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Original Research Article

Wastewater Removal Pollutants Using *Polyethylene terephthalate* Media : Moving Bed Biofilm Reactor

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Abstract

The increasing wastewater necessitates innovative wastewater treatment methods, such as anaerobic MBBR with PET as media, which enhance microbial degradation and biofilm formation. The aim was to analyze the rate of degradation kinetics in anaerobic MBBR reactors for biological wastewater treatment. This research examined three factors: the BOD, COD, and TSS. Domestic wastewater was used in this study. The reactor measured 40 × 40 × 50 cm and had a thickness of 4 mm. The construction was performed using glass material. The operation was performed for 30 days. Microorganisms grew and reproduced on the surface of the plastic bottle cap media during the anaerobic bioreactor seeding process by adding as many local microorganisms as 1.6/70 liters of wastewater. The study revealed that domestic wastewater used for wastewater treatment has BOD, COD, TSS, Ammonia, and Fat contents that exceed the set threshold value. The BOD/COD ratio was 0.55. After acclimatization, the biofilm was fully developed, effectively removing organic contaminants and producing fungal polysaccharides. In conclusion, the study of substrate concentration and degradation kinetics is crucial for system design and operation, emphasizing the need for substrate optimization to enhance microbial activity.

Keywords: Anaerobic; degradation kinetics; wastewater; pollutants, MBBR

1. Introduction

The increasing prevalence of plastic waste, particularly from polyethylene terephthalate (PET) products, poses significant environmental challenges, necessitating innovative wastewater treatment approaches that can simultaneously address plastic pollution and enhance the efficiency of wastewater treatment processes. The use of plastic materials, such as PET bottle caps, as media in anaerobic moving bed biofilm reactors (MBBRs) is a promising strategy for enhancing the microbial degradation of organic pollutants in wastewater. This approach leverages the unique properties of plastic media to promote biofilm formation, which is crucial for effective microbial activity and pollutant removal under anaerobic conditions. The microbial communities that develop on these plastic substrates can significantly influence the degradation processes, enhancing the overall efficiency of wastewater treatment systems (Khatoon et al., 2014).

Moreover, the diversity of bacterial populations associated with different types of plastic media can significantly influence the degradation pathways and efficiency of wastewater treatment systems. For example, research has indicated that varying the carrier materials in biofilm reactors can lead to distinct microbial community structures and functional capabilities, which are essential for the degradation of complex organic pollutants (Zheng et al., 2024). The adaptability of these microbial communities to different environmental conditions, including variations in nutrient availability and salinity, further underscores the potential of using plastic media in anaerobic MBBRs for wastewater treatment (Gagliano et al., 2017; Hudayah et al., 2021).

The selection of BOD, COD, and TSS as key parameters is based on their significance in assessing the organic load and treatment performance of wastewater. BOD measures the amount of oxygen required by microorganisms to decompose organic matter, providing insight into the biodegradable fraction of the wastewater (Subroto et al., 2022). COD quantifies the total amount of oxygen required to chemically oxidize organic and inorganic matter, thus serving as a broader indicator of water pollution (Shahzad et al., 2022). TSS represents the concentration of suspended particles in water, which can affect the efficiency of biological treatment processes by hindering light penetration and oxygen transfer (Subroto et al., 2022). Together, these parameters offer a comprehensive understanding of the wastewater's organic content and the effectiveness of the treatment process.

Research has demonstrated the effectiveness of biofilm reactors in treating industrial effluents, with studies showing significant reductions in COD and BOD using various biofilm carriers (Han et al., 2020; Proano-Pena et al., 2020). For instance, Han et al. reported the potential of biofilm reactors in treating recycled paper wastewater, highlighting the importance of selecting appropriate media for optimal pollutant removal (Han et al., 2020). However, the specific application of PET media in MBBRs remains underexplored, particularly concerning its comparative performance against other materials.

Moreover, while the literature discusses the advantages of different biofilm carriers, such as their surface area and microbial attachment properties, there is limited research focusing on the unique characteristics of PET as a biofilm support medium. The studies conducted on various biofilm carriers, including 3D-printed media and glass beads, suggest that the choice of carrier significantly influences microbial community structure and treatment performance (Proano-Pena et al., 2020). Yet, the specific interactions between PET media and microbial populations in MBBRs, particularly in the context of nutrient removal and pollutant degradation, warrant further investigation.

Another critical gap is the operational parameters that optimize the performance of PET-based MBBRs (Al Hosani et al., 2022; Kawan et al., 2022). While some studies have explored these parameters in relation to other biofilm carriers, the specific implications for PET media in MBBRs have not been thoroughly examined. This presents an opportunity for future research to establish optimal operational conditions that maximize the effectiveness of PET media in removing BOD, COD, and TSS.

