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Treatment of Gas Refinery Wastewater

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ABSTRACT

This study aims to design a laboratory reactor that is suitable for producing organic fertilizer from gases generated by anaerobic bacteria in refinery wastewater, making it applicable in agriculture. The activated sludge reactor designed comprises three main components: primary sedimentation, aeration, and secondary sedimentation along with a sludge return system. It features a glass cubic tank with a capacity of 5000 ml and a sedimentation basin of the same volume. The research examined the impacts of ammonium, phosphorus, and methanol concentrations, inoculation rate, hydrodynamic retention time, and fermentation duration. Wastewater treatment experiments were carried out over a span of 14 days. The findings indicated that biological treatment of the wastewater resulted in a reduction of biochemical oxygen demand (BOD) by 71.27% and total organic carbon (TOC) by 48.98%. Additionally, total dissolved solids (TDS) decreased by 60.96%, and the electrical conductivity (EC) of the water was reduced by 42.6% during this period. Over time, the concentrations of H₂S, NH₃, CH₄, and CO₂ gases in the wastewater decreased by 30.08%, 25.60%, 42.71%, and 95.80%, respectively. The sludge that settled in the wastewater treatment ponds is rich in nutrients and can be transformed into compost fertilizer under specific conditions, making it highly beneficial for agricultural use.

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1. Introduction

Refinery wastewaters consist of complex mixtures of organic and inorganic compounds that present significant environmental challenges [1]. These effluents contain a variety of hydrocarbons, oils, and toxic substances, making their treatment a technological hurdle [2-4]. With the growing global demand for oil, the production of refinery wastewater is expected to increase, underscoring the need for effective treatment solutions [3]. Although several

physical, chemical, and biological methods are utilized for treatment, biological processes are recognized as environmentally friendly and cost-effective options [1, 5]. Recent innovations include the concept of wastewater refineries, which focus on recovering valuable resources such as water, energy, nitrogen, and phosphorus from wastewater, potentially transforming treatment from a cost burden into a profitable opportunity [6, 7]. Nevertheless, no single process can meet all treatment requirements, emphasizing the necessity for ongoing research and development in this area [1].

A variety of methods have been established to eliminate contaminants and recycle wastewater. These methods encompass physical, chemical, biological, and electrochemical processes [8]. Wen et al. [9] conducted a study on biological fertilizers utilizing carbon dioxide gas. They focus on producing biological fertilizers that leverage nitrogen-fixing bacteria, primarily found in plant roots. This type of biological fertilizer is mainly derived from sugars and other plant materials rich in sugar, but its production cost is relatively high. Additionally, biological fertilizers face challenges such as sensitivity to dryness and elevated ambient temperatures, which limits their effectiveness based on climate and cultivation type. In contrast to traditional biological fertilizers, those based on methanotrophic bacteria have the advantage of extracting salts from the soil, thereby enhancing soil fertility. They also demonstrate resilience to environmental conditions like drought and high temperatures, making them increasingly valuable for improving soil health.

Kumar et al. [10] combined biological fertilizers with chemical fertilizers to enhance soil properties for corn cultivation. Their findings indicated that the application of biological fertilizers led to an increase in organic matter as well as higher levels of nitrogen, phosphorus, potassium, and micronutrients in the soil. Additionally, Naeimi et al. [11] examined both the quantity and quality of treated effluents from various sewage treatment plants across different regions in Iran. They explored the potential for using these effluents to irrigate green spaces and parks, concluding that, in accordance with World Health Organization guidelines and Environmental Protection Organization regulations, treated wastewater can be utilized for irrigation purposes. In developing countries, land application of sewage has long been a common practice for disposing of urban wastewater while also supplying water for agricultural needs.

Alishiri et al. [12] investigated the biological treatment of Tabriz petrochemical wastewater, which. The findings revealed that the nitrogen factor had a significant effect on the removal of biological oxygen demand (BOD), accounting for 73.4% of the impact. The addition of folic acid, nitrogen, and phosphorus to Tabriz petrochemical wastewater, which is deficient in these nutrients, not only promoted the growth of these microorganisms but also enhanced other parameters of the effluent.

Fazal et al. [13] explored the treatment of industrial wastewater through the membrane bioreactor (MBR) method. The MBR wastewater treatment process is an integrated system that combines biological treatment (activated sludge) with a submerged membrane system. By uniting sedimentation (clarification), aeration, and filtration units within a single reactor, this method replaces traditional wastewater treatment processes (conventional activated sludge), resulting in a straightforward and efficient system that lowers initial investment costs and reduces operating expenses. This approach substitutes the gravity sedimentation unit with a membrane separation system, yielding numerous benefits, including operational stability, decreased excess sludge production, and significantly enhanced quality of the treated effluent. Therefore, this system is well-suited for a variety of applications in the reuse of treated wastewater for both urban and industrial wastewater treatment [14].

