and BOD increased by the Fenton process after 4 days of treatment; this might be contributed to increasing the concentration of Fe^{2+} in the treated wastewater [26]. It is recommended that using an optimal dose of reagents is very important to meet the desired removal of pollutants for a specific effluent using several experiments [27]. Moreover, in the normal conditions using the dark Fenton, an accumulation of Fe^{3+} and its complication with carboxylate types from the decay of organic matter generally reduces the decay rates of the Fenton reaction [17]. It is recognized that total Kjeldahl nitrogen (TKN) is the whole of [ammonia, organic (*i.e.* Amino acids, humic acids, proteins & urea) and reduced nitrogen] and nitrate. In this study, there was slightly removal efficiency (5.24%) of TKN after biological treatment after 24 h then reached 10.4%

Table 1. Some physicochemical and bacteriological characteristics of the used El-Rahawy drain water

Parameters	Units	Minimum	Maximum	Average
pH	---	6.3	7.3	6.9
TSS	mg/L	12	13.8	12.7
TDS	mg/L	3500	4025	3691.2
COD	mgO_2/L	165	189.7	174
BOD	mgO_2/L	63	72.4	66.4
TKN	mg/L	23.5	27.1	24.8
TP	mg/L	10.9	12.5	11.5
Chloride	mg/L	746.9	858.9	787.6
Sulfate	mg/L	857.2	1006.5	972.8
Total coliform	MPN/100mL	$1.5x10^8$	$2.3x10^9$	$1.5x10^9$
Fecal coliform	MPN/100mL	$1.2x10^7$	$1.4x10^8$	$6.7x10^7$
E. coli	MPN/100mL	$1.1x10^7$	$1.6x10^8$	$6.5x10^7$

Table 2. The average values and removal % of some physicochemical and bacteriological parameters after biological and Fenton reaction treatment after 24 h and 4 days

Parameters	Units	Influent	After 24 h				After 4 days			
			Biological treatment		Fenton reaction		Biological treatment		Fenton reaction	
			Value	R %	Value	R %	Value	R %	Value	R %
pH	---	6.9	7.03	---	6.6	---	6.9	---	6.7	---
TSS	mg/L	12.7	3.2	74.8	0.0	100	1.1	91.3	0.0	100
TDS	mg/L	3691.2	1518.7	58.9	1552.5	75.9	1157.4	68.6	1232	66.6
COD	mgO_2/L	174	116.03	33.3	34.8	80	75.1	56.8	36.8	78.8
BOD	mgO_2/L	66.4	46.4	30.2	7.0	89.4	31.3	52.8	7.2	89.1
TKN	mg/L	24.8	23.5	5.24	2.5	89.9	22.2	10.4	2.4	90.3
TP	mg/L	11.5	9.3	19.1	1.4	87.8	3.6	68.6	1.03	91
Chloride	mg/L	787.6	490.2	37.8	352.8	55.2	343.8	56.3	298.3	62.1
Sulfate	mg/L	972.8	378.6	58.9	382.5	60.6	206.5	78.8	219.3	77.4
Total coliform	MPN /100mL	$1.5x10^9$	$5.5x10^5$	99.96	0.0	100	$1.2x10^4$	99.99	0.0	100
Fecal coliform	MPN /100mL	$6.7x10^7$	$6.0x10^3$	99.99	0.0	100	$2.9x10^2$	99.99	0.0	100
E. coli	MPN /100mL	$6.5x10^7$	$3.1x10^2$	99.99	0.0	100	$2.0x10^2$	99.99	0.0	100

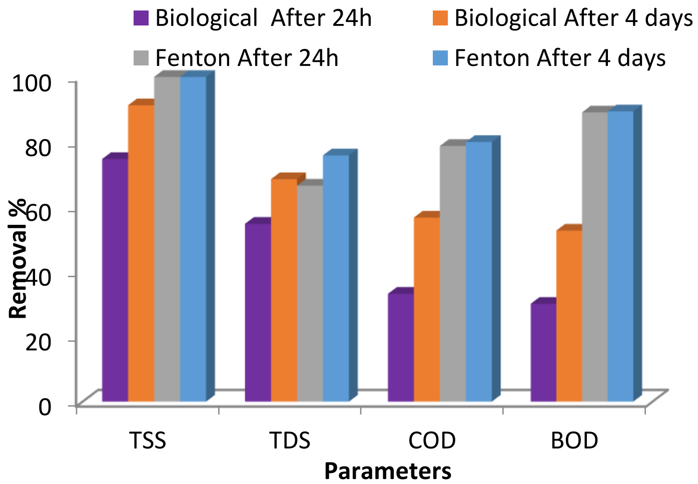

Fig 2. Removal % of TSS, TDS, COD and BOD parameters after biological and Fenton treatment processes