The integration of polyethylene terephthalate (PET) media in Moving Bed Biofilm Reactors (MBBRs) for the removal of pollutants such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Suspended Solids (TSS) presents a significant area of research with notable gaps and potential for innovation. Current literature indicates a growing interest in utilizing various biofilm carriers, including PET, to enhance wastewater treatment efficiency. However, there remains a lack of comprehensive studies specifically addressing the performance of PET media in MBBRs concerning the simultaneous removal of BOD, COD, and TSS. This study aims to explore the potential for improved degradation of organic pollutants in wastewater, contributing to both environmental sustainability and resource recovery.

2. Methods

2.1. Research design

The research method used is pure experimental research by measuring and observing data directly. This research is laboratory scale where the reactor used has a batch system with an anaerobic

system. The Michaelis-Menten equation and linear regression were used to describe a model of the experimental results. The Michaelis-Menten equation is a fundamental model that describes the rate of substrate reactions. This model is particularly relevant in the context of wastewater treatment, where enzymatic processes are used to degrade pollutants. The application of Michaelis-Menten kinetics in wastewater treatment allows quantification of the efficiency of enzymatic reactions, which is essential for optimizing treatment processes. This study examined three factors: the biological oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids. BOD, COD, and TSS are crucial parameters in assessing wastewater organic load and treatment performance. BOD measures oxygen needed for microorganisms to decompose organic matter, COD measures oxygen needed for chemical oxidation, and TSS represents suspended particles in water, affecting biological treatment efficiency.

Domestic wastewater was used in this study. Importance of Domestic Wastewater in Research were 1. Prevalence and Composition: Domestic wastewater constitutes a substantial portion of total wastewater generated globally. It typically contains high levels of organic matter, nutrients (such as nitrogen and phosphorus), and pathogens. Understanding its composition is essential for developing effective treatment strategies. The study indicated that the BOD, COD, TSS, ammonia, and fat contents of the domestic wastewater exceeded regulatory thresholds, highlighting the need for efficient treatment methods. 2. Real-World Application: Research utilizing domestic wastewater provides insights into the performance of treatment technologies under realistic conditions. This is crucial for the scalability and applicability of laboratory findings to full-scale operations. The anaerobic MBBR reactor in this study was designed to mimic real-world scenarios, allowing for the assessment of microbial activity and biofilm formation in a controlled environment.

Although the processing outcome test was conducted for both types of waste water, the first test was conducted using wastewater obtained directly from a pollutant source. The model determination is based on the variables that affect the cause and effect of the decrease in the BOD, COD, and TSS levels. The Michaelis-Menten equation was used to calculate the kinetic parameters by determining the substrate concentrations of BOD, COD, and TSS, which are gradually produced during wastewater treatment. Km is obtained if the initial response speed in the Michaelis-Menten equation is exactly half of the maximum speed using equation (1)

$$Vo = \frac{Vmaks(S)}{S+KM} \quad (1)$$

2.2. Measurements of BOD, COD, TSS, Fat, Ammonia

2.2.1 Biochemical Oxygen Demand (BOD) Measurement

BOD is a measure of the amount of oxygen required by microorganisms to decompose organic matter in water over a specified period, usually 5 days at 20°C. BOD measurement is carried out with the following steps: - Sampling: Water samples are taken from representative locations and stored in tightly closed bottles to prevent gas exchange. - Sample Dilution: The water samples taken are usually diluted with treated (aerated) water to avoid overestimation. Dilution is carried out in an appropriate ratio, for example 1:1, 1:2, or 1:10, depending on the initial BOD concentration. - Initial Dissolved Oxygen Determination: Dissolved oxygen (DO) is measured using a DO meter or Winkler titration before incubation. - Incubation: The diluted sample is left in the dark at 20°C for 5 days. - Final Dissolved Oxygen Determination: After the incubation period, dissolved oxygen is measured again. - BOD Calculation: BOD is calculated using equation (2)

$$BOD_5 = DO_{\{initial\}} - DO_{\{final\}} \quad (2)$$

2.2.2. COD (Chemical Oxygen Demand) Measurement

COD is a measure of the amount of oxygen required to oxidize organic and inorganic materials in water using chemical oxidizing agents. Commonly used methods are the permanganate method and the dichromate method. Here are the steps for COD measurement using the dichromate method: - Sampling: As in BOD measurement, water samples are taken and stored properly. - Reagent Preparation:

