ARTICLE / INVESTIGACIÓN

Studying the efficiency of the multiple biofilters in the reduction of pollutants from wastewater

Khalid Falih Hassan*, Ahmed Aidan Al-Hussieny, Elaff safa Al Deen Hassan, Ruah Abd Almunim
DOI. 10.21931/RB/2022.07.02.55
Water and Environment Directorate, Ministry of Science and Technology, Iraq.
Corresponding author: alrubbay@gmail.com

Abstract: The Microorganisms: yeast *Sporobolomyces yunnanensis*, *Rhodotorula mucilaginosa* and *Kluyveromyces marxianusin* growth of 184× 10^6 ± 15 cell\ml, and bacteria *Pseudomonas aeruginosa* and *Bacillus cereus* in the growth of 9 – 15× 10^8 ± 5 cell\ml, and algae *Chlorella vulgaris* in the growth of 45 × 10^5 ± 5 cell\ ml were selected as a bio filter to reduce organic and nonorganic pollutants from wastewater by COD, TOC, TN and TP concentrations of 455, 151, 9.5 and 31 ppm in treatment period 4, 6 and 18 hr. and pH of 7-8 in 25 Co as a Batch culture system. The yeasts showed ability to reduce COD concentration by 50-64% in 18 hr. treatment period, and the bacteria showed the ability to reduce COD, TOC, TN and TP concentrations by 59-69%, 57-66%, 53-63% and 40-55% in 18 hr. treatment period. At the same time, algae showed efficiency in reducing TN and TP by 81, 82%. The selective microorganisms showed high efficiency as a biofilter to reduce pollutants concentrations from wastewater in three serial steps by a treatment period of 6 hr in each step with a high ability to reduce COD, TOC, TN , and TP concentrations by 83, 91, 100, and 100% in 18 hr. treatment period. Ultimately, this study demonstrated the high efficiency of the multiple biofilters consisting of bacteria, yeasts, and algae in reducing the concentration of pollutants in sewage wastewater.

Key words: Multiple biofilters, Bacteria, Yeast, Algae, COD, TOC, TN.

Introduction

Industrial, agricultural and residential wastewater is one of the main sources of pollution of water bodies after being discharged into rivers without adequate treatment or partial treatment. These wastes contain various organic materials and pathogens that lead to a decrease in the status of natural waters and to the alteration of the balance of the aquatic environment. They also lead to pollution, as the characteristics and composition of the biodegradability of a pollutant are affected by the type of resulting industry and the presence of other pollutants. Environmental conditions such as temperature, humidity and the degree of concentration of the pollutant affect the waters. Taking into account the ability of these organisms to consume nitrogen and phosphorus compounds and carbon and methane compounds[1].

Wastewater treatment processes have evolved in recent years and have been developed with highly efficient technologies to reduce pollutants, such as Biofilteration, Bioshaft, and Moving Bed Biofilm Reactor (MBBR) technology, as international companies and research centers compete to find low-cost and high-efficiency methods for treating industrial and domestic wastewater and recycling this water for agricultural, industrial and other uses. The bio-filter technology is one of the efficient techniques for reducing pollutant concentrations from wastewater. These techniques depend on the metabolic activity of microorganisms to reduce the organic and inorganic pollutants in wastewater and select a group of efficient species in biological treatments according to the type of pollutants[2]. Also, (3) in their study showed that biofilters are highly effective in the biodegradation of organic and inorganic pollutants in wastewater. This effect depends on the type of pollutant and the microorganisms used as biofilters and the ability of these organisms to coexist within specific conditions in the waste sample, and the exploitation of pollutants as a source of energy liberation for its permanence and reproduction.

Bacteria are among the efficient microorganisms in the biological treatment of wastewater, as they can secrete a wide range of decomposing enzymes that break down organic and hydrocarbon materials and convert them into more explicit materials and adsorb many heavy elements on their cell wall; in addition to their spread throughout the water body and their rapid growth. *Bacillus*, *Pseudomonas*, and some *Micrococcus* strains are highly efficient in breaking down dissolved organic matter by their decomposing enzymes[3], and (4); in their study, it was proved that the ability of *Chromatium* and *Bacillus* bacteria to consume sulfates significantly in addition to nitrate and nitrite from polluted water and reduce their concentrations significantly. (5) their study showed the ability of bacteria to reduce the concentration level of phosphates, nitrates, and nitrites from polluted water and convert them into food granules stored inside their cells.