The activated sludge process is a biological treatment method that utilizes the activity of microorganisms to enhance the decomposition rate of organic substances in wastewater. The term "activated sludge" refers to the return of the biological suspension, which contains a mass of microorganisms, that participates in the removal of dissolved organic substances in the aeration basin. When incoming wastewater comes into contact with this sludge, the growth and proliferation of microorganisms lead to the removal of larger quantities of sewage waste, resulting in relatively purified wastewater. Activated sludge is composed of sludge particles generated from raw or settled wastewater during primary treatment (by microorganisms) in aeration tanks, where there is an adequate supply of dissolved oxygen [15].

Ezz et al. [16] created an algae-bacterial granular sludge/digestion/gas decomposition system aimed at effectively managing petrochemical wastewater. They employed the algae-bacterial granular sludge (ABGS) technique to treat petrochemical wastewater contaminated with monoethylene glycol (MEG). The bioreactor containing ABGS demonstrated superior performance compared to the bacterial granular sludge as the MEG loading rate increased, showcasing impressive recovery efficiency.

The application of biological treatment utilizing methanotrophic bacteria is relatively new on a global scale. These microorganisms possess the remarkable ability to withstand harsh environmental conditions, including high temperatures and dryness. They can convert methane into methanol, produce protozoan proteins, consume atmospheric methane, mitigate greenhouse effects, and decompose toxic chemicals [17]. Methanotrophic bacteria are a category of methylotrophs capable of utilizing methane as their sole source of energy and carbon. These methanotrophs are classified into three types: X, II, and I.

Methanotrophs oxidize methane to produce methanol and formaldehyde. Subsequently, formaldehyde is incorporated into the biomass via one of two pathways: the ribulose monophosphate pathway or the serine pathway.

Drip filter systems typically consist of garbage collection, granulation, primary sedimentation, trickle filtration, secondary sedimentation, a disinfection system, and a sludge treatment system, along with components for final disposal. Once the sewage has settled, bacteria begin to break down the settled solids, converting them into biological fertilizer. The aim of this research is to design a laboratory reactor that can convert polluting gases into biofertilizers using methanotrophic bacteria, thereby facilitating wastewater treatment. Alongside the decomposition of organic matter, the study also explores the reduction and removal of gaseous pollutants from refinery effluents. This approach can significantly decrease the pollution load in various types of wastewater.

2. Materials and methods

This research is quantitative, cross-sectional, and analytical, aimed at examining the reduction of pollutants and excess sludge. The objectives were explored using a two-reactor system with active sludge.

The wastewater treatment process utilizes a trickle filter. Initially, the incoming wastewater undergoes primary sedimentation. To facilitate its entry onto the flat beds, the wastewater is channeled through a distribution system that disperses it across plastic flat beds. As the wastewater flows downward through the filter bed, bacteria attached to the surface begin to break down the organic matter. The growth of this biofilm on the filter bed continues as more wastewater enters the system.

Once the biofilm reaches a critical thickness, the outer layers start to peel off and are carried away through the lower drainage system. The sewage and solid materials collected via this drainage are then transferred to a secondary sedimentation tank, where solids are separated from the wastewater (Fig. 1).

In practice, a portion of the treated wastewater is typically recycled back to the trickling filter. This recirculation helps dilute the incoming wastewater and enhances the quality of the final effluent. Implementing this recirculation process leads to a more balanced hydraulic load, improved distribution of wastewater across the bed, reduced clogging, and increased treatment efficiency.

After the wastewater settles, bacteria begin to convert the solids into a stable organic fertilizer that is rich in nitrogen, phosphorus, and potassium. Temperature, pH, and oxygen in the environment can have a great impact on bacterial activity and the biofertilizer production process.

Typically, optimal growth occurs within a specific range of temperature and pH levels. Research indicates that bacterial growth is significantly influenced by pH, with optimal conditions generally falling between 6.5 and 8. Studies have shown that different bacterial strains exhibit specific pH preferences, often related to their natural environment [18]. For instance, *Escherichia coli* demonstrated the fastest generation time at pH 6.8 in Eosin Methylene Blue Agar media [19]. In limnic systems, bacterial growth rates displayed a parabolic relationship with pH, peaking around neutral conditions (pH 6-8) [20]. Interestingly, bacteria can adapt to their environment, with soil bacteria showing optimal growth at pH levels close to their native soil pH [21]. The pH of growth media can change during bacterial cultivation, often converging to a strain-specific value, and the evolution of pH depends on the carbon source used [22].

