JOURNAL OF BIOLOGY EDUCATION
E-ISSN 2656-3436/ P-ISSN 2615-3947
IAIN KUDUS
http://journal.iainkudus.ac.id/index.php/jbe

Assessment of Chemical Oxygen Demand Removal Efficiency and Microbial Dynamics during Aerobically Degradation of Wastewater in Activated Sludge

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ABSTRACT
This study investigated aerobically chemical oxygen demand (COD) removal and microbial dynamics during activated sludge treatment of wastewater. The 12-days experiment used a descriptive analysis to correlate operational parameters of pH, F/M ratio, MLSS, and SVI with COD removal efficiency. Microscopic examination identified temporal shifts in abundances of bacterial flocs, flagellates, ciliates, and rotifers. COD removal declined from 79% on day 0 to 4% by day 12, corresponding with increased SVI and protozoan proliferation. High SVI caused biomass washout, reducing bacterial catabolic capacity. Predominance of bacterivorous protozoa and rotifers likely suppressed floc-forming bacteria responsible for COD biodegradation. Conserving balanced mixed communities through optimized operational conditions appears decisive for continuous treatment performance. This study provides comprehensions on microbial ecological interactions altering temporal variability in activated sludge COD removal.

Keywords: activated sludge, COD removal, microbial ecology

INTRODUCTION

Wastewater is one of the prominent environmental predicaments facing both municipal and industrial segments globally. Municipal wastewater is engendered from domestic activities such as bathing, cleaning, and washing, whereas industrial wastewater originates from manufacturing processes. These wastewaters comprehend upraised levels of organic matter which is typically enumerated as chemical oxygen demand or COD (Khumalo *et al*., 2022). COD is quantified in milligrams per liter (mg/L) and denotes the amount of oxygen required to chemically oxidize the organic matter content in a sample utilizing dichromate under acidic conditions (Tchobanoglous *et al*., 2014). When COD levels in wastewater excessed and released into the environment without further treatment, it can cause eutrophication of surface waters and degradation of water quality (Boguniewicz-Zablocka *et al*., 2020; Harefa *et al*., 2021). Therefore, COD reduction is an imperative decisive factor in wastewater treatment before discharge (Boguniewicz-Zablocka *et al*., 2020; S. S. Lin *et al*., 2021).

Various practices have been reconnoitered for COD removal embracing physical-chemical methods, advanced oxidation processes and biological treatments such as activated sludge, moving bed biofilm reactor (MBBR), sequencing batch reactor (SBR), membrane bioreactor, and ozonation (Khalidi-Idrissi *et al*., 2023; Meena *et al*., 2022). Among them, activated sludge process is more efficient, relatively low operation costs, simple process, and able to treat varied industrial wastewaters (Jagaba, Kutty, Baloo, *et al*., 2022; Jagaba, Kutty, Noor, *et al*., 2022; Khalidi-Idrissi *et al*., 2023; Meena *et al*., 2022; Mirbolooki *et al*., 2017). It employs microorganisms grow as bacterial flocs in an aeration tank to biologically degrade organic matter when sufficient oxygen is provided (Seviour and Nielsen, 2010; Shchegolkova *et al*., 2016). This renovates COD into harmless end-products and abates sludge production from treatment (Alexandre *et al*., 2016).

The quintessential operating parameters that stimulus COD removal performance and microbial community structure embrace mixed liquor suspended solids (MLSS), food to microorganism (F/M) ratio, and sludge volume index (SVI) (Bitton, 2005; Tchobanoglous *et al*., 2014; Waqas *et al*., 2023). The MLSS is the concentration of suspended biomass in the aeration tank measured as mg/L. The F/M ratio defines the amount of food or organic loading applied per unit biomass. The SVI measures the settleability of the activated sludge and higher values indicate poorer settling (Bitton, 2005; Tchobanoglous *et al*., 2014).

COD removal implementation in activated sludge is inspired by microbial community dynamics (Lee *et al*., 2019; S. Liu *et al*., 2023; Shi *et al*., 2021). Floc-forming bacteria are responsible for degradation of organic matter, but their populations can be suppressed by protozoa and rotifers that prey on bacteria (Bitton, 2005; Fyda *et al*., 2015; Sun *et al*., 2018). This decreases the catabolic capacity of activated sludge (Fyda *et al*., 2015). Numerousness of parasitic protozoa also selects for bacterial morphotypes more resistant to predation though less effective for COD removal (Modak *et al*., 2007). Thus, conserving and sustaining a sensible diversified community is notable and influential for sustainable execution in biological COD removal (Chen *et al*., 2021; Shchegolkova *et al*., 2016; Shi *et al*., 2021; Smith *et al*., 2003).

The contemporaneous investigation aimed comprehensively to examine COD removal and associate microbial ecological shifts during 12-days activated sludge treatment of wastewater (Lateef *et al*., 2013) under controlled operational parameters such as MLSS, F/M ratio and SVI (Bitton, 2005). Descriptive analysis correlated these variables to COD removal efficiency. Microscopic examination identified shifts in abundances of bacterial flocs, flagellates, ciliates, and rotifers over time. The experiment was carried out in an Environmental Microbiology Laboratory with an aerobic degradation process within a 10L bioreactor of activated sludge. This provides insights into treatment efficiency fluctuations in relation to changes in microbial community constitutions and their relations.

METHOD

Research Location and Operational Parameters

This study devoted an experimental research design to comprehensively examine the COD removal performance and associate microbial community dynamics under various operational parameters such as Mixed Liquor Suspended Solid (MLSS), Food to

Microorganism (F/M) ratio, and Sludge Volume Index (SVI). The experiment was conducted over 12 days as normally ranged and also yielded to the high treatment efficiency (Lateef *et al.*, 2013) without iteration of experimental measurement conducted in the Environmental Microbiology Laboratory of Department of Environmental Engineering, Faculty of Engineering, King Mongkut's University of Technology Thonburi, Bangkok, Thailand.

