ISSN 2712-0554
Heritage and Sustainable Development
Original Research
Vol. 6, No. 2, October 2024, pp.657-670
https://doi.org/10.37868/hsd.v6i2.769

Assessment of amoxicillin biodegradation using a fluidized bed reactor

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Received Apr. 18, 2024
Revised Aug. 5, 2024
Accepted Aug. 18, 2024

Abstract

In recent decades, emerging pharmaceutical contaminants, such as personal care items, pharmaceuticals, and disinfectants, have increased alarmingly in aquatic ecosystems. The efficacy of wastewater treatment plants in eliminating these contaminants has been demonstrated. This study aims to find an alternative method, such as using the fluidized bed reactor (FBR) with bacteria to remove amoxicillin from wastewater. The active bacteria *Acinetobacter baumannii* and *Klebsiella pneumoniae* were isolated from one of the pharmaceutical factories in Baghdad, Iraq, from Al-Jazeera wastewater, which was used in FBR technology to decompose the antibiotic amoxicillin. The study examined several operational parameters, including pH, total organic carbon (TOC), dissolved carbon dioxide (CO_2) concentration, airflow rate, amoxicillin concentration, and bacterial number, while maintaining a constant temperature. High-performance liquid chromatography (HPLC) was used to find the reaction route. The maximum amoxicillin removal efficiency was 93% and 91% for *Acinetobacter baumannii* and *Klebsiella pneumoniae*, respectively. For both bacterial species, the pH decreased due to the formation of amino acids and CO_2. The suitable operation condition was 5 mg/L of amoxicillin concentration, flow rate of 30 m/s, and the number of bacteria 47 x 10^5 cfm/mL and 40 x 10^5 cfm/mL for *Acinetobacter baumannii* and *Klebsiella pneumoniae*, respectively. After treatment, the number of bacteria increased due to the degradation of amoxicillin. The steady-state time is found at 64 hours. The organic matter reaction pathway ends at the aromatic amino acid lysine, which kills gram-negative bacteria. This method was found to be successful in removing amoxicillin in low concentrations by using *Acinetobacter baumannii*. In the future, treating amoxicillin in wastewater using FBR technology with gram-positive bacteria is recommended.

Keywords: Amoxicillin, Airflow rate, Fluidized bed reactor, Water pollution, Wastewater treatment

1. Introduction

Micropollutants, including hormones, detergents, personal care items, medications, and disinfectants, are a major concern for aquatic ecosystems due to environmental harm [1]. From a few nanograms to several hundred micrograms per liter, researchers have found pharmaceutical quantities in wastewater, surface water, and

groundwater [2, 3]. The sewage network carries antibiotics from human and animal secretions and waste (sweat and urine) to wastewater treatment plants [4, 5], which are difficult to remove completely [6]. The use of antibiotics in conventional treatment reduces bacterial activity. Over time, bacteria adapt to antibiotics, leading to the emergence of antibiotic-resistant bacterial strains [6, 7]. Amoxicillin (AMO), a proven β-lactam antibiotic, effectively treats gram infections [8, 9, 10]. It was shown that AMO may promote the development of antibiotic bacteria [11]. There are many methods used to treat amoxicillin from wastewater, such as UV/H_2O_2 treatment, solar/H_2O_2 treatment [12, 13], adsorption, ozonation [14, 15], electrochemical processes [16], membrane bioreactors [17], and fluidized bed reactors [18], is used to improve wastewater treatment. However, each technology has limitations and disadvantages, such as low removal efficiency and high cost. Many studies have investigated these techniques and found that sorbents from *Moringa oleifera* seeds could effectively remove ibuprofen medication residues from municipal wastewater [19]. Another study found that activated carbon effectively removes diclofenac from wastewater [20]. The ability and efficacy of membrane separation, adsorption, and advanced oxidation processes (AOPs) in eliminating commonly used drugs from Water are explored. The continuous filtration mode was also investigated. Combining filtration (using activated carbon (AC) and micelle-clay granule complexes) with AOPs improves the economy of treating wastewater, which contains recalcitrant pharmaceutical compounds (PhACs) [21]. Using a sulfidogenic fluidized bed reactor to co-treat acidic Water was the subject of research.

Regarding the treatment of batch bioreactors using sulfate-reducing microorganisms. While ibuprofen and diclofenac had some of the highest percentages at 58.6% and 52.3%, respectively, in the bioreactor, naproxen and ketoprofen had clearance rates of 41.9% and 46.6%, respectively [22]. They have tested the biological treatment of the white rot fungus *Trametes versicolor* in two different bioreactors. The stirred tank bioreactor (STB) successfully removed 85 percent of the two naturally occurring pharmaceuticals found in the hospital's wastewater [23]. Using microbial fluidized bed reactors to filter out pharmaceutical pollutants in wastewater: a meta-analysis [24]. Examination of amoxicillin degradation by means of supercritical water gasification (SCWG) in a continuous flow reactor. One study found that SCWG effectively removed 78.4 percent of organic carbon from industrial wastewater [10]. This research aims to treat pharmaceutical wastewater containing the antibiotic amoxicillin using fluidized bed reactor technology, which is a cheap and ecologically beneficial technique. This research stands out because it uses bacteria in the amoxicillin production line of wastewater from the Iraqi pharmaceutical plant Al-Jazeera.

There does not appear to be any research in the literature identifying microorganisms from the amoxicillin manufacturing process.

2. Method

2.1. Chemicals

In this investigation, pure amoxicillin was obtained from the Al-Jazeera factory in Baghdad, Iraq, and all spectral measurements were performed using a single-beam spectrophotometer (UV/VIS), model SP-OPTIMA, Germany, to obtain the maximum wavelength and standard curve.

2.2. Sampling, bacterial isolation, and no. of bacterial calculation

Using closed glass containers, wastewater samples were collected (in October 2022) from the Al-Jazeera pharmaceutical factory in Baghdad, Iraq. They took the samples to a lab to separate the germs. The samples were grown on a nutrient slant and kept until needed. Bacteria were cultured on MacConkey agar in a certain way to identify whether they were gram-positive or gram-negative. The Central Health Laboratory in Baghdad, Iraq, utilized Vitek 2 equipment to confirm the conventional identification of bacterial isolates. One milliliter of effluent was diluted up to a concentration of 10^{-1}-10^{-5} serially using normal saline. We cultured nutrient agar with 0.1 mL of each dilution and incubated the plates at 37 °C for 24 hours. We used the following calculation to get the number of colonies per milliliter [25].