The experiments were designed using a method that focuses on one variable at each stage, allowing for the separate measurement of how different concentrations of each variable affect wastewater treatment efficiency. Optimization tests were conducted after 14 days of device operation. Following this period, laboratory measurements of BOD, chemical oxygen demand (COD), and microbial concentration were taken (Table 1). The variables and their respective concentration levels are as follows:

- i. Inoculum concentration: 0.05, 0.1, 0.15, and 0.2 g.L⁻¹
- ii. Ammonium sulfate concentration: 0.1, 0.2, 0.3, and 0.5 g.L⁻¹
- iii. Sodium phosphate concentration: 0.1, 0.2, 0.3, and 0.5 g.L⁻¹

Table 1. List of tests of the outgoing effluent [23-25]

Testing measurement	The method of measuring	Measuring device
BOD	Biotest	-
COD	Titration	-
TOC	decomposition and burning	Furnace
TDS	Preparation of solution	Oven
pH	Preparation of solution	pH meter
EC	Electron exchange	Conductivity meter
Fe²⁺	photometry	spectrophotometer
Sulfate	photometry	spectrophotometer
Sulfite	Iodometry	-
Hydrogen sulfur	photometry	spectrophotometer
Ammonia	photometry	spectrophotometer
Methane	FID detector	Gas chromatography VARIAN CP-3800
Carbon dioxide	Titration	-

The TOC and TDS are Total Organic Carbon and Total dissolved solids, respectively.

The experiments were structured using a one-variable-at-a-time approach, allowing for the separate measurement of how varying concentrations of each variable impact wastewater treatment, specifically through BOD and COD measurements. Optimization tests were conducted after 14 days of device operation.

2.1. Determination of COD and BOD

The standard potassium dichromate method was utilized for determining COD. In this experiment, 20 ml of wastewater was first added to an Erlenmeyer flask, followed by the addition of 0.4 g of mercury sulfate and 10 ml of potassium dichromate solution. The COD value of the wastewater sample was then determined based on the volume of ferrous ammonium sulfate solution used for titration, facilitated by the Freon reagent. For BOD₅ measurement, the conventional manometric method was employed. A WTW model BOD OxiTop IS6 measuring device, equipped with a pressure sensor, was used in a biochemical incubator at 20°C for 5 days, utilizing simulated wastewater with a measurement range of up to 4 g.L⁻¹.

In this study, the aeration time is calculated based on the retention time. BOD (g.m⁻³) is typically measured from the liquid mixture in the aeration pond over time. The F/M ratio represents the amount of food to microorganisms in grams of BOD per gram of mixed liquor suspended solids (MLSS) per day, while θ denotes the sludge age in days. These parameters are determined as follows [26]:

$$\text{BOD} = \frac{\text{wastewater sample after settling}}{\text{MLSS}} \quad (1)$$

$$\frac{F}{M} = \frac{Q \times \text{BOD}}{V \times \text{MLSS}} \quad (2)$$

$$\theta = \frac{\text{MLSS} \times V}{\text{SS}_o \cdot Q_o + \text{SS}_i \cdot Q_i} \quad (3)$$

where Q (m³.day⁻¹) and V (m³) are amount of sewage flow, and volume of liquid mixture in the aeration pond, respectively.

2.2. Activated sludge reactor structure

The activated sludge reactor features a fixed bed, consisting of a glass cubic tank with a volume of 5 L, where raw sewage is stored. From this tank, the wastewater is transferred via an injection pump to two rectangular plastic cubic tanks, each also with a volume of 5L, which serve as the substrate for bacterial growth.

The incoming wastewater is injected into the reactor by a flow injection pump with a capacity of 1 L.h⁻¹. Aeration is provided by an aquarium air pump with a maximum capacity of 13 L.min⁻¹. The required air supply is adjusted based on the organic load entering the reactor, ranging from 2.5 to 12.5 L.min⁻¹. Mixing in this system is facilitated by the incoming air flow (Fig. 1(b)), and the purified wastewater is ultimately stored in a cubic tank with a volume of 4 L. The entire setup operates at room temperature (20-25°C). Fig. 1 illustrate the discontinuous activated sludge reactor used in this study, along with its associated accessories.

2.3. Bacterial culture and preparation of microbial suspension

In this study, methanotrophic bacteria are used to investigate their ability in biological treatment. The bacteria utilized were sourced from the Pasteur Institute in Tehran. To create the inoculation liquid, a linear bacterial culture is established on Mueller-Hinton agar. In a completely sterile environment, a single colony is selected from the bacterial

culture plate and transferred into 4 ml tubes of Mueller-Hinton broth [27]. The tubes are incubated at 37°C for 4 hours to facilitate bacterial growth. After this period, the turbidity of the bacterial medium is assessed using the McFarland 0.5 standard, which corresponds to a concentration of 1.5×10^8 bacterial colony units per ml [28]. Once the turbidity is adjusted to match the McFarland 0.5 standard, it is used as the inoculating liquid at concentrations of 0.1, 0.15, and 0.2 g.l⁻¹ to proceed with the process.

