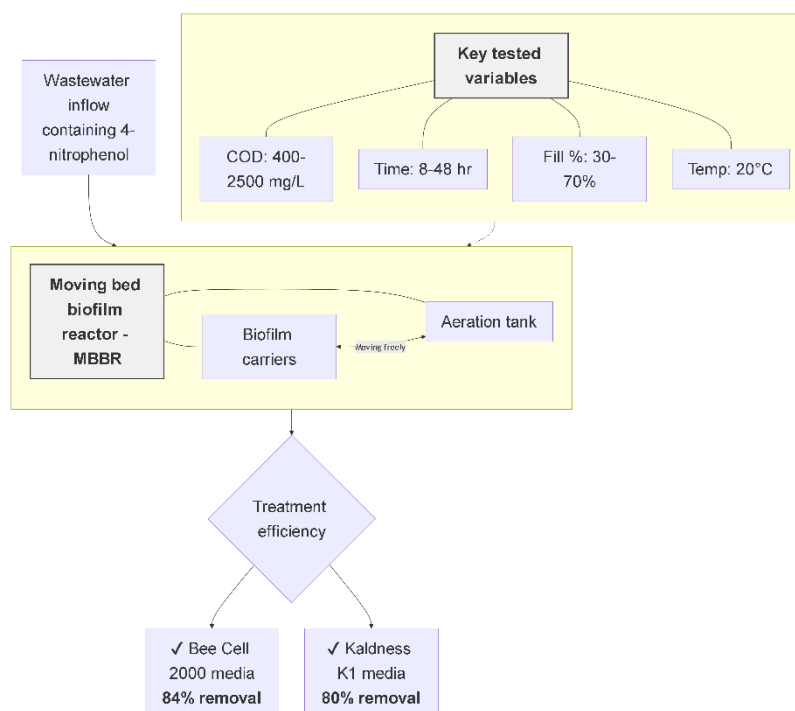


# The role of media type on the performance of the moving bed biofilm reactor in removal of 4-nitrophenol from wastewater

Mahdi Ghaderi, Ali Attarzadeh\* 

Department of Civil Engineering, Qom University of Technology, Qom, Iran.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Oily pollutants, such as 4-nitrophenol, are unavoidable problems. One of the methods employed for treating wastewater polluted with 4-nitrophenol is the use of moving bed biofilm reactors (MBBRs). The present research compared, for the first time, the two common types of media (Kaldness K1 and Bee Cell 2000) in MBBR for treating wastewater polluted with 4-nitrophenol, and determined and reported the optimum conditions for media types and heat. A biofilm layer was first formed on the surface of the media in each reactor, and then the microorganisms were acclimatized to the contaminants. Following that, changes in efficiency with variations in initial COD concentration (400-2,500 mg/L), retention time (8, 12, 24, and 48 h), and filling ratio (30, 50, and 70 %) in the two reactors were determined and compared. The highest efficiencies (84 and 80 %) for the reactor with Bee Cell 2000 media and that with Kaldness K1 media, respectively) were observed. Both reactors containing Bee Cell 2000 media and Kaldness K1 media were very capable of treating wastewater polluted with 4-nitrophenol at 20 °C. Still, the reactor that included the Bee Cell 2000 media yielded better results compared to that containing the Kaldness K1 media.



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## 1. Introduction

Today, wastewater treatment is of great importance in research (Ahmadi *et al.*, 2023). Biological treatment systems are living bacterial environments that remove contaminants by feeding on the nutrients in

\*Corresponding author Email: [Attarzadeh@qut.ac.ir](mailto:Attarzadeh@qut.ac.ir)

the wastewater (Babanezhad *et al.* 2017; Ghaderi, Tamadoni and Mahdizadeh, 2019). The role played by a biological treatment reactor is to prepare suitable conditions for the biological growth of bacteria to remove the contaminants. (Naeemah Bashara and Qaderi, 2024; Khourshidi and Qaderi, 2023). In MBBRs, the entire tank volume is

used for microorganism growth. Furthermore, contrary to activated sludge reactors, MBBRs do not require a return sludge cycle. Based on processes in MBBRs and on their performance in Europe, their use is cost-effective. Installing MBBRs creates special opportunities for reducing the effective space. Other reactors have disadvantages, in these reactors, the uncontrollable growth of the fixed bioparticles cause changes in the hydraulic properties of each particle that is followed by basic changes in the features of the fluid substrate and will influence mass transfer from the substrate into the biofilm (Chen, Sun and Chung, 2008; Melo and Vieira, 1999; Lee, Kang and Lee, 2006; Xiao, Rodgers and Mulqueen, 2007). In MBBRs, because there is no return sludge cycle, there are fewer operational limitations in systems of biofilm growth on surfaces (Attached Biofilm) (Ghaderi, Asadi and Kouhirostamkolaei, 2020; Lee, Kang and Lee, 2006; Xiao, Rodgers and Mulqueen, 2007; Rusten *et al.*, 2000). Media types that float in the aeration tank are used in MBBRs. Biofilms (microbial layers) grow on submerged media and thus increase the floating biological mass that decomposes the organic matter in the wastewater. This method is very desirable for COD, BOD, and nitrogen removal.