No. of bacterial colonies (cfu/mL) = No. of colonies x1mL x (1/dilution factor) (1)

2.3. Bacterial dose and mineral salt medium (MSM)

Multiple methods were used to determine the dose of bacteria, and the optimal method was the dilution method. "The dose given was 1 mL for each 1 L of mineral salt medium (MSM). The medium used contains the following chemicals (g/L) produced by Sigma-Aldrich: K_2HPO_4, 0.5; KH_2PO_4, 0.04; NaCl, 0.1; $CaCl_2.2H_2O$, 0.002; $MgSO_4 \cdot 7H_2O$, 0.02; $(NH_4)_2SO_4$. 0.2; and $FeSO_4$. 0.001. After dissolving every component in 1000 mL of distilled Water, the pH value was adjusted to 7", and the solution was then sterilized by autoclave [26, 27].

2.4. Determination of amoxicillin absorbance

The antibiotic amoxicillin has an absorption wavelength that ranges from 200 to 800 nm. Figure 1 shows the absorbance peaks at 280 nm when measured using the UV-VIS spectroscopic technique. We also found the calibration curve for the absorbance of amoxicillin as a function of its concentration. As shown in Figure 2, the process implements Beer's Law for drug compounds ranging from 1-50 mg/L.

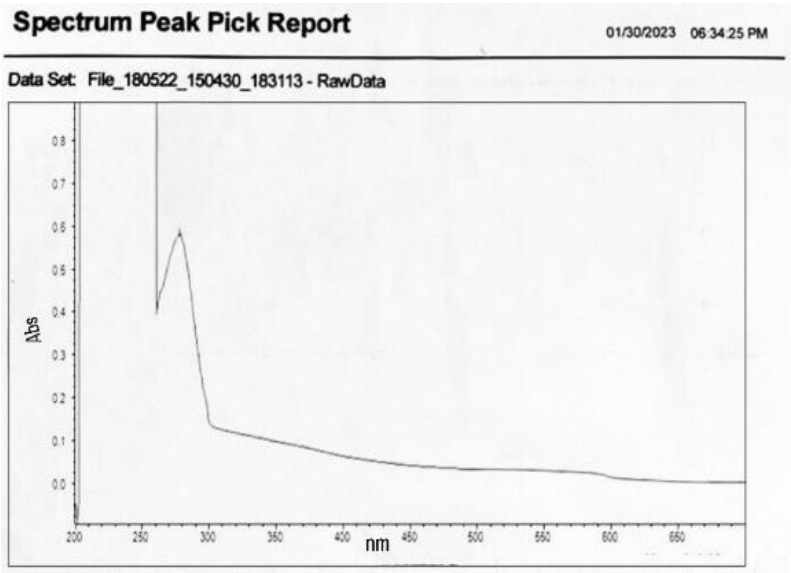

Figure 1. The spectrum of amoxicillin solution (0.01 M) in the spectral range of 200–800 nm

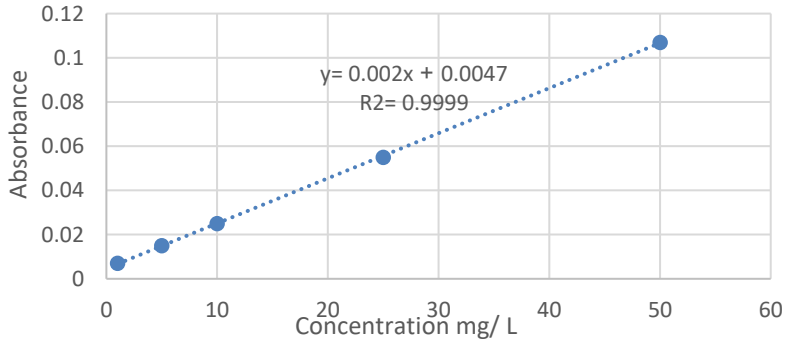

Figure 2. Calibration curves of the spectrum of amoxicillin

2.5. Dissolved CO_2 test

The CO_2 in the solution was allowed to develop before 25 mL of the sample was transferred to a conical flask along with 0.05 mL of 0.1 N sodium sulfate and two drops of methyl orange indicator. Next, we changed the hue of the mixture from red to yellow by titrating it with 0.02 N (NaOH). Titration with two drops of phenolphthalein indicator showed a rosy color [25, 28]. To determine the amount of CO_2, one uses the following equation:

$$Dissolved\ CO_2\ (mg/L) = (Ax\ Bx\ 50x\ 100) / V \quad (2)$$

Where A = mL of NaOH titrant, B = normality of NaOH, V = sample volume in mL.

2.6. Calculation of minimum fluidization velocity Umf

One of the most critical concerns when designing and operating fluidized beds is determining the minimum particle velocity during fluidization [29, 30]. An ascending fluid's drag force is larger than or equal to the mass of the particles, and a surface gas velocity becomes dominant. The effect of fluidization velocity on particle size, density, pressure, and temperature, among other fluid and particle properties [31, 32]. The liquid can move between the adsorbent gaps at modest fluidization velocities, like in a packed bed. When the settled particles start to separate, the flow rate starts to go up.

Further acceleration causes the particles to float on top of the liquid. The minimal fluidization velocity is the abbreviation for this value. Increasing the fluidization velocity beyond (Um) causes the bed to expand since the adsorbent particles are now further apart. Results from stable-state fluidization are gradual and without hiccups [33, 34]. The pressure across the bed drops and stays constant [35, 36, 37]; the lowest fluidization velocity is (Umf=10m/s), as shown in Figure 3. We examine the results of this treatment procedure using a range of fluidization velocities (Umf = 10, 30, and 60 m/s).