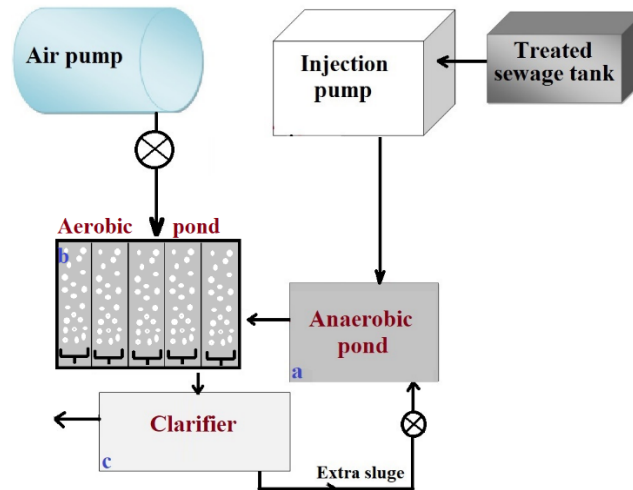


Fig. 1. The structure of the research discontinuous activated sludge reactor

2.4. Coagulants and their mechanism

Coagulation-flocculation is a commonly employed method in wastewater treatment aimed at eliminating suspended particles, colloids, and dissolved pollutants [29]. This process entails the addition of coagulants such as ferric chloride or alum to neutralize charges, followed by flocculation to create larger aggregates [29, 30]. Numerous coagulants, flocculants, and adsorbents have been examined for their efficacy in wastewater treatment. For example, combining bentonite clay with Extradoc P70 has been shown to produce large aggregates containing pollutants, significantly enhancing sedimentation rates and the removal efficiency of resinous substances in phenolic wastewater [31]. The size and settling velocity of the resulting flocs can significantly influence treatment effectiveness. In a dynamic separator, coagulation-flocculation has achieved a removal rate of 95-99% for suspended solids at optimal flow rates [32]. This method is effective in eliminating suspended, colloidal, and certain soluble fractions from wastewater [33].

2.5. Coagulation mechanism

Alum, with the chemical formula $\text{Al}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O}$, is a consumable coagulant. When added to water, it reacts with alkalinity to produce Al^{3+} ions. These Al^{3+} ions neutralize the negative charges of colloidal particles, leading to their coagulation. This mechanism can be summarized in four steps: a) Condensation of the electric double layer, b) Surface adsorption and charge neutralization, c) Trapping of particles in sediment and d) Surface attraction, forming a chemical bridge between particles. To determine the optimal pH and coagulant dosage, two methods have been considered: 1) Zeta potential control, as proposed by Riddick, where the coagulant is used to achieve a zero-zeta potential, and 2) The jar test, in which varying pH levels and coagulant amounts are tested to establish optimal condition.

To effectively manage a treatment plant and monitor the key factors influencing activated sludge treatment, it's crucial to focus on important indicators rather than just pollution intensity parameters. Factors such as nutrients, dissolved oxygen, retention time, pH, toxicity, temperature, mixing, and hydraulic flow should be thoroughly examined.

As a result, the following tests should be conducted and measured daily in the activated sludge system [34]: Inlet sewage flow (Q_i) and outlet flow (Q_o) in m³/day, Temperature of air and sewage, Sludge volume index (SVI), Return flow of sludge and excess sludge, Visual assessment of the aeration and sedimentation pond (including color, transparency, effluent quality, foam presence, turbulence, odor, etc.), Measurement of BOD and COD for the inlet, outlet, and aeration tank, pH levels for the inlet, outlet, and aeration tank, Concentration of suspended solids in the liquid mixture ($MLSS$) in mg.L-1, Dissolved oxygen (DO) levels, Thirty-minute sedimentation test, Levels of nitrogen and phosphorus compounds, Sludge age (θ) and its coverage depth in days, Concentration of suspended solids in excess sludge (SS_i) and in the effluent stream (SS_o) in mg.L-1, Microscopic examinations, Consumption of water, electricity, and fuels.

Some common issues that may occur in the activated sludge system include sludge bulking, foam formation in the aeration basin, odor and noise generation, sludge rising, and low BOD removal. Each of these problems can be influenced by factors such as pH, temperature, food-to-microorganism ratio (F/M), and others.