Bacteria are divided into several classes according to their metabolism and breakdown abilities: (1) hydrolytic bacteria that secrete a group of enzymes that break down polysaccharides, fats, and proteins and convert them into organic acids, amino acids, and other simpler compounds, and (2) fermentative bacteria that work on converting the-

Citation: Falih Hassan K , Aidan Al-Hussieny A, safa Al Deen Hassan E , Abd Almunim R. Studying the efficiency of the multiple biofilters in the reduction of pollutants from wastewater. Revis Bionatura 2022;7(2) 55. http://dx.doi.org/10.21931/RB/2022.07.02.55
Received: 15 March 2022 / **Accepted:** 8 April 2022 / **Published:** 15 May 2022

Publisher's Note: Bionatura stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

se compounds into alcohol and fatty acids. Consequently, acetogenic bacteria convert it into acetate and hydrogen, then degradable bacteria break down these compounds and convert them into carbon dioxide and water[6].

Yeasts are among the efficient microorganisms in the biodegradation of organic pollutants in wastewater; as (7) showed in their study, the efficiency of *Candida* and *Rhodotorula* yeasts in reducing pollutant concentrations and using this yeast within a biofilter with an efficiency of more than 40% in reducing pollutants. As well as (8) demonstrated the efficiency of some species of black yeast isolated from domestic sewage wastewater in breaking down organic pollutants because they possess an effective and highly efficient enzymatic system to break down a wide range of hydrocarbons, aromatic and aliphatic substances.

The current study aims to use the yeast *Sporobolomyces yunnanensis*, *Rhodotorula mucilaginosa* and *Kluyveromyces marxianus*, *Bacillus cereus*, *Pseudomonas aeruginosa* bacteria and *Chlorella Vulgaris* as biofilters for multiple and sequentially to reduce the concentrations of organic and inorganic pollutants from wastewater.

Materials and methods

Collection and preparation of samples

Wastewater samples were collected from the secondary sedimentation stage of the Al-Rustumiyah wastewater treatment plant using polyethylene plastic bottles (5 liters) and transferred to the laboratory. Other wastewater samples were collected using sterile glass bottles (100ml) to isolate and identify bacteria and yeasts.

Isolation, culturing, and identification of yeasts

Yeasts were isolated from sewage water. Potato dextrose agar (PDA) medium was prepared with the addition of the antibiotic chloramphenicol prepared by dissolving 250 mg of chloramphenicol in 250 ml of distilled water, one ml of the sample placed in sterile glass plates, and adding the sterilized solid media then was shaken well, and the plates were incubated at 27 °C for 48 hr. A smear was taken from each colony using a sterile loop and transferred to a new sterile plate containing PDA media by streak plate method and incubated at 27 °C for 48 hr, after which the resulting yeasts were diagnosed by conducting biochemical tests and based on the key of taxonomy[9], *Sporobolomyces yunnanensis*, *Rhodotorula mucilaginosa* and *Kluyveromyces marxianus* were diagnosed.

Isolation and identification of bacteria

One milliliter of sewage was taken from a sewage water sample and planted by spreading a nutrient medium on the plates. The plates were incubated at 37 °C for 24 hours. A smear from each bacterial colony was transferred to the diagnostic media (differential and selective) to differentiate between different bacterial species and obtain pure bacterial colonies, and biochemical tests were used to diagnose the bacteria. *Bacillus cereus* was isolated and identified according to (10), and a pure isolate of *Pseudomonas aeruginosa* was used, diagnosed by VITEK 2 compact system in the Bacteriological Laboratory/Water Research Center.

Algae culturing and diagnosis

Suitable biomass for *Chlorella vulgaris* cells was obtained within liquid cultures based on (11) through the availability of nutrients in sufficient quantities and the use of culturing with the continuous movement to eliminate local environments at a temperature of (25 ± 2 C) and a light intensity of (250 microeinstein /m^2 / s), green algae were diagnosed by preparing slides and examining them at a power of 400 X using a compound light microscope (12) and (13).