Nowadays, the use of media in wastewater treatment is increasing daily, especially in attached growth biological methods, due to their large contact surface area together with their small volume that lead to increased efficiency in wastewater treatment (Ødegaard, Gisvold and Strickland, 2000; Hosseini and Borghel, 2005; Melo and Vieira, 1999).

These media are produced from the best materials, mainly as light blocks with very strong structures in various designs in the form of beehives and egg cartons with vertical, crossing, and oblique channels. Media are used in surface contact areas in degasser towers, as a wave absorber in sedimentation and fat removal tanks, and as a heat transfer surface area in cooling towers. They are made of polyethylene or polypropylene with a specific weight of about 0.92-0.96 g/cm<sup>3</sup>, usually in different shapes with inside cross-sectional walls to enhance their strength and specific surface areas (Ødegaard, Gisvold and Strickland, 2000; Hosseini and Borghel, 2005; Melo *et al.*, 1999).

The type of media and temperature play a very important role in determining the efficiency of the MBBRs. The present research compared, for the first time, two common types of media used in MBBRs for treating wastewater containing 4-nitrophenol, and determined and reported the optimum conditions for each type.

## 2. Materials and methods

The reactors were built on a laboratory scale to perform the tests. The sludge was prepared from wastewater treatment, and then the microorganisms were adapted for decomposing 4-nitrophenol pollution. In the adaptation period, the initial chemical oxygen demand (COD) for the reactors was 300 mg/l, and glucose was used as the initial feed, and its content was reduced gradually and replaced by 4-nitrophenol. COD removal efficiencies were then determined and compared by employing various filling ratios of media, retention times, and initial COD concentrations for both reactors. The effects of removing suspended microorganisms on the removal efficiency of each compound were also studied and compared. C<sub>2</sub>Cl<sub>4</sub>, O<sub>2</sub>Si (silica gel powder); H<sub>2</sub>SO<sub>4</sub> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, HgSO<sub>4</sub>, Ag<sub>2</sub>SO<sub>4</sub> (for measuring COD); H<sub>2</sub>SO<sub>4</sub>, NaOH (for adjusting pH of the solution in the 6.5-7.5 range), ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>); combination of K<sub>2</sub>HPO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub> salts (as a phosphorus source to obtain the COD: N: P ratio of 100:5:1); magnesium sulfate (MgSO<sub>4</sub>·7H<sub>2</sub>O), sodium chloride (CaCl<sub>2</sub>·2H<sub>2</sub>O), iron chloride (FeCl<sub>3</sub>·6H<sub>2</sub>O), and sodium molybdate (Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O) were purchased from Merck Co, (Germany). Spectrophotometer (to measure COD), digital scale (with the precision of 0.001 gram), pH meter, furnace, oven (to determine MLSS), aeration pumps were the main equipment employed in the study.

### 2.1. The reactors

The characteristics of both reactors used in the present research are listed in Table 1.

**Table 1.** Characteristics of the reactors used in the study

Reactor material	Plexiglas
Wall thickness, mm	5
Internal diameter, cm	21
External diameter, cm	22
Effective height, cm	61
Total height, cm	90
Total volume, L	31.156
Effective volume, L	21.117

The present research intended to compare the performance of MBBRs using two different types of media (Kaldness k1 and Bee Cell 2000). Characteristics of the media types are presented in Table 2.

**Table 2.** Characteristics of media types used in this study.

Parameter	Media type	
	Kaldness k1	Bee Cell 2000
Material	High-density polyethylene (HDPE)	Polystyrene (PS)
Shape	Cylindrical	Bee Cell
Color	White	White
Density (kg/m <sup>3</sup> )	123	950
Specific surface area for biofilm growth ( m <sup>2</sup> /m <sup>3</sup> )	500	650

## 2.2. Adaptation of microorganisms to the synthesized wastewater

In all biological treatment systems, the first step in starting the processes is to adaptation of the microorganisms to the pollutant. This is especially of great importance for materials such as 4-nitrophenol that are not readily biodegradable and also have toxic properties because biological oxidation only takes place if the microorganisms are properly adapted to the pollutant; Otherwise, the removal efficiency of organic compounds and wastewater treatment will be very low and practically unsuitable.

The initial COD was 300 mg/L during the adaptation period. In the adaptation, firstly, glucose was injected into both reactors with COD equivalent to 300 mg/L. To adapt the microorganisms to the synthetic wastewater, wastewater containing different concentrations of 4-nitrophenol was then injected in 11 steps. COD equivalent to 300 mg/L was injected into the reactors in the second stage; i.e., the selected shares of the organic load consisting of 4-nitrophenol and glucose were 10 and 90 percent, respectively. The ratio of the organic load (4-nitrophenol) to glucose injected into the reactors was then raised to 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, and 100:0, and the COD removal efficiency at the end of retention time (24 h) was determined in each stage. As shown in Fig. 1, it took the microorganisms in the reactors 45 days to adapt to the 4-nitrophenol.