Sludge age, also known as cell retention time, is a performance index that relates to the food-to-microorganism ratio. While the retention time of the liquid mixture (aeration time) in the aeration pond typically ranges from 3 to 30 hours, the duration of biological solids in the system is significantly longer, lasting several days. When sampling wastewater containing suspended solids in a suspension form, it is crucial to ensure that the collected sample accurately represents the actual concentration of these materials at the time of sampling. After thoroughly mixing the effluent, the sampling device should be inserted into the effluent stream at the top, middle, and bottom, and filled accordingly. The sample should be withdrawn slowly to avoid the introduction of air bubbles.

All samples are collected using sterile equipment and placed in sterile sampling containers. For effluent sampling, a sterile syringe is utilized, and samples are taken from each of the three designated areas to ensure accuracy. A total of approximately 42 samples were collected in sterilization containers. Following collection, the samples are promptly transported to the laboratory for enrichment and isolation testing. Table 2 presents the specifications of the prepared wastewater along with the physicochemical characteristics studied.

Table 2. Characteristics of the effluent entering the activated sludge reactor

Measurement parameter		Measurement parameter	
pH	8.7	EC ($\mu\text{s.cm}^{-1}$)	8200
COD (ppm)	640	TDS (ppm)	7260
BOD (ppm)	275	H ₂ S(ppm)	0.79
VS ¹	906	TKN ² (ppm)	339
Alkalinity (ppm) as CaCO ₃	1195.9	TVFAs ³ (ppm) as Acetate	29

All data correspond to the average value from three replicates (n = 4). ¹Volatile solid, ²Total Kjeldahl nitrogen, and ³Total volatile fatty acids.

3. Results and discussion

The wastewater treatment process includes various stages such as pre-treatment, biological treatment, and chemical treatment. Each of these stages plays an important role in removing pollutants and returning water to the natural cycle. In this study, data related to wastewater treatment will be analyzed to evaluate the performance of treatment systems, the effectiveness of the methods used, and their environmental impacts. This information can contribute to improving treatment processes and developing innovative solutions in refinery wastewater management.

3.1. The inoculation levels

The findings regarding the impact of varying inoculation levels on cell proliferation in wastewater are summarized in Table 3. The results indicate that as the amount of microorganism inoculation increases, the cell concentration in the wastewater also rises. Specifically, an inoculation rate of 0.2 g of dry cells per liter of wastewater resulted in the highest cell population, reaching 4.2 g of dry cells per liter. Notably, the cell population does not exceed this level under any conditions, making 0.2 g of dry cells per liter the optimal economic inoculation amount. This is because, with an increase in cell population, the optimal growth conditions for the microorganisms diminish, causing the cell concentration to lose its upward trend.

Table 3. The effect of changes in the amount of inoculation on cell proliferation

Inoculum size (g.L ⁻¹)	Final biomass concentration (g.L ⁻¹)
0.1	2.7
0.15	3.8
0.20	4.2

3.2. Ammonium concentration

The results of the investigation into the impact of nitrogen source concentration on wastewater treatment are presented in Table 4, as referenced in Table 2. Ammonium sulfate is crucial in several wastewater treatment processes. However, elevated sulfate levels in wastewater can negatively impact anaerobic-oxic (AO) treatment systems, hindering the degradation of COD and ammonia nitrogen [35]. On a positive note, ammonium sulfate can improve sludge dewater ability by decreasing capillary suction time and specific resistance to filtration. It accomplishes this by breaking down cell membranes, transforming bound water into free water, and precipitating proteins. As observed, increasing the concentration of ammonium sulfate up to 0.3 g.L⁻¹ results in a decreasing trend in the amount of oxygen required for microbial and chemical decomposition. This indicates the economic range for the consumption of this substance in the removal of sewage pollution using this method.

Table 4. The effect of changes in nitrogen source concentration on the amount of wastewater treatment

C _{Ammonium sulfate} (g.L ⁻¹)	BOD (ppm)	COD (ppm)
0.1	58	102
0.2	45	93
0.3	37	77
0.5	39	82

3.3. Phosphate concentration

The biological removal of phosphorus in wastewater treatment is affected by several factors. One key factor is biomass concentration, with optimal removal occurring at 0.2 mg.L⁻¹ (see Table 5) which is consistent with the results of Sidat et al. [36]. The initial concentration of total phosphorus and the dose of active sludge significantly influence removal efficiency through processes such as assimilation, hydrolysis, and adsorption [37]. Additionally, dissolved oxygen levels affect microbial growth, with higher concentrations resulting in increased biomass production [38]. To optimize phosphorus removal economically and environmentally, it's essential to analyze the relationship between chemical dosing and phosphorus discharge [39]. These studies underscore the complexity of phosphorus removal in wastewater treatment, highlighting the need to consider various factors, including biomass concentration, initial

phosphorus levels, dissolved oxygen, and chemical dosing, to enhance the process for both economic and environmental advantages.