Chemical tests

The chemical factors (the chemical oxygen requirement (COD), total organic carbon (TOC), total phosphorous (TP), and total nitrogen (TN)) were measured before and after treatment according to (14).

Counting of bacterial cells number

Cell numbers of *Pseudomonas aeruginosa* and *Bacillus cereus* were calculated by preparing McFarland 0.5 solution according to (15).

Counting of yeast and algae cells number

The numbers of yeast and algae cells were calculated in 1 ml of broth using the Hemocytometer (Neubauer counting chamber)[16] and depending on the equation:

Number (cells/ml) = Number of cells in 4 squares x 4 x 10

The multiple biofilters

Three isolates of yeast *Sporobolomyces yunnanensis*, *Rhodotorula mucilaginosa* and *Kluyveromyces marxianus* were used as a mixture of the first stage of the biofilter to reduce the concentration of pollutants from sewage wastewater in pH 5-6 and covering the bottles with foil aluminum to prevent the light. *Bacillus cereus* and *Pseudomonas aeruginosa* were used in the second stage as a mixture in pH conditions 7-8, and *Chlorella vulgariswas* used in the last stage of the biofilter in pH 7-8.

Experiments

Yeasts *Sporobolomyces yunnanensis*, *Rhodotorula mucilaginosa* and *Kluyveromyces marxianus* were used with a growth density of 184 x 10^6 ± 15 cells/ml, while *Bacillus* and *Pseudomonas* were used at a growth density ranging 9-15 x 10^8 ± 5 cells/ml and *Chlorella vulgaris* at a growth density of 45 x 10^5 ± 5 cells/ml to reduce pollutant concentration in a sample of sewage water in terms factors the chemical requirements for oxygen (COD), total organic carbon (TOC), total phosphorous (TP), and total nitrogen (TN) using of the glass cylinders, which are contained a sterile wastewater sample (1 liter) each separately, and 20 ml of the broth of the selected microorganisms were added for each cylinder with the period treatment of 4, 6 and 18 hrs.; also it was used a wastewater sample without any addition as the control. The factors COD, TOC, TN, and TP were measured before and after treatment and for each cylinder to know the efficiency of these selected microorganisms in the treatment processes.

The selected microorganisms were used as multiple biofilters using graduated glass cylinders containing a sterile sewage wastewater sample (1 L), and 20 ml of broth for each yeast were added to them at a treatment period of 6 hrs. The treated samples were taken and filtered with filter paper (0.45 µ) and added 20 ml of each bacteria to them at a treatment period of 6 hrs. The samples were filtered and 20 ml of broth algae were added at a treatment period of 6 hrs. The factors COD, TOC, TN, and TP were measured before and after the treatment, and a wastewater sample was used as a control.

Calculations

The percentage of concentration reduction % = initial concentration- final concentration / initial concentration x 100.

Results and discussion

Efficiency of yeasts *Sporobolomyces yunnanensis*, *Rhodotorula mucilaginosa* and *Kluyveromyces marxianus* in reducing pollutants concentration from sewage wastewater

Table (1) shows the reduction of pollutant concentration from sewage wastewater for the control in terms of the factors COD, TOC, TP, and TN at a treatment period of 4, 6, and 18 hrs., the concentration of the factors before treatment was 455, 151, 9.5 and 31 ppm. The concentration of the factors became 454, 151, 9.2 and 29, respectively, after 4 hrs. After 6 hrs. of the treatment period, the concentration of the factors became 452, 144, 8.7 and 29.5 ppm, respectively, and after 18 hrs. The concentration of the factors COD, TOC, TP, and TN became 440, 128, 8.5 and 26 ppm with a reduction percentage of 3, 15, 10 and 16%, respectively.