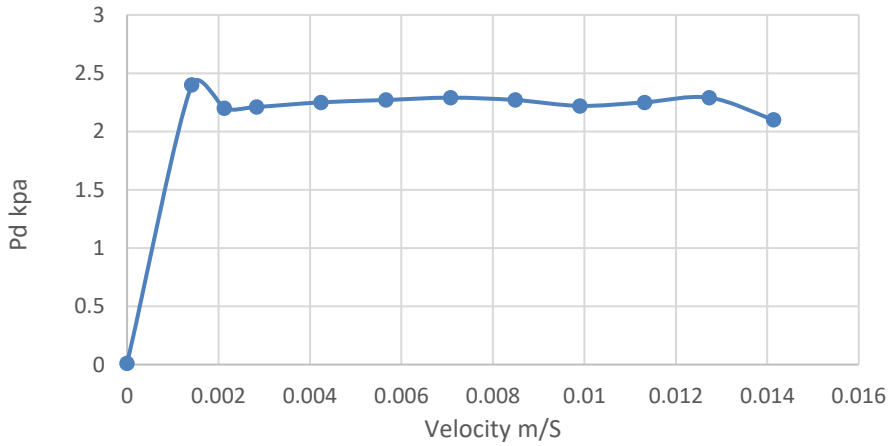

Figure 3. Minimum fluidization velocity

2.7. The pilot plant apparatus

An experimental fluidized bed reactor (FBR) was manufactured and used in this study. Figure 4 illustrates the components of this station.

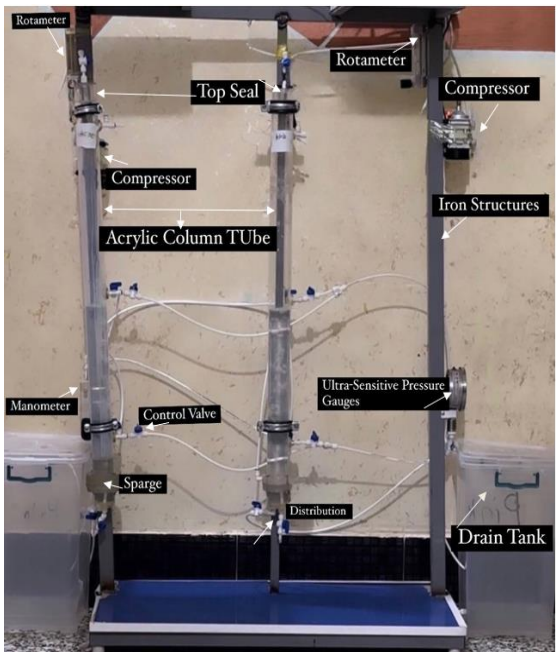

Figure 4. The pilot plant FBR

Figure 5. The pilot plant's gas spargers

The apparatuses and equipment of the FBR included:

1. The iron structure with consistent dimensions to carry the contents of the FBR.
2. At the base of the station's flange is a column-shaped transparent acrylic tube with a 5 cm internal diameter, 6 cm external diameter, 1 m height ideal for bacterial growth, and 5 mm thickness. Two columns form the reactor's core column, which holds reaction materials.
3. There are two distributors for air regulation within the liquid column and two valves for receiving the samples.
4. Use a monometer device. To measure the pressure generated by the liquid in different areas along the experimental time.
5. A gas flowmeter (panel rotameter) is used to regular the amount of air entering during a unit of time.
6. Compressors are used for air supply.
7. Two tanks (drain) used for processing.
8. Spargers between two pieces of the flange; there are 69 holes with a diameter of 2 mm, totaling 2.17 cm^2, which is 10.6% of the pipe section's overall area, as displayed in Figure 5. Since the amount of air is constant over time, these spargers' primary function is to allow air to enter at various speeds and bubble sizes.

2.8. Procedures

Synthesized wastewater containing amoxicillin was the subject of a series of experiments. The research took place in a reactor with a fluidized bed. The operation condition variables included a 1 mL dose of bacteria (in this study, two types of bacteria were used individually), different concentrations of amoxicillin (5, 30, 50 mg/L), and different velocities (10, 30, 60 m/s). The system was operated continuously for 64 hr until it reached the steady-state phase. The samples were subjected to spectrophotometer tests at 280 nm to determine the removal rate based on the amount of absorption, to determine the amoxicillin concentration, and to confirm these results to the HPLC spectrum for all samples to determine the reaction pathway and the TOC concentration and pH.

3. Results and discussion

3.1. Bacterial isolate collection and identification

Pharmaceuticals and other chemicals are abundant in industrial effluent. This study identified amoxicillin-biodegrading bacteria from Al-Jazeera factor wastewater. Vitek 2 detected *Acinetobacter baumannii* and *Klebsiella pneumoniae* in wastewater. In pairs or singles, "gram-negative, non-motile, non-spore-forming, non-lactose fermenter *Acinetobacter baumannii* resembles a coccobacillus [38]. As illustrated in Figure 6, *Klebsiella pneumoniae* is gram-negative, lactose-fermenting, facultatively anaerobic, encapsulated, rod-shaped, and non-motile" [39].