Table 5. The effect of changes in phosphorus source concentration on the amount of wastewater treatment

C Sodium phosphate (g.L ⁻¹)	BOD (ppm)	COD (ppm)
0.1	44	78
0.2	29	53
0.3	36	66
0.5	41	72

3.4. Methanol concentration

The findings regarding the impact of methanol concentration as a growth stimulant for microorganisms on wastewater treatment are shown in Table 6. It is evident that increasing methanol concentration does not lead to significant changes in the values of two parameters, BOD and COD. Consequently, methanol concentration appears to have minimal effect on wastewater treatment.

Table 6. The effect of changes in methanol concentration as a stimulus for the growth of microorganisms on the amount of wastewater treatment

C Methanol (g.L ⁻¹)	BOD (ppm)	COD (ppm)
0.1	43	63
0.2	42	62
0.3	41	60
0.5	39	59

3.5. pH

The pH level of the culture medium significantly influences the growth of methanotrophic bacteria. For the majority of these bacteria, optimal pH ranges from 5 to 7, while extreme pH values can reduce efficiency. At the ideal pH, the specific growth rate reaches its peak. Typically, pH is adjusted using soda and ammonium sulfate. However, to provide a nitrogen source and neutralize acidic metabolites, the presence of ammonia ions in the reaction medium is essential. As shown in Table 7, pH levels during wastewater treatment have been decreasing.

Table 7. pH changes during wastewater treatment

	Initial amount	7 days	14 days
pH	8.8	7.9	6.8

3.6. The iron, sulfate and sulfide ions concentration

The composition of the culture medium components significantly affects both the quality and quantity of methanotrophic bacteria produced. The suitability of the cultivation environment is crucial for the growth rate, efficiency, and stability of these bacteria. High levels of soluble salts, including sulfate and sulfide, along with certain amounts of iron in water, contribute to hardness and pose challenges for water usage, especially in industrial applications.

Table 8. Changes of iron, sulfate and sulfide ions during wastewater treatment

	Initial amount	7 days	14 days
Fe ²⁺ (ppm)	8.80	7.9	6.8
SO ₃ ²⁻ (ppm)	7.80	6.5	5.6
SO ₄ ²⁻ (ppm)	3.73	2.9	1.4

Over the 14 days of biological wastewater treatment, the levels of iron, sulfate, and sulfide showed a decreasing trend, with reductions of 58.09%, 28.20%, and 62.46%, respectively.

3.7. Methanotrophic bacteria function

The biochemical efficiency of the purification system is influenced not only by the quantity of bacteria present but also by the physical condition of the bacterial mass. A reduction in the percentage of organic nitrogen alters the physical properties of the flocs, causing them to become denser and decreasing the contact surface area between the bacteria and their environment. A key indicator for assessing sludge characteristics is the sludge volumetric index, which measures the volume occupied by one gram of sludge (based on dry matter) after it has settled for 30 minutes in a laboratory sedimentation container.

Table 9 presents an analysis of various sludge parameters over a 14-day period. Activated sludge systems are vulnerable to operational disturbances that can impact floc structure and the content of filamentous bacteria. Conditions such as low dissolved oxygen levels ($<2.0 \text{ mg.L}^{-1}$) and extended sludge age (14 days) encourage the proliferation of filamentous bacteria, resulting in sludge bulking. The growth of filaments occurs when environmental conditions favor their development over zoogloal microorganisms, with dissolved oxygen levels within the floc often being a limiting factor. The properties of sludge, including its settling characteristics, residual turbidity, and suspended solids content, are affected by the structure of the flocs and the length of the filaments. A filament length of $107 \mu\text{m.ml}^{-1}$ is used to differentiate between bulking and non-bulking sludge. Strategies to control bulking include ensuring sufficient dissolved oxygen levels and utilizing aerobic selectors with short hydraulic residence times [42]. Additionally, image analysis and chemometric techniques are emerging as effective tools for monitoring activated sludge systems and identifying operational challenges [43].

COD represents the amount of oxygen needed for the chemical breakdown of organic materials in wastewater, and this value is lowered through wastewater treatment. As shown in the results (Fig. 2), over the 14 days of wastewater treatment, the COD parameter exhibited a downward trend, decreasing by 77.18%.