Table (2) shows the reduction of pollutants concentration from wastewater in terms of factors COD, TOC, TP, and TN using *Sporobolomyces yunnanensis* yeast for a treatment period of 4, 6, and 18 hours, as the concentration of factors before treatment was 455, 151, 9.5 and 31 ppm, respectively. Consequently, after 4 hrs. of the treatment period, the concentrations became 451, 138, 9.2, and 29 ppm, respectively, and then after 6 hrs. of the treatment period, the concentration of factors became 377, 128, 9.2, and 28.7 ppm, respectively. While, the concentrations of factors became 225, 92, 7.4 and 26 ppm after 18 hrs. The reduction percentage was also 50.5, 39, 22 and 16%, respectively.

Table (3) shows the reduction of pollutants concentration from wastewater in terms of factors COD, TOC, TP, and TN using *Rhodotorula mucilaginosa* yeast at a treatment period of 4, 6, and 18 hrs., as the concentrations of factors before treatment were 455, 151, 9.5, and 31 ppm, respectively. The concentration became 455, 140, 9.5, and 31 ppm, respectively, after 4 hrs. of treatment also after 6 hrs. of treatment, the concentrations became 365, 126, 8.7, and 29 ppm, respectively, while the concentrations of COD, TOC, TP and TN became 210, 82, 6.6 and 22.5 after 18 hrs. of treatment with the reduction percentage of 54, 46, 30.5 and 27%, respectively.

Table (4) shows the reduction of pollutant concentrations from wastewater in terms of the factors COD, TOC, TP, and TN using *Kluyveromyces marxianus* yeast for a treatment period of 4, 6, and 18 hrs. The concentrations of the factors before treatment were 455, 151, 9.5 and 31 ppm, respectively. The concentrations became 450, 148, 9, and 30 ppm, respectively, after 4 hrs. of treatment. However, the concentrations of factors became 360, 122, 6.4, and 30 ppm, respectively, after 6 hrs. of treatment. Whilst, the concentrations became 162, 35, 4.6, and 25 ppm after 18 hrs. With the reduction percentage of 64, 77, 32, and 19%, respectively.

The current study showed the high efficiency of the selected yeasts in reducing the concentration of the chemical oxygen requirement of sewage wastewater with a rate ranging from 50-64% compared to the control during a treatment period of 18 hrs. The results illustrated the high ability of the yeast *Kluyveromyces marxianus* to reduce the concentration of pollutants from the wastewater in terms of the factors COD, TOC, TP, and TN compared to the control. The results matched with the study (14) where they showed the ability of the yeast Kluyveromyces marxianus on the production of a group of effective enzymes in biodegradation processes such as β-galactosidase, inulinase, β-polygalacturunases, and a group of peroxidase enzymes, and the current study agreed with (15) as they showed the ability of *Rhodotorula mucilaginosa* to break down the complicated

Factors	Concentration before treatment (ppm)	Concentration after treatment (ppm)			The reduction percentage % after 18 hrs.
		4hrs.	6hrs	8hrs.	
COD	455	454	452	440	3
TOC	151	151	144	128	15
TN	9.5	9.2	8.7	8.5	10
TP	31	29	29.5	26	16

Table 1. Reducing the concentration of pollutants from wastewater for the control in terms of COD, TOC, TP, and TN at a treatment period of 4, 6, and 18 hrs.

Factors	Concentration before treatment (ppm)	Concentration after treatment (ppm)			The reduction percentage % after 18 hrs.
		4hrs.	6hrs	8hrs.	
COD	455	451	377	225	50.5
TOC	151	138	128	92	39
TN	9.5	9.2	9.2	7.4	22
TP	31	29	28.7	26	16

Table 2. Reducing the concentration of pollutants from wastewater in terms of factors COD, TOC, TP, and TN using *Sporobolomyces yunnanensis* yeast for a treatment period of 4, 6, and 18 hours.

Factors	Concentration before treatment (ppm)	Concentration after treatment (ppm)			The reduction percentage % after 18 hrs.
		4hrs.	6hrs	8hrs.	
COD	455	455	365	210	54
TOC	151	140	126	82	46
TN	9.5	9.5	8.7	6.6	30.5
TP	31	31	29	22.5	27

Table 3. Reducing the concentration of pollutants from wastewater in terms of factors COD, TOC, TP and TN using yeast *Rhodotorula mucilaginosa* for a treatment period of 4, 6 and 18 hours.