## 2.3. Parameters studied in the main experiments

At the end of the adaptation period, and taking the formation of biofilms on media into consideration, the main experiments were performed, the COD content was regularly increased, and the removal efficiency of the contaminant was determined. During this stage, the effects of various incoming COD concentrations (400, 500, 600, 800, 1000, 1500, 2000, and 2500 mg/L) were studied in the reactor containing Kaldness K1 media, and also the effects of various incoming COD concentrations (400, 500, 600, 800, 1000, 1500, 2000, 2500 and 3000 mg/L) were studied in the reactor containing Bee Cell 2000 media. Considering the higher ability of the reactor containing the Bee Cell 2000 media than the reactor containing the Kaldness K1 media, the total period for performing the experiments was shorter for the reactor containing the Bee Cell 2000 media compared to the reactor containing the Kaldness K1 media.

## 2.4. Sampling from the reactors

At the end of the retention time in the reactors, the aeration pumps were turned off for the media and the suspended sludge to settle to the bottom of the reactor. Following that, about 100 mL of the supernatant wastewater was extracted through the related valve and used for COD analysis (APHA, AWWA and WEF, 2012).

## 2.5. Kinetics of the biological reactions

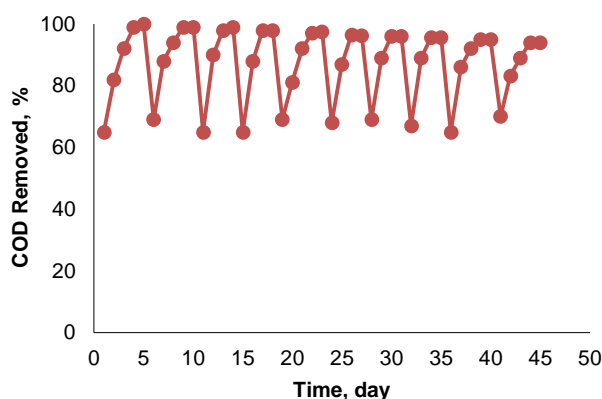
Using models is usual in environmental investigations (Ebrahimi and Qaderi, 2021; Dabbaghi *et al.*, 2021). The first-order kinetic model and the Grau and Stover-Kincannon kinetic models were employed to study the kinetics of biological reactions of the reactors in the removal of 4-nitrophenol, and their correlation coefficients were compared (Qaderi, Ayati and Ganjidoust, 2011; Delnavaz, Ayati and Ganjidoust, 2009).

## 3. Results and discussion

### 3.1. COD removal efficiencies during the adaptation period

As shown in Fig. 1, the removal efficiency declined every time the concentration of 4-nitrophenol was raised, the efficiency improved with loading repetition, and, eventually, a stable stage was reached. In

general, the efficiencies of the stable state were not significantly different at various concentrations during the adaptation period. Fig. 1 indicates that the reactor containing Bee Cell 2000 media exhibited higher removal efficiency and also reached the stable state earlier than the reactor containing Kaldness K1 media each time the concentration was increased. Moreover, the removal efficiency decreased less in the reactor containing Bee Cell 2000 media compared to that containing Kaldness K1 media.

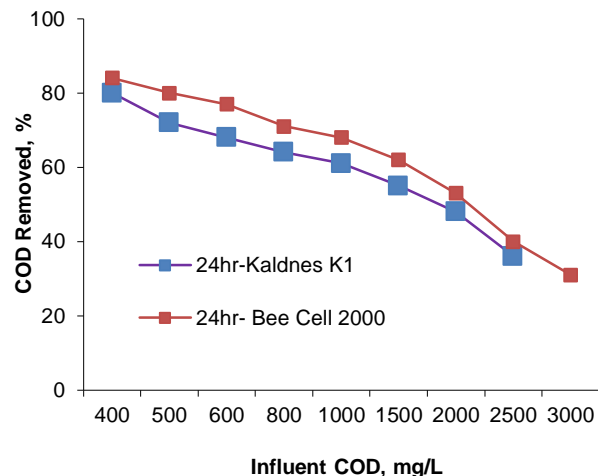


**Fig. 1.** COD removal efficiencies during the adaptation period (COD<sub>in</sub> = 300 mg/L, retention time 24 h, Temperature = 20 °C).

In general, the adaptation period of the microorganisms to the 4-nitrophenol took 45 days. The highest decrease in the steady state removal efficiency for two successive loading operations was 1.6 percent, and it took 4 days on average to reach the stable state each time increases in loading were required.

### 3.2: Effects of initial concentration on the COD and 4-nitrophenol removal efficiencies

In Fig. 2, COD removal efficiencies with increases in the initial concentration of 4-nitrophenol at a retention time of 24 h for the two reactors were determined and compared.