Table 9. The values and operating parameters for aerobic processes at different times

Day	BOD (ppm)	MLSS (mg.L^{-1})	F/M (mg.L^{-1})	Q/day	Aeration time (h)	Return percentage %
Initial amount	640	0.46	110.04	6	15	75
7	339	0.25	59.83	8	15	72
14	146	0.12	28.72	10	15	69

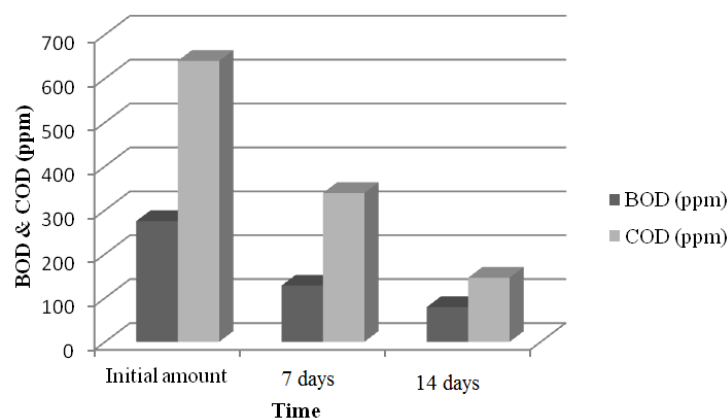


Fig. 2. BOD and COD parameters during wastewater treatment

To determine the height of the sludge bed, a sampling method is employed using bubble pumps. The ideal thickness of the sludge bed in secondary sedimentation typically ranges from 0.4 to 0.8 cm. To maintain the balance of nutrients for microorganisms and the average cell lifespan, surplus active sludge produced throughout the day should be removed. The most common approach for this is to discharge sludge from the return sludge system, as it requires both sludge excretion pumps and provides better sludge management. The discharged sludge is directed into condenser for consolidation before being sent to the initial settling tanks. The resulting mixed is then transferred to the primary settlement pond for further mixing and settling.

Total organic carbon is a reliable indicator for estimating organic matter content in various environmental contexts (Fig. 3). TOC has been found to be a potential index for organic content in effluents, showing good correlation with COD measurements [44]. Research on total organic carbon (TOC) trends in aquatic environments shows mixed results. Factors affecting effluent TOC concentrations include biological treatment processes, influent composition, and sewer separation rates, particularly in certain discharge zones [45]. In this study, the TOC parameter shows a decreasing trend from 1187 to 581 ppm over the course of 14 days of wastewater treatment, and this reduction of 48.98% can be attributed to the chemistry of liquid aerosol water [46].

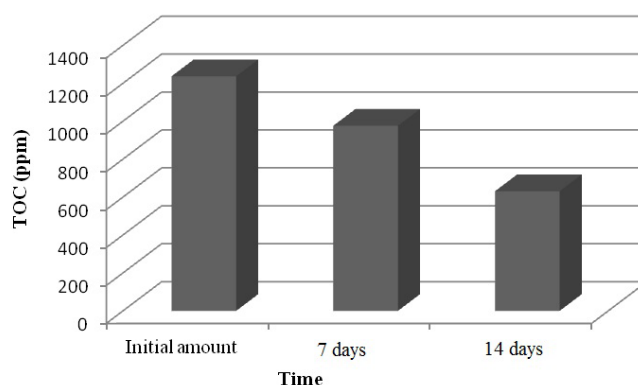


Fig. 3. TOC parameter during wastewater treatment

Total Dissolved Solids, which indicates the quantity of dry residues, has a significant impact on the movement, chemical transformation, and ionization of materials. It is essential for evaluating the suitability of water for human and livestock consumption, as well as for agricultural and industrial applications. Electrical Conductivity of water indicates the presence of conductive solutes. Given the direct correlation between electrical conductivity, TDS, and dissolved salts in water, measuring EC is crucial for monitoring wastewater quality.

The relationship between TDS and EC is a vital component of water quality assessment. The TDS/EC ratio is affected by factors such as salinity content and material composition [47]. The findings from this study reveal a linear relationship between TDS and EC, both showing a decreasing trend (Table 10). Specifically, their values decreased by 60.96% and 48.58%, respectively. It's important to note that the relationship between Total Dissolved Solids (TDS) and Electrical Conductivity (EC) in water is complex and not always linear.

Table 10. TDS and EC parameters during wastewater treatment

Measurement parameter	Initial amount	7 days	14 days
TDS (ppm)	7360	4641	2873
EC ($\mu\text{s}\cdot\text{cm}^{-1}$)	8200	5841	4216

However, for highly concentrated solutions with EC values below 85,000 $\mu\text{S}/\text{cm}$, a linear relationship is more accurate [48]. In such cases, a simple linear equation ($\text{TDS} = k_e \times \text{EC}$) is often utilized.

The composition of the culture medium components significantly affects both the quality and quantity of methanotrophic bacteria produced. The suitability of the cultivation environment is crucial for the growth rate, efficiency, and stability of these bacteria. High levels of soluble salts, including sulfate and sulfide, along with certain amounts of iron in water, contribute to hardness and pose challenges for water usage, especially in industrial applications. Over the 14 days of biological wastewater treatment, the levels of iron, sulfate, and sulfide showed a decreasing trend, with reductions of 58.09%, 28.20%, and 62.46%, respectively.