Factors	Concentration before treatment (ppm)	Concentration after treatment (ppm)			The reduction percentage % after 18 hrs.
		4hrs.	6hrs	8hrs.	
COD	455	455	365	210	54
TOC	151	140	126	82	46
TN	9.5	9.5	8.7	6.6	30.5
TP	31	31	29	22.5	27

Table 4. Reducing the concentration of pollutants from wastewater in terms of factors COD, TOC, TP and TN using *Kluyveromyces marxianus* yeast for a treatment period of 4, 6 and 18 hours.

organic compounds associated with heavy metals in the contaminated water due to its effective enzymatic system. A study (16) proved that the use of yeasts in wastewater treatment processes has an influential role in the biodegradation of organic waste. At the same time, its efficiency is less in the process of reducing the concentrations of nitrogen and phosphorous compounds due to its nutrition system and the effectiveness of its enzymes in fermentation.This was proven by the current study results, as the percentage of reduction in the concentration of nitrogen and phosphorous compounds did not exceed 12-32%.

Efficiency of *Bacillus* and *Pseudomonas* bacteria in reducing pollutants concentration from sewage wastewater

Table (5) shows the reduction of pollutants concentration from wastewater in terms of the factors COD, TOC, TP, and TN using *Bacillus cereus* at a treatment period of 4, 6, and 18 hours, as the concentrations of the factors before treatment, were 455, 151, 9.5 and 31 ppm, respectively. While the concentrations became 450, 147, 8.5, and 29 ppm, respectively after 4 hrs. of treatment, however after 6 hrs. of treatment, the concentration of COD, TOC, TP, and TN factors became 325, 130, 7.8, and 21 ppm, respectively, and after 18 hrs of treatment, the concentrations became 185, 65, 3.5 and 16.5 ppm with a reduction rate of 59, 57, 63 and 46%, respectively.

Table (6) shows the reduction of pollutants concentration from wastewater in terms of factors COD, TOC, TP, and TN using *Pseudomonas aeruginosa* for a treatment period of 4, 6, and 18 hrs., as the concentrations of factors before treatment were 455, 151, 9.5, and 31 ppm, respectively. After 4 hours of treatment, they became 435, 150, 9.5, and 28.7 ppm, respectively; however, the concentrations became 320, 122, 6.6 and 18 ppm after 6 hours of treatment. At the same time, the concentrations after 18 hrs. of treatment became 140, 52, 4.5, and 14 ppm with a reduction rate of 69, 66, 53, and 55%, respectively.

The results of the current study illustrated the high efficiency of the selected bacteria in reducing the concentration of pollutants from the sewage wastewater during the treatment period of 18 hours compared to the control, as the reduction percentage of the concentration of the chemical oxygen requirement for *Bacillus* and *Pseudomonas* was 59-69%, the total organic carbon was 57-66%, as well as for total nitrogen was 53-63%, and total phosphorous was 40-55% compared to the control. So, the results matched with study (18), where they demonstrated the ability of Bacillus to secrete several enzymes that destroy organic and hydrocarbon compounds with high efficiency and use in treating water contaminated with oil derivatives. Also, the results of the current study are in agreement with the study (19); it was showed the efficiency of microorganisms in the biodegradation of organic pollutants, as they demonstrated the ability of *Bacillus* bacteria to produce a group of peroxidase enzymes that decompose and destroy organic materials and aliphatic and aromatic compounds with high efficiency. The results of the current study also coincided with study (20), as they showed in their study the high enzymatic efficiency of *Pseudomonas* bacteria in destroying many organic compounds and hydrocarbon pollutants, including the cytochrome group and the biphenyl dioxygenase group, which have a wide range and high efficiency in the biodegradation of organic pollutants and benzene compounds. The current study's results also agreed with (21) as they showed in their study the ability of bacteria *Pseudomonas* produces a wide range of enzymes that break down hydrocarbons and benzene compounds and their ability to biodegrade these compounds with high efficiency.