The reagent used is potassium dichromate ($K_2Cr_2O_7$) in an acidic medium (usually with sulfuric acid). - Oxidizing Reaction: The water sample is mixed with the reagent and heated in an acidic condition for a certain time (usually 2 hours). - Titration: After the reaction is complete, the remaining unreacted potassium dichromate is titrated using sodium thiosulfate solution ($Na_2S_2O_3$) to the specified end point. - COD Calculation: COD is calculated based on the amount of potassium dichromate that reacts with the water sample. The COD calculation using equation (3)

$$\mathrm{COD} = \{(V_{\{Na2S2O3\}} \times N_{\{Na2S2O3\}} \times 8000)\}\{V_{\{sample\}}\}$$

Where:

$V_{\{Na2S2O3\}}$ is the volume of sodium thiosulfate used,

$N_{\{Na2S2O3\}}$ is the normality of the sodium thiosulfate solution, and

($V_{\{sample\}}$ is the volume of the water sample.

2.2.3. TSS (Total Suspended Solids) Measurement

TSS is a measure of the total solids suspended in water, which can be measured by the filtration method. Here are the steps for TSS measurement:

- ✓ Sampling: Water samples are taken and mixed evenly to ensure representativeness.
- ✓ Filtration: The water sample is filtered using filter paper with suitable pores (usually 0.45 microns) to separate the suspended solids from the water.
- ✓ Drying: The filter paper containing the solids is dried in an oven at 105°C for 1-2 hours until constant weight.
- ✓ Weighing: After drying, the filter paper is weighed to determine the weight of the suspended solids.

  TSS is calculated using equation (4)

$$\mathrm{TSS} = \{(W_{\{solids\}})\}\{V_{\{sample\}}\} \times 1000$$

  Where

  ($W_{\{solids\}}$): is the weight of the solids remaining on the filter paper (in mg)

  ($V_{\{sample\}}$): is the volume of the water sample (in liters)

2.2.4. Fat Measurement

The gravimetric method is used to measure fat. This procedure involves precipitation of fat using a solvent, followed by weighing. The steps are

- ✓ Precipitation: The wastewater sample is mixed with a suitable solvent to precipitate the fat.
- ✓ Filtration: The mixture is then filtered to separate the fat from the liquid phase.
- ✓ Drying and Weighing: The precipitated fat is dried and weighed to determine the fat concentration.

2.2.5. Ammonia Measurement

The method used is spectrophotometry. This method involves measuring the absorbance of light at a specific wavelength. The general procedure is:

- ✓ Sample Preparation: A wastewater sample is taken and filtered to remove solid particles.
- ✓ Chemical Reaction: The sample is mixed with a reagent that will react with ammonia to form a colored compound.
- ✓ Measurement: The absorbance of the solution is measured using a spectrophotometer at a suitable wavelength. T

  The ammonia concentration can be calculated based on a calibration curve.

2.3. Reactor Set Up and operation

The reactor measured 40 × 40 × 50 cm and had a thickness of 4 mm. The construction was performed using glass material. The reactor had a capacity of 80 liter capacity. PVC pipes were considered

when designing wastewater input and output. The operation was performed for 30 days. The primary tank held 1.6 L local microorganisms, 70 L wastewater, and 8.4 L anaerobic reactor material. The PET plastic bottle cap medium was 3 cm in diameter and 28.26 cm a surface area. The reason for taking this size is by looking at previous research that successfully reduced the pollutant load by using a reactor with a volume of 0.8 liters (Paul and Hall, 2021). then we expanded the volume to 80 liters with the aim of seeing whether a reactor with a larger size is able to be a good reactor in this wastewater treatment system. This will also be used for the development of a real-scale reactor. The combined surface area of all media and each medium (500 plastic bottle caps per reactor × 28.26 cm^2) was 14,130 cm^2. The reactor is located in a greenhouse. To ensure that the reactor ran correctly, a reactor design test was conducted to ensure compliance with the study conditions and methods. The reactor is then filled with bioactivators, which are generated from fermented fruit peel waste that has become liquid and contains bacteria and yeasts. "Local microbes" is the term used to describe this fermented liquid. The incorporation of local bacteria in bioreactor systems for the production of bioactivators from fermented fruit peel waste offers numerous advantages, including ecological adaptation (Manzano-Gómez et al., 2023), functional diversity (Cai et al., 2021), cost-effectiveness (Rodriguez-Gonzalez et al., 2021), sustainability (Lyu et al., 2024), and enhanced fermentation performance (Duran-Cruz et al., 2024). Each data point was plotted on a database-created graph following the data collection. Detailed description of the media and reactor is shown in Table 1.