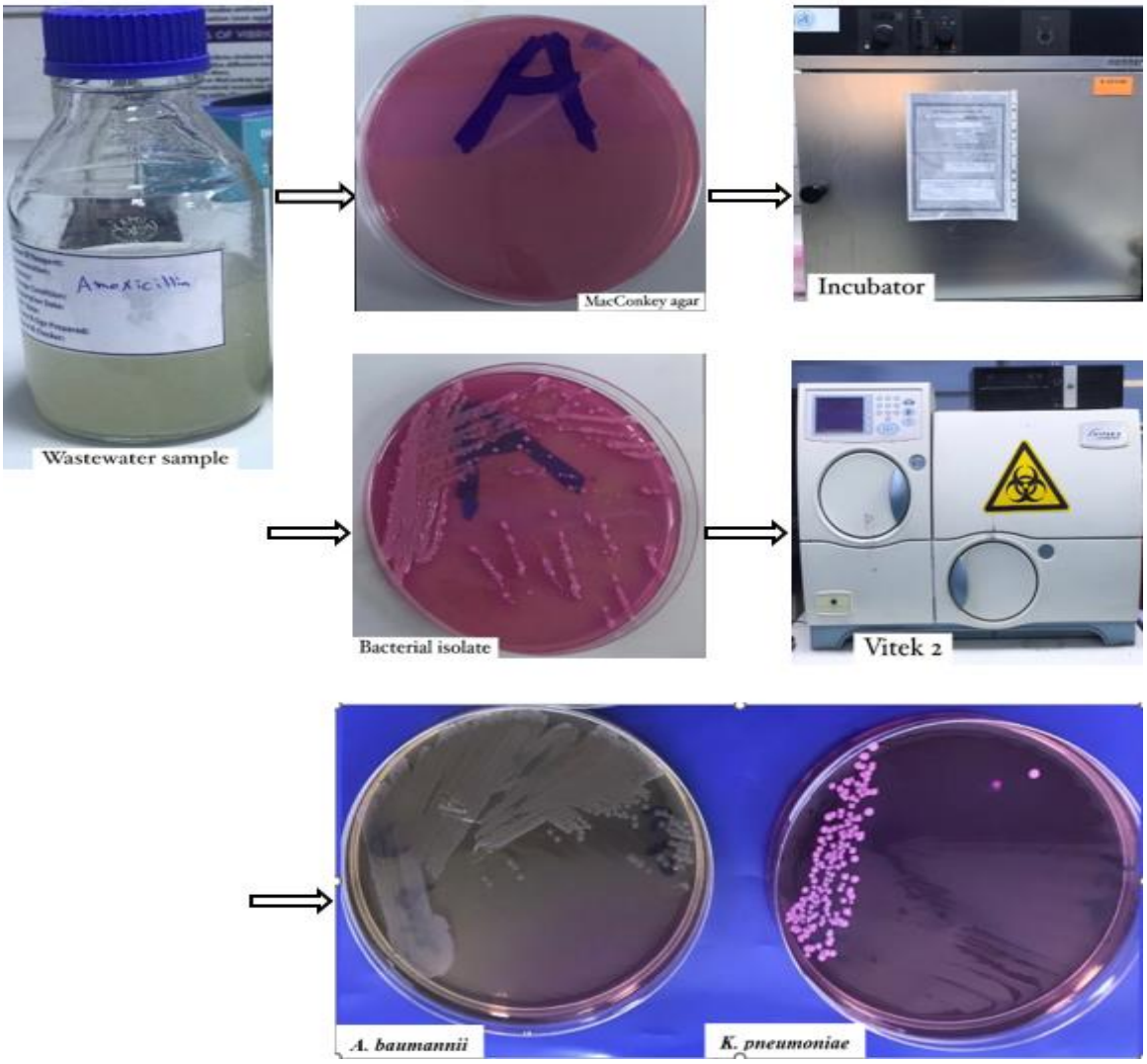

Figure 6. Isolation of *Acinetobacter baumannii* and *Klebsiella pneumoniae* from pharmaceutical wastewater

3.2. Different antibiotic concentrations

The experiments were conducted in two sets, one with *Acinetobacter baumannii* and another with *Klebsiella pneumoniae*. In each set, three different concentrations of amoxicillin were used (5, 30, and 50 mg/L) while maintaining constant air velocity (30 m/s) and pH 7 "at room temperature." The findings illustrated in Figures 7 and 8 are related to *Acinetobacter baumannii*. Maximum removal efficiency of amoxicillin and TOC by using 5, 30, and 50 mg/L concentrations of the antibiotic was attained at 84%, 85%, 52.31%, 47%, and 27.12%, 30%, respectively. The removal efficiency of amoxicillin and TOC by using *Klebsiella pneumoniae* were 62%, 69%, 40.33%, 19%, and 22%, 12%, respectively, as shown in Figures 9 and 10. TOC measures the amount of organic carbon in the sample used in the analysis (every six hours), while measurement of amoxicillin concentration (every four hours). The removal efficiency of amoxicillin and TOC increased with time. However, removal efficiency decreases with increased amoxicillin concentrations due to the degradation of amoxicillin by *Acinetobacter baumannii* and *Klebsiella pneumoniae,* which releases carbon dioxide and water [40, 41]. According to the results of the carbon dioxide levels before and after treatment, as shown in Figures 11 and 12, CO_2 concentration increased with time due to the oxidation of amoxicillin by bacteria. pH values decreased for the two types of bacteria due to the degradation of organic matter into organic acids. Another reason can be the dissolution of CO_2 and the formation of carbonic acid over time, as shown in Figures 13 and 14. Table 1 shows the change in the number of bacteria throughout the experiment. The increase in the number of bacteria reflects the decomposition process of amoxicillin, as it produces both amino acids and carbon dioxide. The reaction course was examined using an HPLC device. Increased carbon dioxide production was observed at a

concentration of 5 mg/L, indicating increased bacterial activity. At concentrations of 30 mg/L and 50 mg/L, bacterial activity decreased, perhaps due to a change in pH. At these higher concentrations, it acts as an antibacterial, which kills bacteria before removing them. The readings show that the pH is slightly different for both types of bacteria. Hence, this technology works well to treat wastewater containing low concentrations of antibiotics.

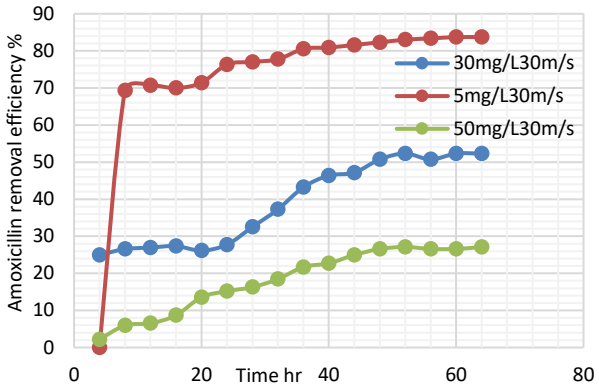

Figure 7. Amoxicillin removal percentage with time in FBR with *Acinetobacter baumannii*