Efficiency of *Chlorella vulgaris* in reducing pollutants concentrations from sewage wastewater

Table (7) shows the reduction of pollutants concentration from wastewater in terms of the factors COD, TOC, TP, and TN using *Chlorella vulgaris* for a treatment period of 4, 6, and 18 hrs., as the concentrations of factors before treatment, were 455, 151, 9.5 and 31 ppm, respectively, however, the concentrations became 410, 148, 8, and 29 ppm, after 4 hrs. of treatment. While after 6 hrs. of treatment, the concentrations of the factors became 388, 133, 5.2, and 20

Factors	Concentration before treatment (ppm)	Concentration after treatment (ppm)			The reduction percentage % after 18 hrs.
		4hrs.	6hrs	8hrs.	
COD	455	450	325	185	59
TOC	151	147	130	65	57
TN	9.5	8.5	7.8	3.5	63
TP	31	29	21	16.5	46

Table 5. Reducing the concentration of pollutants from wastewater in terms of COD, TOC, TP, and TN using Bacillus cereus for a treatment period of 4, 6, and 18 hours.

Factors	Concentration before treatment (ppm)	Concentration after treatment (ppm)			The reduction percentage % after 18 hrs.
		4hrs.	6hrs	8hrs.	
COD	455	435	320	140	69
TOC	151	150	122	52	66
TN	9.5	9.5	6.6	4.5	53
TP	31	28.7	18	14	55

Table 6. Reducing the concentration of pollutants from wastewater in terms of factors COD, TOC, TP, and TN using *Pseudomonas aeruginosa* for a treatment period of 4, 6 and 18 hours.

Factors	Concentration before treatment (ppm)	Concentration after treatment (ppm)			The reduction percentage % after 18 hrs.
		4hrs.	6hrs	8hrs.	
COD	455	410	388	270	40
TOC	151	148	133	95	37
TN	9.5	8	5.2	1.8	81
TP	31	29	20	5.6	82

Table 7. Reducing the concentration of pollutants from wastewater in terms of factors COD, TOC, TP and TN using *Chlorella vulgaris* for a treatment period of 4, 6 and 18 hours.

ppm, respectively, and after 18 hrs. of treatment, the concentration of the factors COD, TOC, TP, and TN became 270, 95, 1.8 and 5.6 ppm with the reduction rate of 40, 37, 81 and 82%, respectively.

The current study results showed the ability of *Chlorella vulgaris* to reduce the concentration of nitrogenous and phosphorous compounds from sewage wastewater with a reduction rate of 81 and 82% compared to the control.

The current results agreed with (22) in their study to reduce the concentration of nitrogen and phosphorous nutrients from sewage wastewater, as they demonstrated the ability of *Chlorella vulgaris* to reduce total nitrogen concentrations by 87% and total phosphorous by 98% to benefit from them in growth and proliferation, as they are essential elements for algae growth. The current results agree with study (23). They clarified the essential and influential role of using this alga in wastewater treatment processes because of its high ability to reduce nitrogen and phosphorous nutrients concentrations and exploit them in growth and proliferation.

Efficiency of the multiple biofilters in reducing pollutants concentration from sewage wastewater

Table (8) shows the percentage of reducing the concentration of pollutants from wastewater in terms of the factors COD, TOC, TP, and TN for the control treatment by using the multiple biofilters at a treatment period of 18 hrs., as the concentrations of the factors before treatment were 560, 172, 25 and 42 ppm, respectively. As well as, after 18 hours of treatment, distributed in three stages (6 hrs. for each stage). The results showed an increase in COD concentration, which became 820 ppm, while the concentration of TOC, TP, and TN were 168, 13.5, and 39 ppm, with a reduction percentage of 2.10 and 7%, respectively.

Table (9) shows the reduction of pollutants concentration from wastewater in terms of the factors COD, TOC, TP, and TN using the multiple biofilters for a treatment period of 18 hours, as the concentration of the factors before treatment were 560, 172, 25 and 42 ppm, respectively. In addition, it was distributed into three phases (6 hrs. for each phase); after 18 hrs. of treatment, the concentration of the factors became 0, 15, 95, and 0 ppm with the reduction percentage of 83, 91, 100, and 100%, respectively.