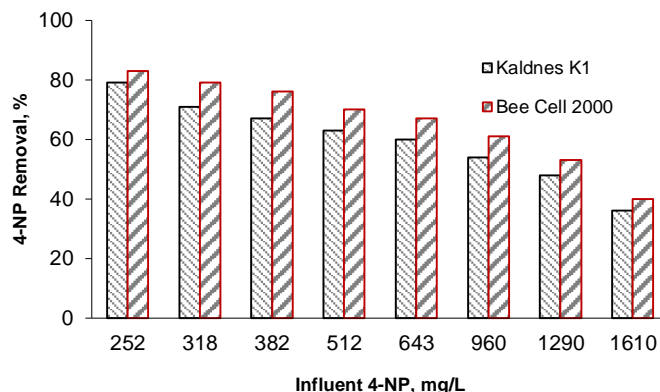


**Fig. 2.** Comparison of COD removal efficiency at various influent COD concentrations for the retention time of 24 h in the reactors containing Bee Cell 2000 and Kaldness K1 media (Retention time: 24 h, filling ratio: 50%, temperature: 20 °C)

As shown in Fig. 2, removal efficiencies of both reactors declined with increases in the initial concentration of 4-nitrophenol at the retention time of 24 h, and, for each concentration, the difference in COD removal efficiency was slight. For example, at COD = 400 mg/L, removal efficiency was 84 percent in the reactor with Bee Cell 2000 media and 80 percent in that with Kaldness K1 media, whereas they were 68 and 61 percent and 40 and 36 percent at COD = 1000 mg/L and at COD = 2500 mg/L, respectively. Therefore, results demonstrate that COD removal efficiency at the retention time of 24 h in the reactor containing Bee Cell 2000 media was slightly higher than that for the reactor with Kaldness K1 media. Fig. 3 reveals that 4-nitrophenol removal efficiencies for the two reactors with Bee Cell 2000 media and Kaldness K1 media were not significantly different, and the largest difference in 4-nitrophenol removal efficiencies was observed at an influent 4-nitrophenol concentration of 382 mg/L. As shown in Fig. 3, 4-nitrophenol removal efficiency in both reactors declined when influent

4-nitrophenol increased from 252 to 1610 mg/L, and this decrease was greater in the reactor with Kaldness K1.

At influent 4-nitrophenol = 252 mg/L, removal efficiency in the reactors containing Bee Cell 2000 media and Kaldness K1 media were 83 and 79 percent, respectively. When influent 4-nitrophenol increased to 643 mg/L, the efficiencies declined to 67 and 60 %, respectively.

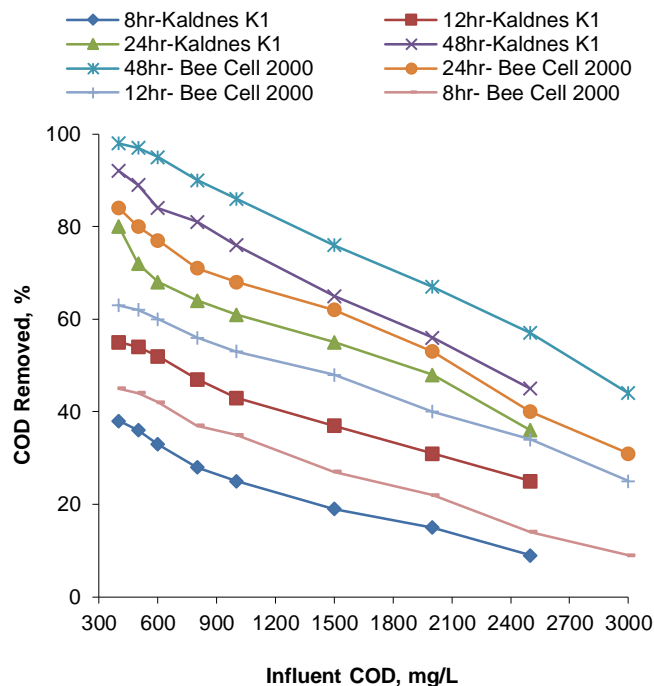


**Fig. 3.** Comparison of 4-nitrophenol removal efficiency at various influent 4-nitrophenol concentrations in reactors containing Bee Cell 2000 and Kaldness K1 media (Retention time: 24 h, filling ratio: 50 %, Temperature: 20 °C).

Finally, at influent 4-nitrophenol = 1610 mg/L, removal efficiencies reached 40 and 36 percent, respectively. The other previous researches confirm these results, and this method is better than previous studied methods (Moghadam and Qaderi, 2019; Taghizadeh, Yousefi Kebria and Qaderi, 2019; Sheikholeslami, Yousefi Kebria and Qaderi, 2020). In other methods, this behavior was confirmed, such as adsorption (Haydar *et al.*, 2003; Tang *et al.*, 2007), solvent extraction (Anitha, Kavitha and Palanivelu, 2011), chemical oxidation (Xiong *et al.*, 2019) Catalytic membrane separation (Wang *et al.*, 2018).