Table 1. Description of media and reactors

Variabel	Description
Media	
Media height	1.5 cm
Diameter	3 cm
Total media in the reactor (plastic bottle caps)	500 pieces
Surface area of media	28.26 cm^2
The total surface area of the media	14,130 cm^2
Media type	PET
Main Reactor	
Material	Glass
Length	40 cm
Wide	40 cm
Heigth	50 cm
Volume	80.000 cm^3 / 80 L
Gas Tank	
Height	15 cm
Diameter	40cm
Volume	6280 cm3 / 6,28 litre

Microorganisms grow and reproduce on the surface of plastic bottle cap media during the anaerobic bioreactor seeding process by adding as many local microorganisms as 1.6 liters / 70 liters of wastewater. The purpose of this procedure is to accelerate the growth of microorganisms in wastewater. During acclimatization, the biofilm structure on the biofilter media thickened. When the concentration of contaminants decreased, the acclimatization process was complete. The time required for the decomposition of organic compounds in wastewater is reduced by the presence of local microorganisms. Biofilm formation occurs on the 3rd to 15th days in polyethylene media, which has a service life of more than 15 years. Further details of the anaerobic reactor are presented in Figure 1.

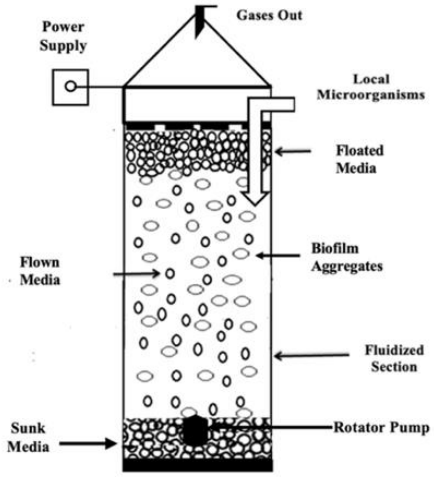


Figure 1. MBBR Anaerobic reactor using bottle caps media

2.3.1. Scanning electron microscopy (SEM)

Biofilms measuring 1 cm were cut for examination using scanning electron microscopy (SEM). The samples were fixed by formaldehyde impregnation. To prevent shrinkage, the biofilm samples were soaked in 20, 40, 60, 80, and 100% ethanol for various times before drying. Using an ion-spraying device, the dried samples were sputtered with gold to increase their conductivity, and SEM imaging was performed. A high-energy electron beam was fired by an electron cannon into the conductive samples placed on the SEM sample stage while the samples were under vacuum. Three-dimensional images were created by amplifying the electron beam signal and sending it to the monitor.

The preparation and examination of biofilms using scanning electron microscopy (SEM) involve several critical steps that enhance the quality and reliability of the imaging results. The fixation of biofilm samples with formaldehyde is essential for preserving the structural integrity of the biofilm matrix and the microbial cells within it. Formaldehyde acts as a cross-linking agent, stabilizing proteins and cellular structures, which is crucial for maintaining the natural architecture of the biofilm during subsequent processing and imaging. This preservation is particularly important given the complex and often delicate nature of biofilms, which are composed of diverse microbial communities embedded in a self-produced extracellular matrix (Dassanayake et al., 2020).

To prevent shrinkage during the drying process, the samples are subjected to a series of ethanol treatments (20%, 40%, 60%, 80%, and 100%). Ethanol dehydration is a common practice in microscopy, as it gradually removes water while minimizing osmotic shock that could lead to structural collapse. This step is vital for ensuring that the biofilm retains its three-dimensional architecture, which is critical for accurate morphological analysis. The sputtering of gold onto the dried samples enhances their conductivity, which is necessary for effective SEM imaging. Non-conductive samples can lead to charging effects during imaging, resulting in poor-quality images and inaccurate interpretations of the biofilm structure (Dassanayake et al., 2020).

The SEM function in this study serves multiple purposes. Primarily, it allows for high-resolution imaging of the biofilm's surface morphology and architecture, providing insights into the spatial arrangement of microbial cells and the extracellular matrix. This imaging capability is crucial for understanding the biofilm's structural characteristics, which can influence its resistance to antimicrobial agents and its overall pathogenicity. Additionally, SEM can reveal details about the biofilm's thickness, density, and the presence of specific microbial species, which are essential for evaluating the biofilm's functional properties and its interactions with the surrounding environment (Dassanayake et al., 2020).