The trend of decreasing levels of iron, sulfate, and sulfide during biological wastewater treatment can be attributed to several factors. Methanotrophic bacteria play a crucial role in converting sulfate to sulfide, which subsequently precipitates with iron to form iron sulfide compounds. This process effectively removes both iron and sulfide from wastewater. The formation of biological iron sulfide composites occurs in distinct phases. Additionally, the dosage of iron in wastewater can enhance the removal of sulfide [49].

Table 11. Examination of exhaust gases during wastewater treatment

Measurement parameter	Initial amount	7 days	14 days
H₂S (ppm)	25.0	20.8	17.3
NH₃ (ppm)	7.8	4.6	3.1
CH₄ (ppm)	4.9	3.2	1.4
CO₂ (ppm)	4.2	2.7	0.8

Methanotrophic microorganisms utilize gaseous substrates dissolved in liquid, making gas-liquid mass transfer a critical factor in their metabolism. The low solubility of gases like methane and hydrogen can lead to significant transfer limitations and overconcentration in anaerobic processes. These limitations present challenges for the large-scale production of value-added products from methanotrophs, highlighting the need for efficient reactor designs to address gas-liquid mass transfer issues [50].

Improvements in bioreactor design are essential for enhancing solid-gas mass transfer and tackling the poor solubility of methane [51]. Research has demonstrated that uncoupling gas-liquid and liquid-microbe resistances can shift the limiting resistance from the gas-liquid interface to the liquid-microbial interface, which in turn affects the apparent half-saturation value. Understanding these mass transfer dynamics is crucial for optimizing biochemical reactor design and evaluation, especially in methanotrophic systems that utilize gaseous substrates.

4. Conclusion

In microalgae-bacteria systems, longer hydraulic retention times (6–10 days) lead to improved organic matter and nutrient removal, as well as better biomass settling [52]. Methanotrophs play crucial roles in environmental methane oxidation and act as biofilters in anaerobic environments. These bacteria show promise in the bioremediation of heavy metals and organic pollutants due to their broad-spectrum methane monooxygenase enzymes [53]. Methanotrophs can be applied in field-scale methane mitigation technologies and wastewater treatment systems for nitrogen removal [54]. They are also effective in transforming various hydrocarbons, including aromatic compounds and halogenated aliphatic, making them valuable for bioremediation in methane-containing environments. As anthropogenic methane emissions increase, the importance of methanotrophs as a global methane sink is expected to grow.

According to the results, the maximum biofertilizer bacterial biomass of 4.2 g.L⁻¹ was obtained at a hydraulic retention time of 1.6 minutes, with an inoculum size of 0.2 g.L⁻¹. Based on the results of sludge conversion into biofertilizer, the amounts of iron, sulfate and sulfide showed a decreasing trend of 58.09, 28.20 and 62.46% respectively during two weeks of biological treatment, which resulted in a decrease of 42.6% in electrical conductivity. Based on the results of sludge conversion into biofertilizer, the amounts of iron, sulfate, and sulfide exhibited decreasing trends of 58.09%, 28.20%, and 62.46%, respectively, during the two weeks of biological treatment. This reduction contributed to a 49.14% decrease in electrical conductivity. The parameters BOD, COD, and TOC also showed decreasing trends. Total dissolved solids (TDS) significantly affect the movement, chemical transformation, and ionization of substances, playing a crucial role in determining the suitability of water for human and animal consumption, agriculture, and industry. In this study, TDS demonstrated a decreasing trend of 60.88%. Methanotrophic bacteria produced biological fertilizer by oxidizing methane and other gases. During the 14 days of wastewater treatment, decreased the concentrations of H₂S, NH₃, CH₄, and CO₂ gases in the wastewater.

Ethical Approval

The study is done through computational methods of chemistry, which is not related to human and animal studies.

Consent to Participate

The authors declare our informed consent to participate in this research.

Consent to Publish

The authors give our consent for the publication of identifiable details, which can include photograph(s) and/or videos and/or case history and/or details within the text (“Material”) to be published in the Journal and Article.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

Study concept and design: Leila Mahdavian, and Hojjat Saki. Analysis and interpretation of data: Leila Mahdavian, and Hojjat Saki. Drafting of the manuscript: Leila Mahdavian, and Hojjat Saki. Critical revision of the manuscript for important intellectual content: Leila Mahdavian, and Hojjat Saki. Statistical analysis: Leila Mahdavian, and Hojjat Saki. Administrative, technical, or material support: Leila Mahdavian, and Hojjat Saki and Supervision: Leila Mahdavian, and Hojjat Saki.

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Availability of data and materials

The authors confirm that the data supporting the finding of this study are available within the article and its Supplementary material.

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