### 3.3. Effect of retention time on COD and 4-nitrophenol removal efficiencies

COD removal efficiency at various retention times for the two reactors containing Bee Cell 2000 and Kaldness K1 media with increases in influent COD concentration were studied and compared (Fig. 4).

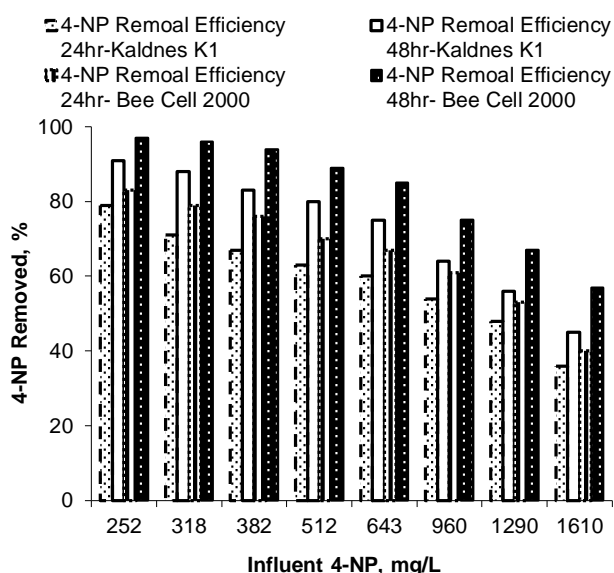


**Fig. 4.** Comparison of changes in COD removal efficiency at various influent CODs and retention times in the reactors containing Bee Cell 2000 and Kaldness K1 media (Filling ratio: 50%, temperature: 20 °C).

As shown in Fig. 4, the removal efficiency for both reactors declined with increases in influent COD concentration. At a retention time of 8 h, COD removal efficiency in the reactor with Bee Cell 2000 media was higher than the reactor with Kaldness K1 media. At a retention time of 12 h, the removal efficiency of the reactor with

Kaldness K1 media was less than that of the reactor with Bee Cell 2000 media. When the retention time was raised to 24 h, removal efficiencies of both reactors increased. at a retention time of 48 h, the removal efficiency of the reactor with Bee Cell 2000 media was much higher than that of the reactor with Kaldness K1 media.

Furthermore, at the retention time of 48 h, removal efficiency declined less with increases in concentration in the reactor with Bee Cell 2000 media: removal efficiency in the reactor with Bee Cell 2000 media declined from 98 to 57 percent when concentration increased from 400 to 2500 mg/L, whereas in the reactor with Kaldness K1 media removal efficiency decreased from 92 to 45 percent at retention time of 48 h. This means that it can reach specific removal efficiencies in a shorter time in the reactor with Bee Cell 2000 media compared to the reactor with Kaldness K1 media. This difference in removal efficiency was greater at high COD concentrations compared to low COD concentrations: at a retention time of 48 h and COD concentration of 400 mg/L, removal efficiencies in the reactors containing Bee Cell 2000 media and Kaldness K1 media were 98 and 92 percent, respectively. At a concentration of 1500 mg/L, removal efficiencies were 76 and 65 percent, and at a concentration of 2500 mg/L, removal efficiencies were 57 and 45 percent. Therefore, it can be seen that the difference in removal efficiency between the two reactors was greater at the higher influent COD concentrations. In Fig. 5, 4-nitrophenol removal efficiencies were compared for the two reactors at retention times of 24 and 48 h.



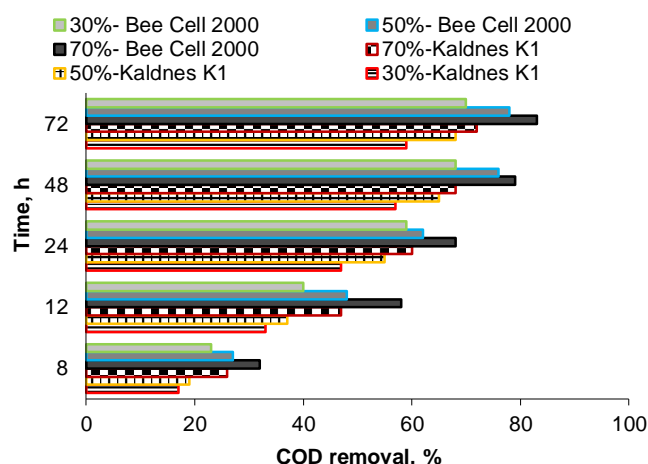
**Fig. 5.** Comparison of changes in 4-nitrophenol removal efficiency at various 4-nitrophenol concentrations and at the retention times of 24 and 48 h in the reactors with Bee Cell 2000 and Kaldness K1 media (Filling ratio: 50%, temperature=20 °C).