3. Result and Discussion

The BOD concentration decreased from 197 mg/L on Day 0 to 31 mg/L by Day 30, indicating a removal efficiency of approximately 84.3%. This significant reduction reflects the biofilm's ability to metabolize organic matter effectively. Similarly, the COD concentration showed a decline from 354 mg/L to 107 mg/L over the same period, resulting in a removal efficiency of about 69.8%. The reduction in COD is crucial as it indicates the effectiveness of the reactor in degrading organic pollutants. The TSS concentration decreased from 127 mg/L to 39 mg/L, achieving a removal efficiency of approximately 69.1%. This reduction highlights the reactor's capability to settle and remove suspended solids effectively. More Detail can be seen in table 2.

Table 2. BOD, COD, and TSS Outlet

BOD Outlet	COD Outlet	TSS Outlet	Day	Temperature	pH
197	354	127	0	25.0	6.7
190	353	123	1	30.0	6.7
180	333	115	2	32.0	6.7
185	346	127	3	32.0	6.7
177	340	119	4	32.0	6.7
183	344	120	5	33	7.0
180	339	125	6	33	7.0
185	341	118	7	33	7.0
176	334	120	8	33	7.0
179	342	113	9	33	7.0
167	331	114	10	33	7.0
165	334	119	11	33	7.0
163	307	97	12	32.0	6.7
152	290	91	13	32.0	6.7
141	278	83	14	32.0	6.7
131	258	78	15	32.0	6.7
126	242	77	16	34	6.7
116	222	73	17	34	7.5
108	225	75	18	34	7.5
97	187	63	19	34	7.5
84	154	57	20	34	7.5
75	145	51	21	34	7.5
65	136	49	22	34	7.5
54	127	47	23	34	7.5
44	118	43	24	35	7.7
34	109	40	25	35	7.7
32	108	40	26	35	7.7
32	108	40	27	35	7.7
31	107	40	28	35	7.7
31	107	39	29	35	7.7
31	107	39	30	35	7.7

Table 3 shows that domestic wastewater used as an inlet source for wastewater treatment has BOD, COD, TSS, Ammonia, and Fat contents that do not meet the requirements and exceed the set threshold value. The BOD/COD ratio was 0.55. Therefore, this waste material is suitable for various biological applications. If the BOD/COD ratio is between 0.3 and 0.6, the most suitable treatment is biological treatment (Ali Ch. Bader et al., 2022).

Table 3. Characteristics of wastewater used as influent in wastewater treatment systems

Parameter	Unit	Value	Standard
BOD	mg/l	197	30
COD	mg/l	354	100
TSS	mg/l	133	30
pH	-	6.5	6-9
Oils and Fats	mg/l	7.1	5
Ammonia	mg/l	29.2	10

Following the SEM investigation, the biofilm that developed on the surface of the plastic bottle cap medium is depicted in Figure 2. SEM images were taken at magnifications of 500X and 5,000X. A 500X magnification of the biofilm developed on the carrier media is shown in Figure 2a. A well-formed bacterial biofilm was visible on the media surface, according to the SEM measurements. One free-floating bacterium landing on the surface, bacterial cell aggregation and attachment, bacteria growing and dividing to form a biofilm, the formation of a mature biofilm, and some biofilms spreading to loosen free-floating bacteria for additional colonization are the five steps involved in biofilm growth. After the acclimatization stage, the biofilm was fully developed, and organic contaminants were effectively removed. Figure 2b shows mature biofilms that produce distinctive fungal polysaccharides. At this point, cells begin to disintegrate and return to their planktonic state, sticking to fresh surfaces to form new biofilm layers.

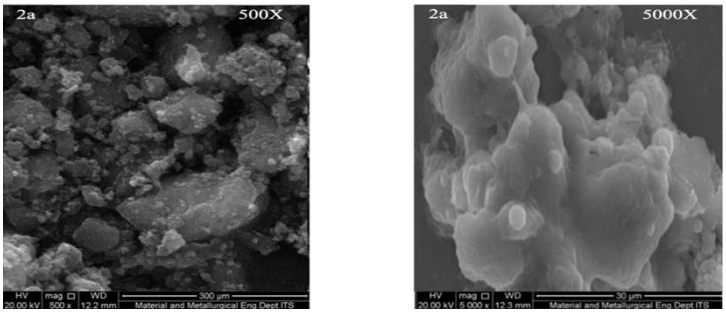


Figure 2. SEM analysis of the biofilm

The existence of a well-formed bacterial biofilm was confirmed by SEM investigation of the surface of the plastic bottle cap medium. Bacterial cell aggregation, growth and division, mature biofilm formation, spreading, and free-floating bacterial landing are the five steps in this process (Zhao et al., 2023). Following acclimation, the biofilm efficiently eliminated organic pollutants and the mature biofilm adhered to new surfaces and produced fungal polysaccharides. By efficiently eliminating organic pollutants, biofilms that form on the surface of the plastic bottle cap media can contribute to better water quality. Maintaining the purity and quality of water depends on the biofilm formation process (Waqas et al., 2023). Additionally, this procedure helps stop the spread of illnesses caused by organic pollutants and improve environmental health. Therefore, the production of biofilms is a successful method for managing water quality (Papciak et al., 2022).