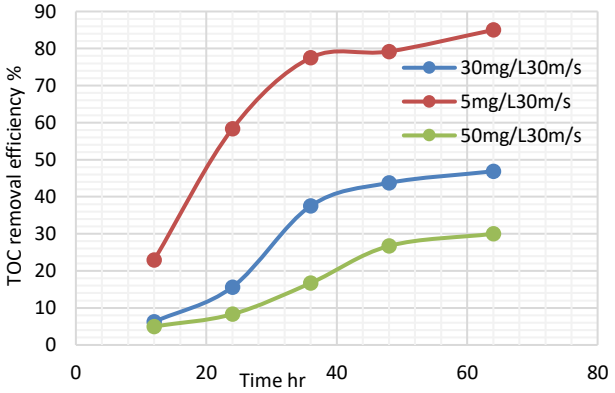

Figure 8. TOC removal percentage with time in FBR with *Acinetobacter baumannii*

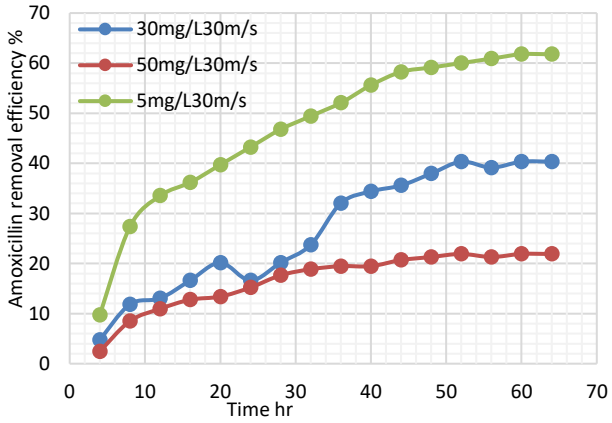

Figure 9. Amoxicillin removal percentage with time in FBR with *Klebsiella pneumoniae*

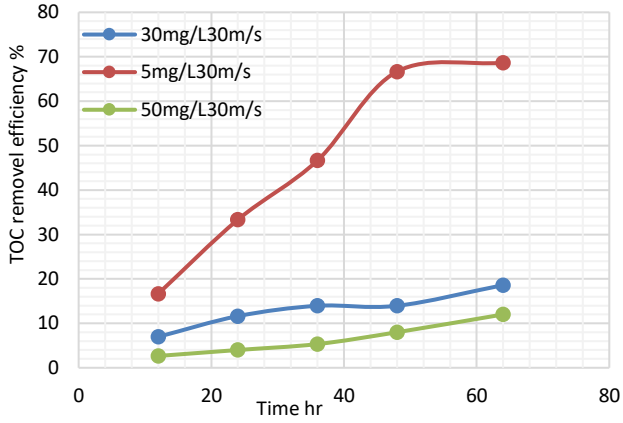

Figure 10. TOC removal percentage with time in FBR with *Klebsiella pneumoniae*

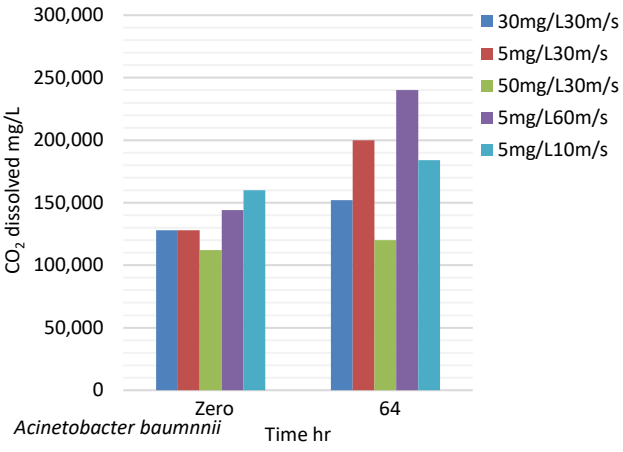

Figure 11. Percentage of CO_2 removal with time in FBR for amoxicillin with *Acinetobacter baumannii*

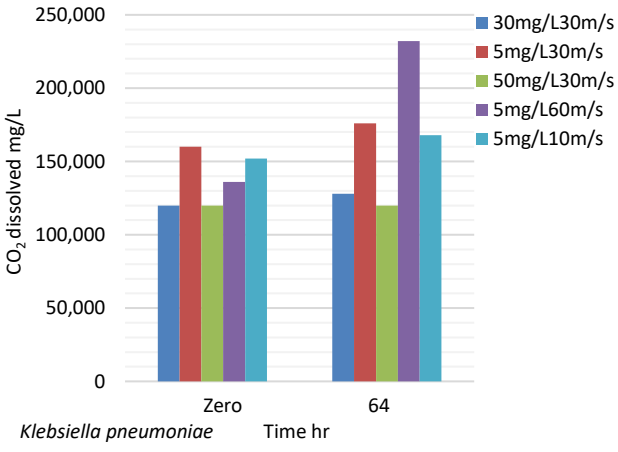

Figure 12. Percentage of CO_2 removal with time in FBR for amoxicillin with *Klebsiella pneumoniae*