As shown in Fig. 5, removal efficiencies of both reactors declined with increases in influent 4-nitrophenol concentration, with the difference that this reduction was smaller in the reactor with Bee Cell 2000 media. Furthermore, the difference in removal efficiencies at retention times of 24 and 48 h was greater in the reactor with Bee Cell 2000 media compared to that with the Kaldness K1 media; i.e., removal efficiency of the reactor containing Bee Cell 2000 media increased more with increase in retention time than that containing the Kaldness K1 media. Fig. 5 shows that the best 4-nitrophenol removal efficiency was achieved in the reactor with Bee Cell 2000 media at the retention time of 48 h. In other previous research, the effect of retention time was investigated, and similar results were reported. However, certain disadvantages limit the application of other methods. For example, physical processes like adsorption do not eliminate the pollutants but rather transfer them from one phase to another, which imposes additional treatment (Zhao *et al.*, 2010). Moreover, conventional chemical processes, besides being reliant on expensive chemical oxidants, can lead to secondary pollution (Yan *et al.*, 2020; Zhao and Kong, 2018).

### 3.4. Effect of media filling ratio on COD removal efficiency

Fig. 6 studied and compared COD removal efficiency at various filling ratios and at retention times of 8, 12, 24, 48, and 72 h for the two reactors containing Bee Cell 2000 and Kaldness K1 media. As shown in Fig. 6, the removal efficiencies of both reactors improved with

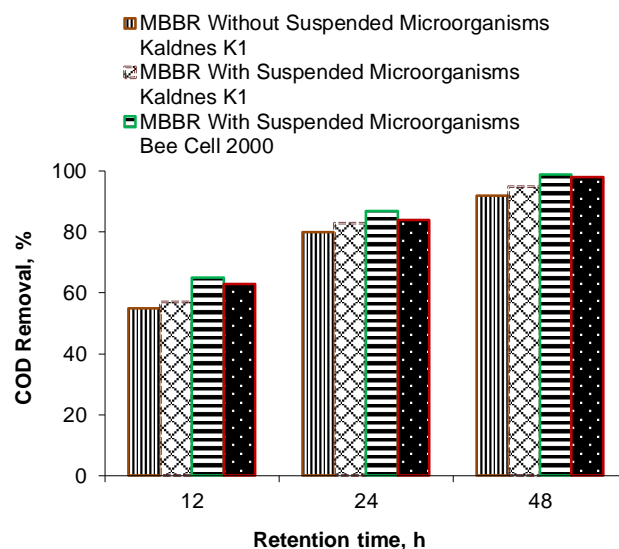
increases in filling ratios. The Fig. 6 reveals that the best retention time for both reactors was 48 h. At this retention time, removal efficiencies at 30 and 70 percent filling ratios for the reactors with Bee Cell 2000 and Kaldness K1 media were 68 and 79 percent and 57 and 68 percent, respectively. Due to the large number of media at the 70 percent filling ratio and the lack of effective movement by the media, and also because of the small difference in removal efficiency at the filling ratios of 50 and 70 percent, the 50 percent filling ratio was the best one for the reactors. At the retention time of 48 h and filling ratio of 50 percent, COD removal efficiencies in the reactors with the Bee Cell 2000 and Kaldness K1 media were 76 and 65 percent, respectively. This suggests that, at a given retention time (48 h), the reactor containing Bee Cell 2000 media at a 50 percent filling ratio had higher removal efficiency than the reactor containing Kaldness K1 media under the same conditions. Similar results were reported in previous research (Qaderi, Ayati and Ganjidoust, 2011).



**Fig. 6.** Comparison of COD removal efficiencies at various filling ratios and retention times in the reactors with Bee Cell 2000 and Kaldness K1 media (COD: 1500 mg/L, Temperature: 20 °C).

### 3.5. Effect of suspended microorganisms on COD removal efficiency

Fig. 7 shows removal efficiencies in the two states of presence or absence of suspended microorganisms at retention times of 12, 24, and 48 h in both reactors.



**Fig. 7.** Comparison of the effect of suspended microorganisms on the removal efficiency of 4-nitrophenol in the reactors containing Bee Cell 2000 or Kaldness K1 media (COD = 400 mg/L, filling ratio: 50%, temperature=20 °C).

According to Fig. 7, the difference in removal efficiency between the two states of before and after removing the suspended microorganisms in both reactors, was negligible. This suggests that the wastewater was mainly treated by the attached microorganisms. As shown in the Fig. 7, removal efficiencies at retention times of 12, 24, and 48 h for the two states of before and after removal of the suspended

microorganisms from the reactors were (65, 63 %), (87, 84 %), and (99, 98%) for the reactor with the Bee Cell 2000 media and (57, 55%), (83, 80%), and (95, 92%) for the reactor with Kaldness K1 media, respectively.

Therefore, the removal of 4-nitrophenol takes place by microorganisms attached to biofilm carriers. Other previous research about MBBR confirmed these results (Qaderi, Ayati and Ganjidoust, 2011).