Figure 3. Stages of biofilm development

Biofilm growth was quite fast in reactors, and on days 1 and 2, a layer of mucus with an uneven yellow plaque began to appear on the surface of the media. There was a significant difference in biofilm formation in reactors. The biofilm layer appeared quite solid, and even on day 15 and above. However, on day 10, reactors plaque and a layer of brownish-yellow mucus formed evenly over the entire surface of the media. biofilm formation tends to be centered on the inner layer of the plastic bottle cap, which is protected by the friction and movement of other media. This prevents the destruction of biofilms formed by friction from media that move against each other. The biofilm formed very densely on day 30, when almost the entire surface of the medium was covered by the biofilm. The rapid growth of biofilm in the reactors can be attributed to several factors: - Nutrient Availability: The presence of nutrients in the reactor media likely facilitated microbial growth, leading to the initial formation of the mucus layer. - Surface Characteristics: The plastic material of the reactor may have provided a favorable surface for microbial adhesion, contributing to the uneven distribution of biofilm. - Hydrodynamic Conditions: The movement of media within the reactor may have created microenvironments that favored biofilm development in certain areas, particularly where shear stress was minimized. The transition from an uneven yellow plaque to a more uniform brownish-yellow layer indicates a shift in microbial community structure and metabolic activity. The dense biofilm observed on day 30 suggests a mature community capable of withstanding environmental stresses. The following are the results of the Michaelis-Menten equation modeling. Under anaerobic conditions, there is a decrease in the rates of BOD, COD, and TSS. For more details, see Figure 4 a,b,c.

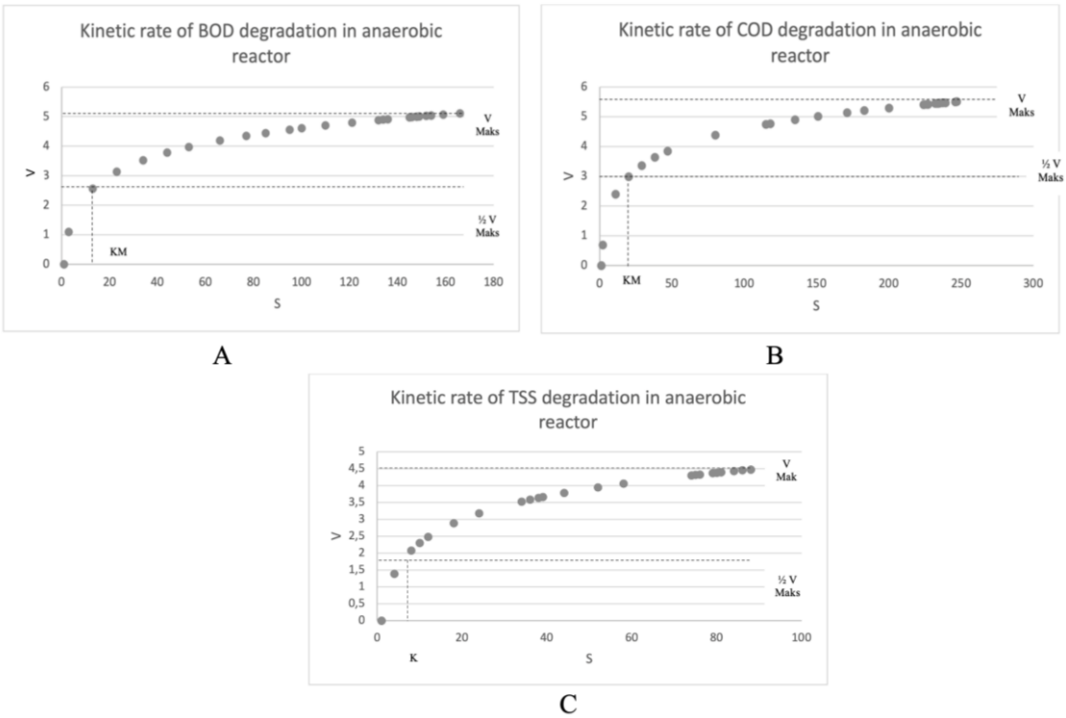


Figure 4. The kinetic rate of degradation of BOD (a), COD (), and TSS (c) using the Menten model