The results of the current study showed the high ability of the biofilter used to reduce the concentrations of organic and inorganic pollutants from sewage wastewater and the high efficiency of the microorganisms used within this filter during 18 hrs. of the treatment period, also the nitrogen and phosphorous compounds were removed from the wastewater sample at a rate of 100%, as the results of the current study agreed with (3) in their study demonstrated the ability of biofilters to reduce the concentration of pollutants from wastewater if appropriate microorganisms were used which can biodegrade when the appropriate conditions were provided for the growth of these organisms, and the results of the current study matched with the study (25), as they proved the ability of the biofilter is eliminated of the total nitrogen from sewage waste by 100% and reduced organic pollutants in terms of total organic carbon measurement by

Factors	Concentration before treatment (ppm)	Concentration after 18hrs. treatment ppm)	The reduction percentage % after 18 hrs.
COD	560	820	-----
TOC	172	168	2
TN	15	13.5	10
TP	42	39	7

Table 8. Reducing the concentration of pollutants from wastewater in terms of the factors COD, TOC, TP, and TN for the control treatment using the multiple biofilters for 18 hours.

Factors	Concentration before treatment (ppm)	Concentration after 18hrs. treatment ppm)	The reduction percentage % after 18 hrs.
COD	560	95	83
TOC	172	15	91
TN	25	0	100
TP	42	0	100

Table 9. Reducing the concentration of pollutants from wastewater in terms of factors COD, TOC, TP, and TN using the multiple biofilters for a treatment period of 18 hours.

95%. The results also matched with (26) and (27), as they proved in their study that the efficiency of biofilters depends on the selection of the species of microorganisms used to treat industrial and domestic wastewater used as biological filters and appropriate conditions provide for the continuity of their activity. The reduction of pollutants concentration from wastewater is based on these microorganisms' metabolism and degradation activity.

Conclusions

The current study demonstrated the high efficiency of the multiple biofilters consisting of bacteria, yeasts, and algae in reducing the concentration of pollutants in sewage wastewater in terms of COD, TOC, TP, and TN with reduction rates of up to 100%.

Conflicts of interest

The authors declare no discrepancy of interest related to this article.

Acknowledgments

We are thankful to the Bacteriological Laboratory/Water Research Center/Water and Environment Directorate / Ministry of Science and Technology / Iraq. Also, we did not get the financial source of the study.

Bibliographic references

1. Bell KY, Wells MJ, Traexler KA, Pellegrin ML, Morse A, Bandy J. Emerging pollutants. Water Environment Research. 2011 Oct;83(10):1906-84..
2. Mara, D. and Horan, N. Handbook of Water and wastewater Microbiological treatments. Academic Press, New York.2003؛
3. Chaudhary DS, Vigneswaran S, Ngo HH, Shim WG, Moon H. Biofilter in water and wastewater treatment. Korean Journal of Chemical Engineering. 2003 Nov;20(6):1054-65.
4. Leigh MB, Prouzová P, Macková M, Macek T, Nagle DP, Fletcher JS. Polychlorinated biphenyl (PCB)-degrading bacteria associated with trees in a PCB-contaminated site. Applied and Environmental Microbiology. 2006 Apr;72(4):2331-42.
5. Tonolla M, Peduzzi S, Hahn D, Peduzzi R. Spatio-temporal distribution of phototrophic sulfur bacteria in the chemocline of meromictic Lake Cadagno (Switzerland). FEMS microbiology ecology. 2003 Feb 1;43(1):89-98.
6. Lin Y, Kong H, He Y, Kuai L, Inamori Y. Simultaneous nitrification and denitrification in a membrane bioreactor and isolation of heterotrophic nitrifying bacteria. Japanese Journal of Water Treatment Biology. 2004 Sep 15;40(3):105-14.
7. Gerardi MH. Wastewater bacteria. John Wiley & Sons; 2006 Apr 20. Pp. 78 – 95.
8. Rabah AB, Ibrahim ML, Ijah UJ, Manga SA. Assessment of the efficiency of a yeast biofilter in the treatment of abattoir wastewater. African Journal of Biotechnology. 2011;10(46):9347-51.
9. Woertz JR, Kinney KA, McIntosh ND, Szaniszlo PJ. Removal of toluene in a vapor□phase bioreactor containing a strain of the dimorphic black yeast Exophiala lecanii□corni. Biotechnology and bioengineering. 2001 Dec 5;75(5):550-8.
10. Kurtzman C, Fell JW, Boekhout T, editors. The yeasts: a taxonomic study. Elsevier; 2011 May 9.
11. Jennifer MP, PCB Gibson JR. A colour Atlas of Bacillus species.1983
12. Al- Husseiny , A .A. ,Hussein H.T. and Hmood, A.H.Increase algae culture by using various ways by different media culture .Journal of the college of basic education. 2014.20(84) :121-142.
13. Edward G. B. and David C. S.Freshwater Algae Identification and Use as Bioindicators. Printed in Great Britain by Antony Rowe, Ltd. Chippenham, Wilts.pp 285.2010
14. Prescott,G.W. Fresh water algae .the pictured Key nature Series . U niversity of ontana . pp 240.1978
15. McFarland, J. Nephelometrer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc. 14: 1176-1178.1907.
16. Prescott, L.M.; Harly, J.P. and Klien, D.A. Microbiological. 5th ed- London. MC Graw Hill companies.2002
17. APHA. Standard method for the examination of water and wastewater, 21th ed. American public Health Association, American water works Association and water pollution control federal, Washington, D.C.2012
18. Gustavo, G. F. Elmar, H.; Christoph, W. and Andreas, K. G. The yeast Kluyveromyces marxianus and its biotechnological potential. Appl Microbiol Biotechnol .2018 79:339–354.