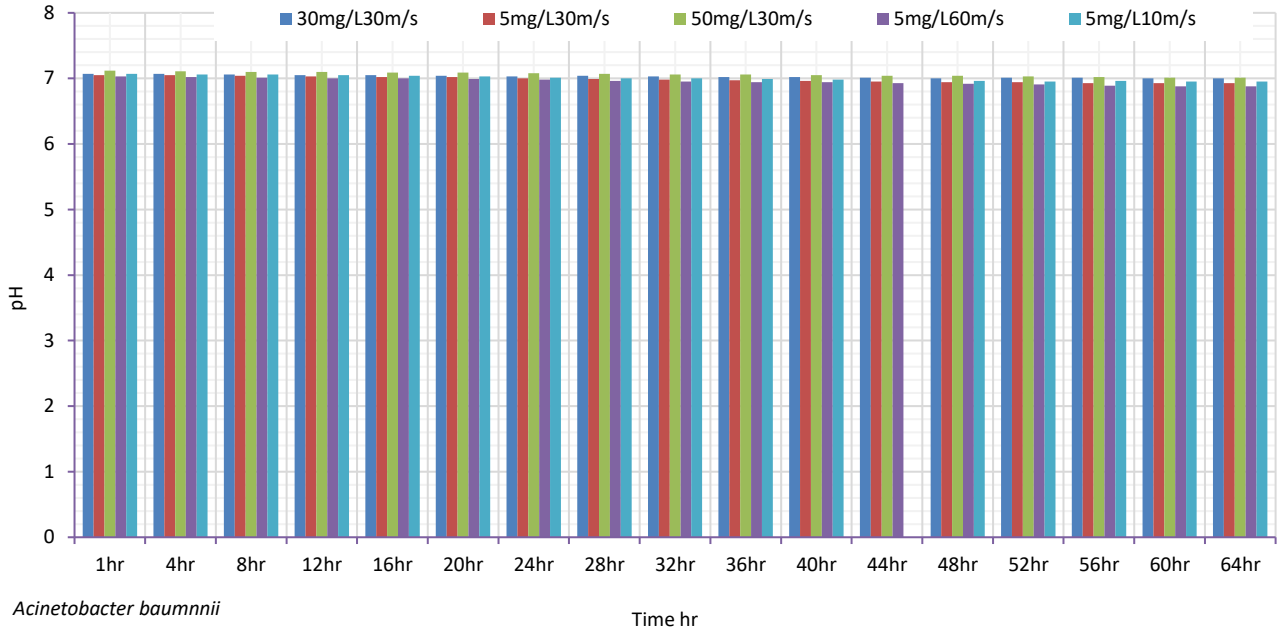

Figure 13. Percentage of pH removal with time in FBR for amoxicillin with *Acinetobacter baumannii*

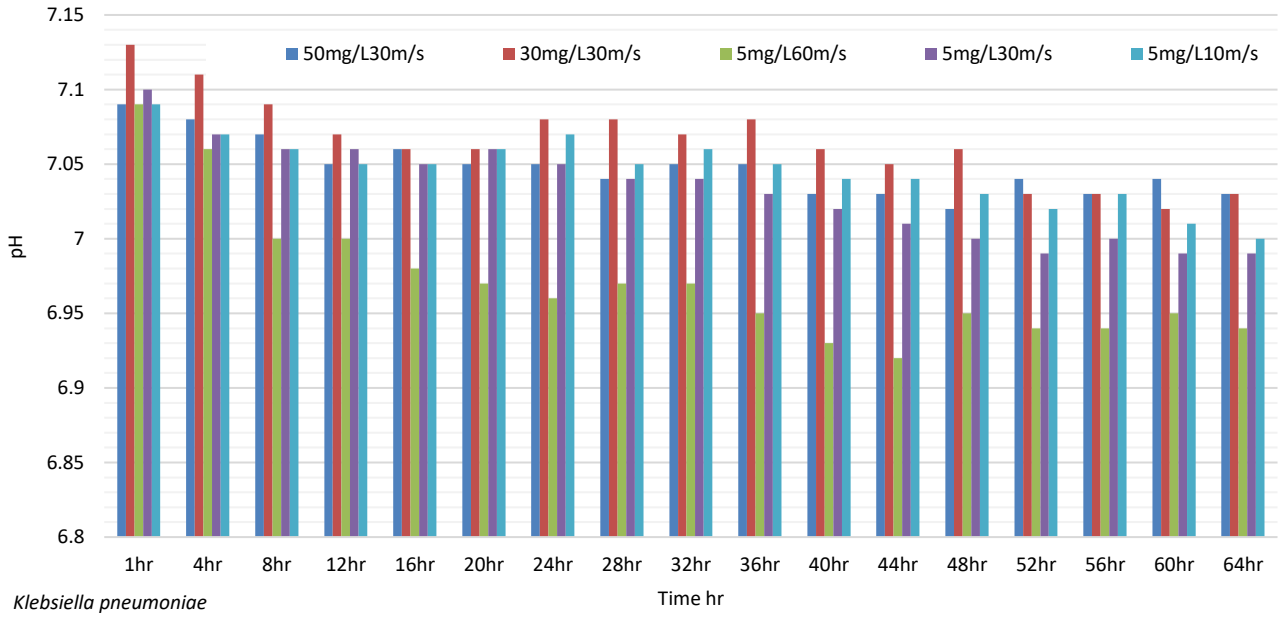

Figure 14. Percentage of pH removal with time in FBR for amoxicillin with *Klebsiella pneumoniae*

Table 1. Number of bacterial isolates before and after treatment

Bacterial isolate	No. of bacteria (cfu/mL)
Klebsiella pneumoniae	$30x10^5$- 40 $x10^5$
Acinetobacter baumannii	31x 10^5- $47x10^5$

3.3. Different airflow rates

These experiments were conducted by varying the air speed (10, 30, and 60 m/s), holding all other variables constant: amoxicillin concentration (5 mg/L), pH 7, and at room temperature. Two types of bacteria, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, were used. Figures 15 and 16 show the results of these

experiments related to the bacteria *Acinetobacter baumannii*. A decrease in the concentrations of amoxicillin and TOC was observed with time, while the removal efficiency increased with time. The removal percentage at a concentration of 5 mg/L showed values of 65%, 75%, 84%, 85%, and 93%, 97.4% at speeds of 10, 30, and 60 m/s, respectively. The results shown in Figures 17 and 18 associated with *Klebsiella pneumoniae* show that amoxicillin and TOC concentrations decrease, and their removal efficiency increases over time. The removal rates at a concentration of 5 mg/L were 59.29%, 67%, 62%, 69%, and 91%, 94% at speeds of 10, 30, and 60 m/h, respectively. Due to the high velocity, the drug substance gets closer to the bacteria that feed on it, which increases the removal efficiency. However, higher speed also leads to higher oxygen levels, which helps bacteria in the bacteriological decomposition process [42].

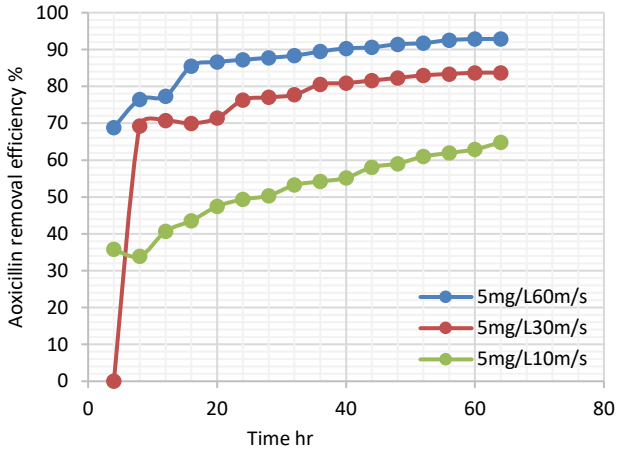

Figure 15. Amoxicillin removal percentage with time in FBR with *Acinetobacter baumannii*