Figure 4 A shows that at a substrate concentration (BOD) below 20, the degradation kinetic rate was still 2.6 or ½ V. The degradation kinetic rate increased at substrate conditions (BOD) above 20 to reach 5.2. This indicates that a high substrate concentration (BOD) increases the substrate degradation kinetic rate (BOD). More details can be seen in Figure 4 A. Figure 27 B shows that at substrate concentration (COD) below 20 the degradation kinetic rate is still at 2.9 or ½ V. The degradation kinetic rate increased under substrate conditions (COD) above 20, reaching 5.8. This shows that a high substrate concentration (COD) increased the substrate degradation kinetic rate (COD). more details can be seen in Figure 4 B. Figure 4 C shows that at substrate concentrations (TSS) below 20 the degradation kinetic rate is still at 2.25 or ½ V. the degradation kinetic rate increases at substrate conditions (TSS) above 20 to reach 4.5. This shows that high substrate concentrations (TSS) increased the substrate degradation kinetic rate (TSS). Further details are presented in Figure 4 C.

The kinetic behavior of anaerobic reactors is intricately linked to substrate concentration, particularly in terms of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Suspended Solids (TSS). Understanding these relationships is crucial for optimizing degradation processes that occur within anaerobic systems. Research indicates that as substrate concentrations increase, microbial activity and degradation efficiency also increase, particularly when concentrations exceed a threshold of approximately 20 g/L. This observation aligns with the model, which describes how reaction rates are influenced by substrate concentration until a saturation point is reached, beyond which additional increases in substrate yield diminishing returns in reaction rates (Sun et al., 2013).

The model serves as a foundational framework for understanding microbial kinetics in anaerobic digestion. It posits that the specific growth rate of microorganisms is a function of the concentration of a limiting substrate and is typically expressed as a hyperbolic relationship. As substrate concentration increases, the growth rate approaches a maximum, reflecting the saturation of microbial metabolic pathways (Mani et al., 2016). This model has been validated in various studies focusing on anaerobic digestion processes, including those involving complex organic matte. For example, the degradation of volatile fatty acids (VFAs) in anaerobic digesters has been effectively modeled using kinetics, demonstrating the model's applicability across different types of organic substrates (Mani et al., 2016).

Moreover, the interaction between the substrate concentration and microbial community dynamics is a critical factor in the efficiency of anaerobic reactors. High substrate availability can lead to increased microbial diversity and activity, which in turn enhances the degradation of organic matter(Chen et al., 2022). For example, studies have shown that the presence of complex substrates can promote a more diverse microbial community, which is beneficial for the breakdown of various organic compounds(Chen et al., 2022). This complexity is essential for maintaining a stable reactor performance, especially under varying operational conditions.

The relationship between the substrate concentration and degradation kinetics in anaerobic digestion is a critical aspect that influences the efficiency of biogas production. Anaerobic digestion is a complex biochemical process involving various microbial communities that convert organic matter, primarily methane and carbon dioxide, into biogas. The concentration of substrates, often measured in terms of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), plays a significant role in determining degradation kinetics. At low substrate concentrations, specifically below a BOD of 20, microbial activity tends to stabilize at a low degradation rate, indicating substrate-limited conditions in which microbial growth is constrained by the availability of organic matter.

As the substrate concentration increased beyond this threshold, the degradation kinetic rate increased significantly. This phenomenon can be attributed to the enhanced metabolic activity of the microbial community, which effectively utilizes available substrates. For example, studies have shown that when BOD levels rise above 20, there is a corresponding increase in the degradation kinetic rate, which is also reflected in COD analyzes (Su et al., 2023). This trend underscores the importance of optimizing substrate concentrations to enhance biogas production and overall reactor performance, as

higher substrate availability can lead to improved microbial activity and, consequently, greater methane yields (Jia et al., 2016).

The dynamics of microbial communities in anaerobic digesters are influenced by substrate concentrations, which affect not only the rate of degradation, but also the composition of the microbial populations involved in the process. For example, higher substrate concentrations can lead to shifts in the relative abundance of specific bacterial and archaeal lineages, which are crucial for the methanogenic phase of anaerobic digestion (Vanwonterghem et al., 2016). The presence of certain methanogens, such as Methanosarcina and Methanoculleus, has been linked to the ability of the microbial community to adapt to varying substrate loads, thereby enhancing the overall efficiency of the digestion process (Dottorini et al., 2021).