19. France, Anne D. R. ; Soraya, S. A. ; Versiane, A. L. and Renata, G. Rhodotorula mucilaginosa Isolated from the Manganese Mine Water in Minas Gerais, Brazil: Potential Employment for Bioremediation of Contaminated Water. Springer. Water Air Soil Pollut,2020 231:527.
20. Bahafid, W.; Joutey, N. T.; Asri, M.; Sayel, H.; Tirry, N. and Ghachtouli, N. El. Yeast biomass: An alternative for bioremediation of wastewater. In Yeast – Industrial Applications. (pp. 269–289).2017.
21. Khalid, S. Lavania, B. and Naresh, K. Bioremediation of Total Petroleum Hydrocarbons (TPH) by Bioaugmentation and Biostimulation in Water with Floating Oil Spill Containment Booms as Bioreactor Basin. Int. J. Environ. Res. Public Health . 18, 2226.2021
22. Ławniczak, Ł.;Wo´zniak-Karczewska, M.; Loibner, A.P.; Heipieper, H.J.; Chrzanowski, Ł. Microbial Degradation of Hydrocarbons—Basic Principles for Bioremediation: A Review. Molecules . 2020 .25, 856.
23. Davies, J.I. and Evans, W.C. Oxidative metabolism of naphthalene by soil Pseudomonads: the ring-fission mechanism. Biochem J.2019. 91:251–261.
24. Ojewumi ME, Okeniyi JO, Ikotun JO, Okeniyi ET, Ejemen VA, Popoola AP. Bioremediation: Data on Pseudomonas aeruginosa effects on the bioremediation of crude oil polluted soil. Data in brief. 2018 Aug 1;19:101-13.
25. Singh R, Birru R, Sibi G. Nutrient removal efficiencies of Chlorella vulgaris from urban wastewater for reduced eutrophication. Journal of Environmental Protection. 2017;8(01):1.
26. Jesús, A.; Juan, A.; Beatriz, J. and Juan, C. Chlorella vulgaris, a microalgae important to be used in Biotechnology: a review. Food Science and Technology. 2020..11.1-7.
27. Boon AG, Hemfrey J, Boon K, Brown M. Recent Developments in the Biological Filtration of Sewage to Produce High□Quality Nitrified Effluents. Water and Environment Journal. 1997 Dec;11(6):393-412.
28. Shahriari T, Shokouhi M. Assessment of Bio-Trickling Filter Startup for Treatment of Industrial Wastewater. International Journal of Environmental Research. 2015 Apr 1;9(2).
29. Tawfik A. Polyurethane trickling filter in combination with anaerobic hybrid reactor for treatment of tomato industry wastewater. Polyurethnae. India: InTech. 2012 Aug 29:355-77.