### 3.6. Kinetics of the biological reactions

Models have key factors in environmental investigations (Ebrahimi Ghadi, Qaderi and Babanezhad, 2019; Yavari and Qaderi, 2020; Khalegh and Qaderi, 2019). Three models were employed to study the kinetics of the reactions in both reactors containing Bee Cell 2000 and Kaldness K1 media to see if the results followed them. To start, the first order model was investigated. The  $(S_0-S)/HRT = k_1S$  diagram, which shows the slope of the  $k_1$  line, was drawn. Results obtained from the reactor with Kaldness K1 media had a correlation coefficient of 0.5364 after linearization. Results indicate that the results obtained from the reactor with Bee Cell 2000 media showed a correlation coefficient of 0.4172. This shows that the reactor containing Kaldness K1 media followed the first-order model better than that containing the Bee Cell 2000 media (Figs. 8 and 9).

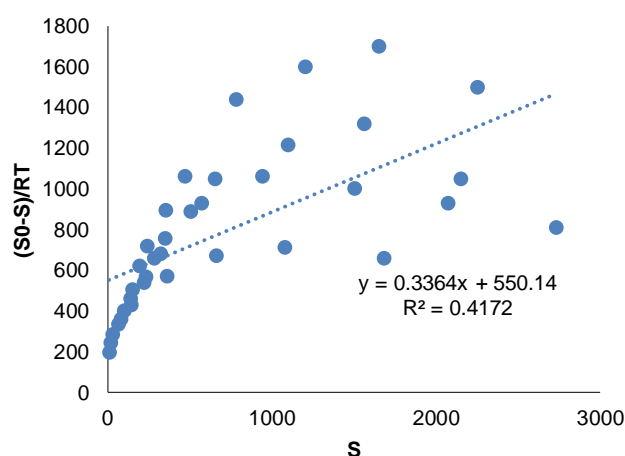


Fig. 8. First-order model for Bee Cell 2000 media.

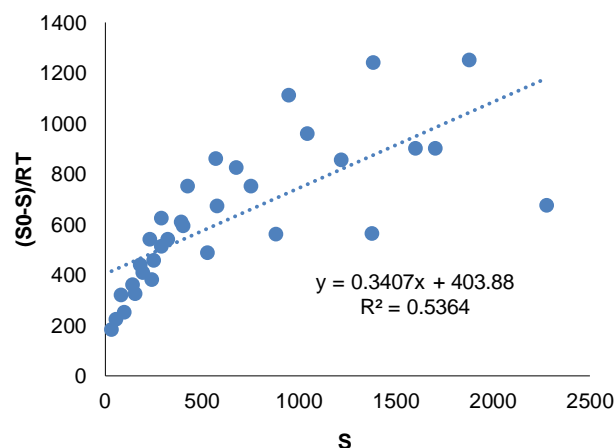


Fig. 9. First-order model for Kaldness K1 media.

The Grau second-order model was studied next. The  $(S_0-\Theta H)/(S_0-S)$  Diagram was drawn against  $\Delta H$ , and the correlation coefficient was obtained. As results indicate, the correlation coefficients for the reactor containing Kaldness K1 media and Bee Cell 2000 media were 0.7189 and 0.6842, respectively. This demonstrates that the reactor with Kaldness K1 media followed the Grau second-order model better than the one with Bee Cell 2000 media. This model was better than the first-order model for both reactors because it had a higher correlation coefficient (Figs. 10 and 11).

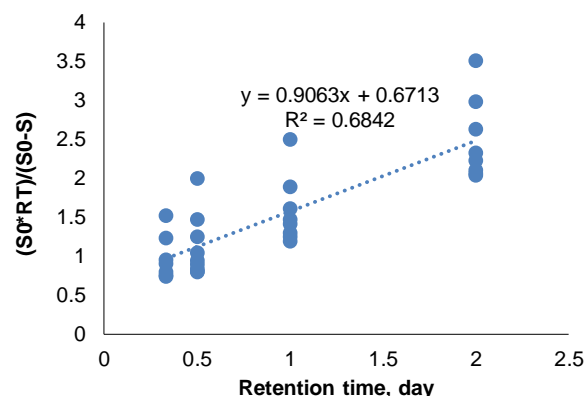


Fig. 10. Grau second-order model for Bee Cell 2000 media.

The Stover-Kincannon second-order model was the third studied model. The  $\Delta H/(S_0-S)$  Diagram was drawn against  $\Delta H/S_0$ . The correlation coefficients for the reactor with Kaldness K1 media and Bee Cell 2000 media were 0.9599 and 0.9698, respectively. In this model, the correlation coefficient for the reactor with Kaldness K1 media was less than that for the reactor with Bee Cell 2000 media (but the coefficients for both reactors were higher compared to the other two models). As indicated by the results, the Stover-Kincannon second-order model had the highest correlation coefficients for both reactors (Figs. 12 and 13).