Moreover, the interaction between substrate concentration and microbial community dynamics is further complicated by factors, such as temperature, retention time, and the presence of inhibitory compounds. For example, elevated concentrations of volatile fatty acids (VFAs), resulting from substrate overloading, can inhibit methanogenesis, leading to a decline in biogas production. Conversely, optimizing the operational conditions, including the acclimatization of microbial consortia to higher substrate levels, can mitigate these inhibitory effects and promote a more stable and efficient anaerobic digestion process (Khafipour et al., 2020).

In addition to the direct effects of substrate concentration on microbial kinetics, substrate type also plays a crucial role in determining the efficiency of anaerobic digestion. Different organic materials, such as food waste, agricultural residues, and animal manures, exhibit varying biodegradability and methane potential. For example, co-digestion strategies that combine substrates with complementary characteristics can enhance the overall performance of anaerobic digesters by improving nutrient balance and microbial interactions (Naphtali et al., 2022). This approach not only maximizes biogas production but also contributes to the stabilization of the digestion process under fluctuating operational conditions.

The implications of substrate concentration on anaerobic digestion extend beyond biogas production to include the quality of the digestate produced. Digestate, which is the residual material after anaerobic digestion, can serve as a valuable biofertilizer; however, its quality is influenced by the substrate composition and the efficiency of the degradation process. High concentrations of certain substrates can lead to the accumulation of undesirable compounds in the digestate, such as pathogens and heavy metals, which may pose risks to soil health and crop production (Zieliński et al., 2021). Therefore, careful management of substrate concentrations is essential not only for optimizing biogas yields but also for ensuring the safety and efficacy of the resulting digestate.

The use of anaerobic conditions also has several advantages, particularly in reducing greenhouse gas production. Therefore, the selection of waste treatment conditions must be adjusted according to the type of waste and final purpose of the process. In addition, anaerobic conditions can also reduce unwanted odor emissions from organic waste (González et al., 2022; Zhu et al., 2021). Selecting the right conditions will ensure the overall efficiency of the waste treatment process (Ćetković et al., 2023; Singh et al., 2023).

The presence of certain inhibitors in wastewater can also affect the degradation kinetics in anaerobic reactors (Khan et al., 2021; Komolafe et al., 2021). These inhibitors can be heavy metals, toxic chemicals, or high concentrations of certain compounds that can inhibit the activity of microbes responsible for breaking down organic matter (Angon et al., 2024). Therefore, it is important to carefully monitor and control the composition of wastewater entering the anaerobic reactor to ensure efficient treatment. By identifying and reducing the presence of inhibitors, operators can improve overall system performance and achieve better treatment results (Mathur et al., 2024).

The average temperature in the reactor is known to be 33.1°C, which allows for the development of mesophilic bacteria (Yin et al., 2014). After being turned on, the anaerobic reactor was operated in batches, and acclimatization in the anaerobic reactor was achieved after 10 days as indicated by a consistent decrease in COD, BOD, and TSS. The simultaneous metabolic activity that occurs during the

anaerobic process is responsible for this, as the CO_2 produced as a by-product is partially dissolved in the wastewater to form bicarbonate, which increases the capacity of the wastewater to buffer pH (Khadaroo et al., 2020).

The formation of CO_2 in this process is very helpful in stabilizing the overall pH, and the average pH in the reactor is 7.2. The high organic removal rate of the anaerobic co-digestion reactor can be attributed to its neutral pH value (7.2), which is effective for biodegradation and/or decomposition processes of the organic materials. This is in agreement with studies that have found pH to be a key factor for the success of the biodegradation process (Darwin et al., 2021).

4. Conclusions

The study demonstrates significant reductions in BOD, COD, and TSS concentrations in an anaerobic reactor over a 30-day period, indicating effective organic matter removal by the biofilm. BOD decreased from 197 mg/L to 31 mg/L (84.3% removal), COD from 354 mg/L to 107 mg/L (69.8% removal), and TSS from 127 mg/L to 39 mg/L (69.1% removal). The BOD/COD ratio of 0.55 suggests suitability for biological treatment. SEM analysis revealed a well-formed bacterial biofilm on the plastic bottle cap medium, which underwent five growth stages, enhancing pollutant degradation. The study highlights the influence of substrate concentration on degradation kinetics, with increased rates observed above 20 g/L for BOD, COD, and TSS. Optimal conditions, including nutrient availability and reactor design, facilitated rapid biofilm development, crucial for maintaining water quality and mitigating organic pollutant spread. The findings underscore the importance of managing substrate concentrations and operational parameters to enhance anaerobic digestion efficiency and biogas production while ensuring digestate quality.

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