after 4 days of the biological treatment. Regarding the Fenton process, TKN reached to 89.4% removal after 24 h, and this percentage increased to 90.3% after four treatment days. Rocher et al. [28] found that 97% of ammonia (NH_3) was oxidized through the biofiltration process. Additionally, after sedimentation, the removal efficiency of organic pollutants reached between 80 and 90%, and suspended solids values were low (25 mg/L).

The removal efficiency of total phosphorus (TP), chloride, and sulfate are enhanced by biological treatment followed by the Fenton process after 24 h followed by 4 days treatment in the desigened system (Table 2). In this study, the efficient removal of some physicochemical and bacteriological parameters in El-Rahawy drain water is increased by biological treatment after 4 days in comparing to after 24 h, it may be due to the added bioaugmented bacterial strains are grown well and produced more much biodegrading enzymes than that after 24 h. The removal efficiency of organic and inorganic pollutants from wastewater by biological treatment is enhanced by the addition of exogenous bacterial consortia, while the increased enzymes produced accelerate the biodegradation process. These enzymatic reactions play an essential role in the wastewater treatment process through the bioaugmentation of the complex pollutants and converting them into pure, nontoxic compounds [29].

Concerning the bacteriological parameters, the counts of total and fecal coliforms and *E. coli* in the influent were $1.5x10^9$, $6.9x10^7$, and $6.5x10^7$ MPN/100 mL, respectively (Table 2). The removal efficiency of total and fecal coliforms and *E. coli* after biological treatment after 24h was 99.96, 99.99, and 99.99%, respectively, while the removal efficiency of total and fecal coliforms and *E. coli* was reached to 99.99% after biological treatment after 4 days. Complete removal (100%) after treatment by Fenton reaction was observed for total and fecal coliforms and *E. coli* (Table 2). In this study, bioaugmentation with a cooperative bacterial consortium exhibited an extraordinary ability for total and fecal coliforms and *E. coli* removal. This bioaugmented substance contained lactic acid bacteria that were antagonistic against other microorganisms, including enteric pathogens [30]. In previous studies, the removal of total and fecal coliforms from wastewater in batch reactors by the effect of the bioaugmented bacteria mixture was confirmed by comparing the removal efficiency of coliforms with negative control reactors (no augmented bacteria were added). They found that coliform counts in control increased between 120% after 24 h and 800% after 7 days, and these studies attributed this increase to the absence of augmented bacteria that could have antagonistic effects against the coliforms group [31, 32]. The water quality standards specified in Egyptian law 48/1982 for COD, BOD, TDS, DO, turbidity, and TSS are as following, ≤30 mg/L, ≤20 mg/L, ≤500 mg/L, ≥5 mg/L, ≤50 mg/L, and ≤20 mg/L, respectively [33, 34]. In this study, the effluent water quality after biological treatment and Fenton reaction do not meet the applicable water quality standards specified in Egyptian law 48/1982 in concerning to COD, BOD, and TDS. Likewise, the TSS and fecal coliform the effluent water quality meets law 48/1982 (Table 2).

4 Conclusion

El-Rahawy drain receives a considerable volume of polluted water that comes from wastewater treatment plants, domestic and agricultural. Biological augmentation technology using adding bacterial strains consortium for drainage wastewater is active and cost-effective for the degradation of organic and inorganic pollutants. Fenton reaction is promising technique, costly and does not require space for the treatment of the El-Rahawy drain. Biological treatment followed Fenton reaction leads to improve the quality of treated effluent, reduce some physicochemical and bacteriological parameters. From the obtained results, it can be found that the level of some physic-chemical and fecal coliform items has been decreased in treated effluent and become within the limit of Law 48 for wastewater quality for reuse in agricultural purposes.

5 Conflicts of interest

There are no conflicts to declare

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