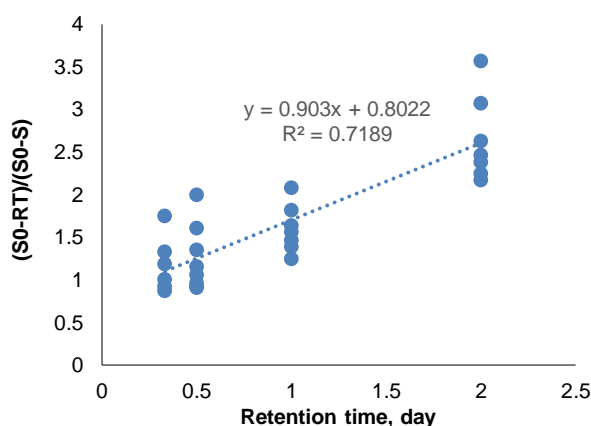


Fig. 11. Grau second-order model for Kaldness K1 media.

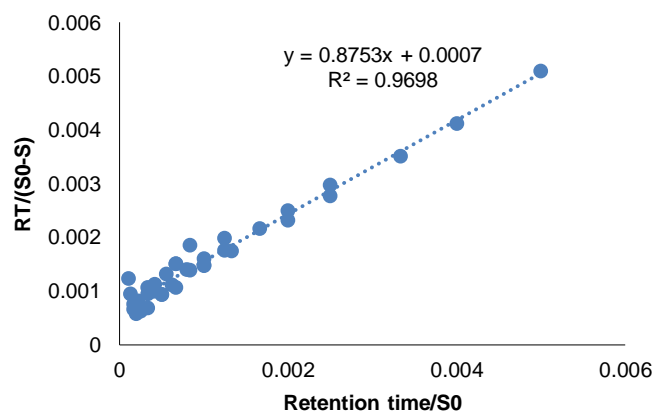
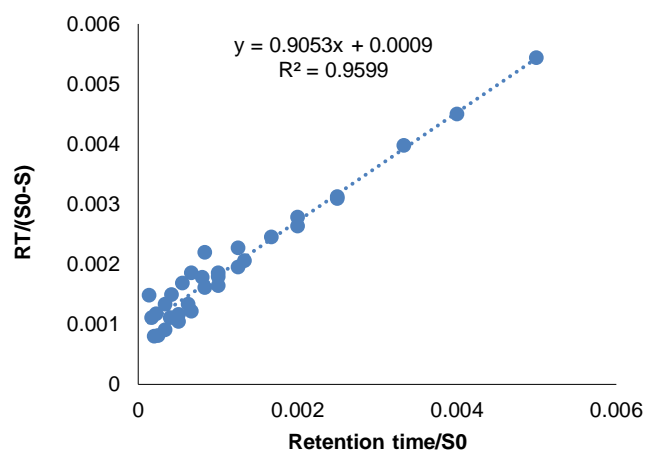


Fig. 12. Stover-Kincannon second-order model for Bee Cell 2000 media.

These results suggest that the 4-nitrophenol removed in the present research was a complicated one. Moreover, a reduction in treatment rate happens with the passage of time in reactions with the second-order model because the 4-nitrophenol not degraded in the early stages of the process, is not readily biodegradable. Previous research reported similar results (Qaderi, Ayati and Ganjidoust, 2011; Farzinfar and Qaderi, 2022).





**Fig. 13.** Stover-Kincannon second-order model for Kaldness K1 media.

#### 4. Conclusions

Results of experiments on the wastewater containing 4-nitrophenol indicated that the adaptation period was 45 days. COD removal efficiency in both reactors declined with increases in the influent 4-nitrophenol concentration, but this reduction was less in the reactor with Bee Cell 2000 media. Removal efficiencies improved for both reactors with increases in retention time. The best retention time for both reactors was 48 h because it sufficiently improved the efficiency. In general, at all retention times (8, 12, 24, and 48 h), the removal efficiency of the reactor with Bee Cell 2000 media was higher than that with Kaldness K1 media. Furthermore, removal efficiencies at filling ratios of 30, 50, and 70 percent and at various retention times were investigated. In both reactors, removal efficiency improved with increases in filling ratio, considering the large number of media at high filling ratios and also the lower removal efficiency when the filling ratio increased from 50 to 70 percent, as compared to its increase from 30 to 50 percent. The optimum filling ratio was 50 percent. Reaction kinetics was investigated by employing the first-order model, the Grau second-order model, and the Stover-Kincannon second-order model, and their correlation coefficients were obtained after linearization and drawing the diagrams. For both reactors, the Stover-Kincannon second-order model had the highest coefficient. Based on the studies and the obtained results, both the reactor containing Bee Cell 2000 media and that containing Kaldness K1 media were very capable of removing 4-nitrophenol, but the reactor with Bee Cell 2000 media yielded better results than that with Kaldness K1 media in removing this contaminant.

#### Author Contributions

Mahdi Ghaderi: Methodology, Data Curation, Writing original draft.  
Ali Attarzadeh: Conceptualization, Methodology, Review and editing of original draft, Supervision.

#### Conflict of Interest

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.

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#### Data Availability Statement

All the data are available in the paper.

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