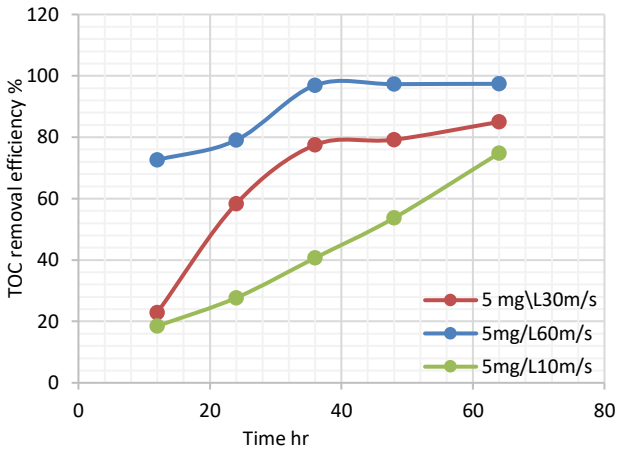

Figure 16. TOC removal percentage with time in FBR with *Acinetobacter baumannii*

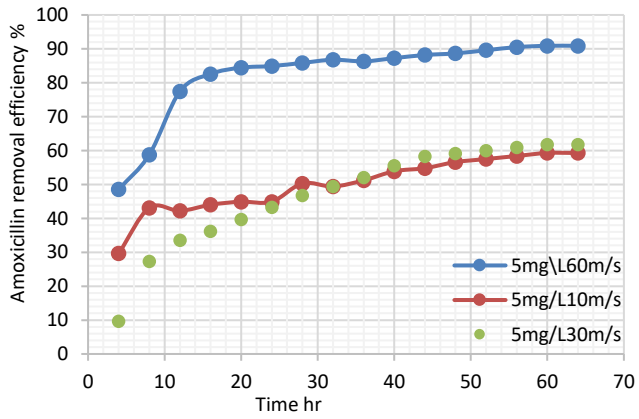

Figure 17. Amoxicillin removal percentage with time in FBR with *Klebsiella pneumoniae*

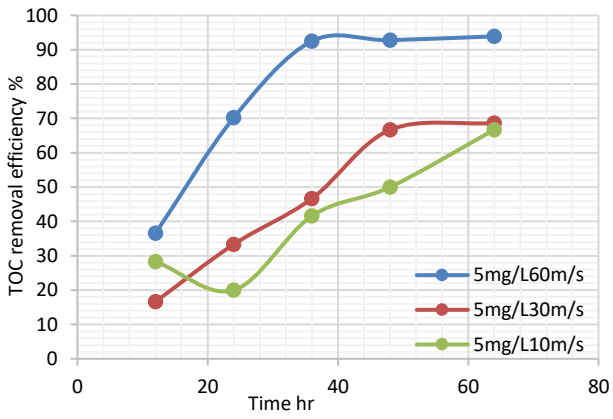

Figure 18. TOC removal percentage with time in FBR with *Klebsiella pneumoniae*

3.4. Reaction pathway

The reaction route identifies amoxicillin breakdown byproducts and explains why the process stopped with both bacteria. The *Klebsiella pneumoniae* chemical route includes aspartic acid, glutamic acid, serine, glysine, alanine, cystine, valine, methionine, isoleucine, d-leucine, and lysine. For *Acinetobacter baumannii*, the pathway included "aspartic acid, glutamic acid, serine, histidine, glysine, therionine, arginine, alanine, tyrosine, cystine, valine, methionine, phenylalanine, isoleucine, leucine, and lysine. Aromatic amino acids are tonic and invigorating [34]. Bacteria need it for metabolism, growth, and tissue repair. Figure 19 (A, B, and C) shows several unnamed peaks. Both species of bacteria ended their chemical pathways with lysine. It stopped the process by killing gram-negative bacteria like *Klebsiella pneumoniae* and *Acinetobacter baumannii* [43].

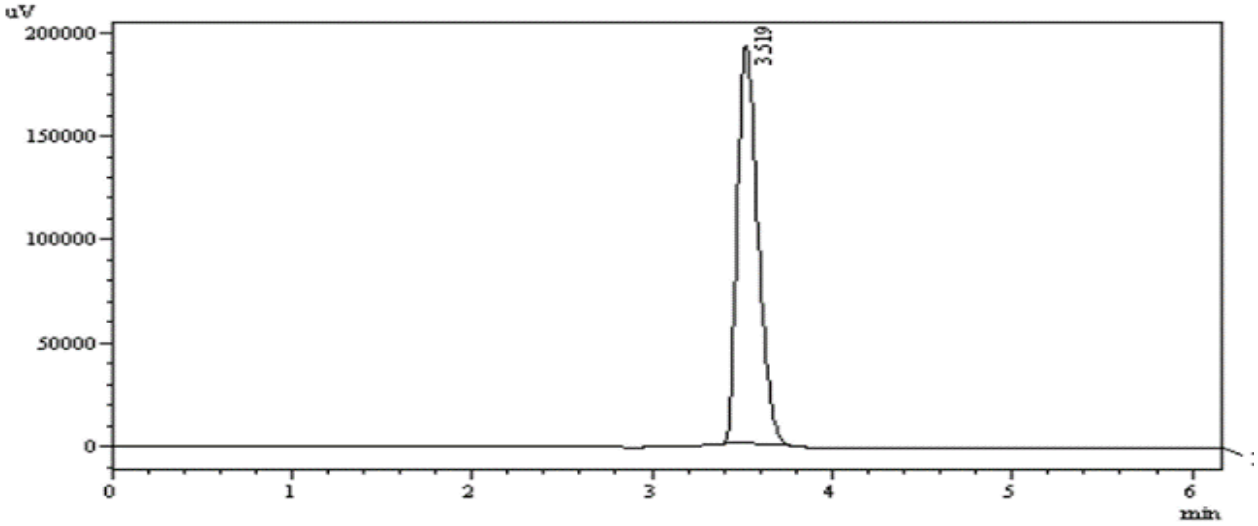

Figure 19a. HPLC spectra of amoxicillin degradation in FBR before treatment (control)

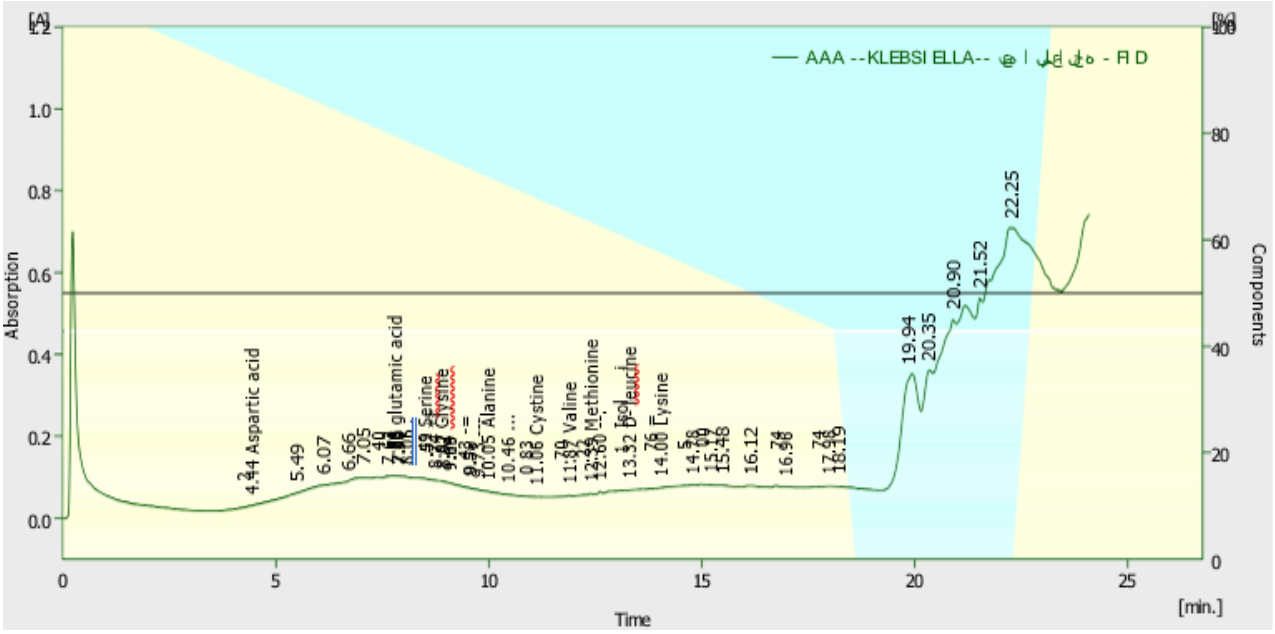

Figure 19b. HPLC spectra of amoxicillin degradation in FBR - *Klebsiella pneumoniae*

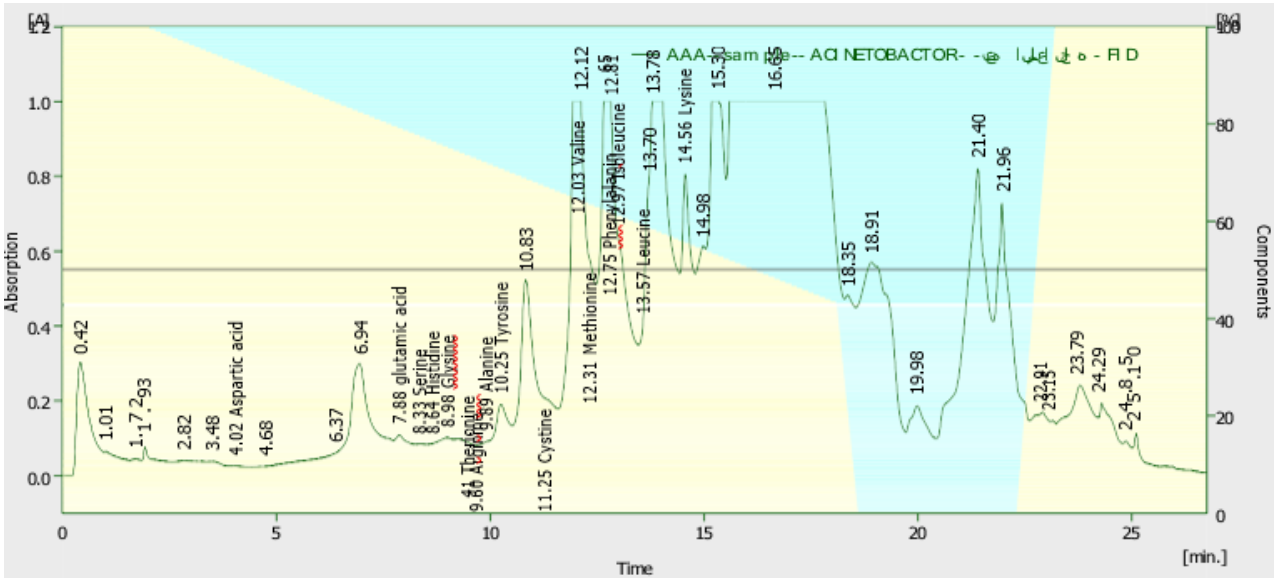

Figure 19c. HPLC spectra of amoxicillin degradation in FBR - *Acinetobacter baumannii*

4. Conclusions

The study concludes that biological treatment using FBR is an effective way to eliminate pharmaceutical contaminants like amoxicillin. In addition, it was found to be extremely effective at removing contamination at low concentrations of 5 mg/L, with removal rates of up to 93% when employing *Acinetobacter baumannii* and 91% when using *Klebsiella pneumoniae*. So, *Acinetobacter baumannii's* removal efficiency is higher than *Klebsiella pneumonia*'s. At a lower pH, amoxicillin degraded into amino acids, and the concentration of carbon dioxide rose with both kinds of bacteria over time. The appearance of lysine at the end of the reaction path for both bacteria indicates that the reaction has stopped because it suppresses the action of the bacteria and kills them because they are gram-negative. FBR is the best choice for removing pharmaceuticals and other contaminants from wastewater.

Declarations

The authors declare that they have no known financial or non-financial competing interests in any material discussed in this paper.

Funding

No funding was received from any financial organization to conduct this research.

Acknowledgments

Thank you to everyone who contributed to the completion of this study, and I thank the staff of the laboratories of the Civil Department of the University of Technology for paving the way for work and providing all necessary facilities.

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