Materials

Wastewater sample was obtained from a full-scale municipal wastewater treatment plant (WWTP) in Bangkok and acclimatized in the lab for 12 days prior to experiment. To represent domestic wastewater, vitamilk (soy milk), urea, NaH_2PO_4, and $FeCl_3$ were added to the municipal wastewater sample.

Aerobic Degradation Process

The aerobic degradation experiments start with adding 4 L of water to level the volumes inside the 10 L reactor, 5 mL Vitamilk (soy milk), 100 mL seed from Thung Khru WWTP then add supplements which including 11 mL urea, 4 mL NaH_2PO_4, 2 mL $FeCl_3$ and mixed all components well, configure the air diffuser and aeration device to the reactor. At day 0, conduct the measurement of several operational parameters such as MLSS, pH, SV_{30}, microscopic examination and the measurement of COD at 0, 3. 6, 9, and 24 hours. The procedure still continues until day 12 while the reactor is aerating, every single day before the measurement of several operational parameters is conducted, turn off the air diffuser for aeration, wait until the sludge settle down for half an hour, after which withdraw about 1/3 -1/2 of the supernatant from the reactor, continue with refilling the 4 L waste water sample from Thung Khru WWTP, and repeat this process every day, and investigate all operational parameters required every 2 days, which is the collection sample should be done before withdrawing. On the last day of the investigation, which is day 12, conduct the same measurement of several operational parameters such as MLSS, pH, SV_{30}, microscopic examination, and the measurement of COD at 0, 3. 6, 9, and 24 h. The data from measurement of several operational parameters such as MLSS, pH, SV_{30}, microscopic examination and the measurement of COD at 0, 3. 6, 9, and 24 h on days 0 to 12 will be used to generate a COD generation curve along time on days 0 and 12.

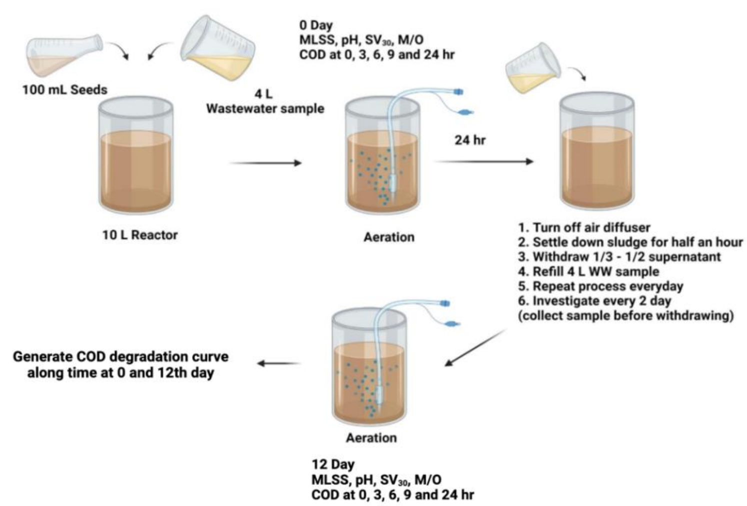


Figure 1. Aerobic degradation test

COD Measurement

Samples were taken from each reactor bottle at 0, 3, 6, 9, and 24 hours. COD concentration was measured using standard closed reflux titrimetric method to determine the percentage of COD removal as shown in Figure 2. The closed reflux, titrimetric method is a standard technique for measuring chemical oxygen demand (COD) in wastewater samples. It involves chemically oxidizing the organic matter, with dichromate serving as the oxidant under acidic conditions. The amount of dichromate consumed is proportional to the COD (Baird *et al*., 2017).

Firstly, to analyze "soluble COD", the samples are filtered through a 0.45-mm filter before analysis to remove biological interference. To begin, 10 mL of filtrated sample is added to a COD reagent vial containing 6 mL 0.25N potassium dichromate solution and 15 mL sulfuric acid reagent which contains Ag_2SO_4 and conc. H_2SO_4 (for blank, only conc. H_2SO_4 is used) is added, composed of silver sulfate in concentrated sulfuric acid. The sample reagent flask is placed into an oven and for 2 hours with temperature of 150°C to complete oxidation (Baird *et al*., 2017).

After cooling, the contents are titrated with standardized 0.1 N ferrous ammonium sulfate (FAS) to determine the residual dichromate. During titration, 3 drops ferroin indicator is added, causing a color change from blue green to reddish brown at the endpoint. A blank with deionized water is concurrently analyzed (Baird *et al*., 2017).

The COD in mg/L is computed as shown in Equation 1 where A and B are ferrous ammonium sulfate volumes for the blank and sample, respectively, and N is the normality of ferrous ammonium sulfate. Digestion causes chloride interference in the analysis of COD (Baird *et al*., 2017).

The COD measurement (Baird *et al*., 2017) can be computed by

$$\text{COD (mg/L)} = \frac{(B - A) \times N \times 8000}{\text{mL sample}} \quad (1)$$

A = mL of FAS titrant used for sample
B = mL of FAS titrant used for blank
N = Normality of FAS from standardization

Then, the normality of FAS (N) (Baird *et al*., 2017) in Equation 1 can be assessed by

$$\text{Normality of FAS} = \frac{6\text{mL} \times 0.1\text{N}}{\text{mL FAS used}} \quad (2)$$

Hence, COD removal efficiency (Baird *et al*., 2017) can be calculated by

$$\text{COD removal (\%)} = \frac{\text{COD}_{\text{initial}} - \text{COD}_{\text{remaining}}}{\text{COD}_{\text{initial}}} \quad (3)$$

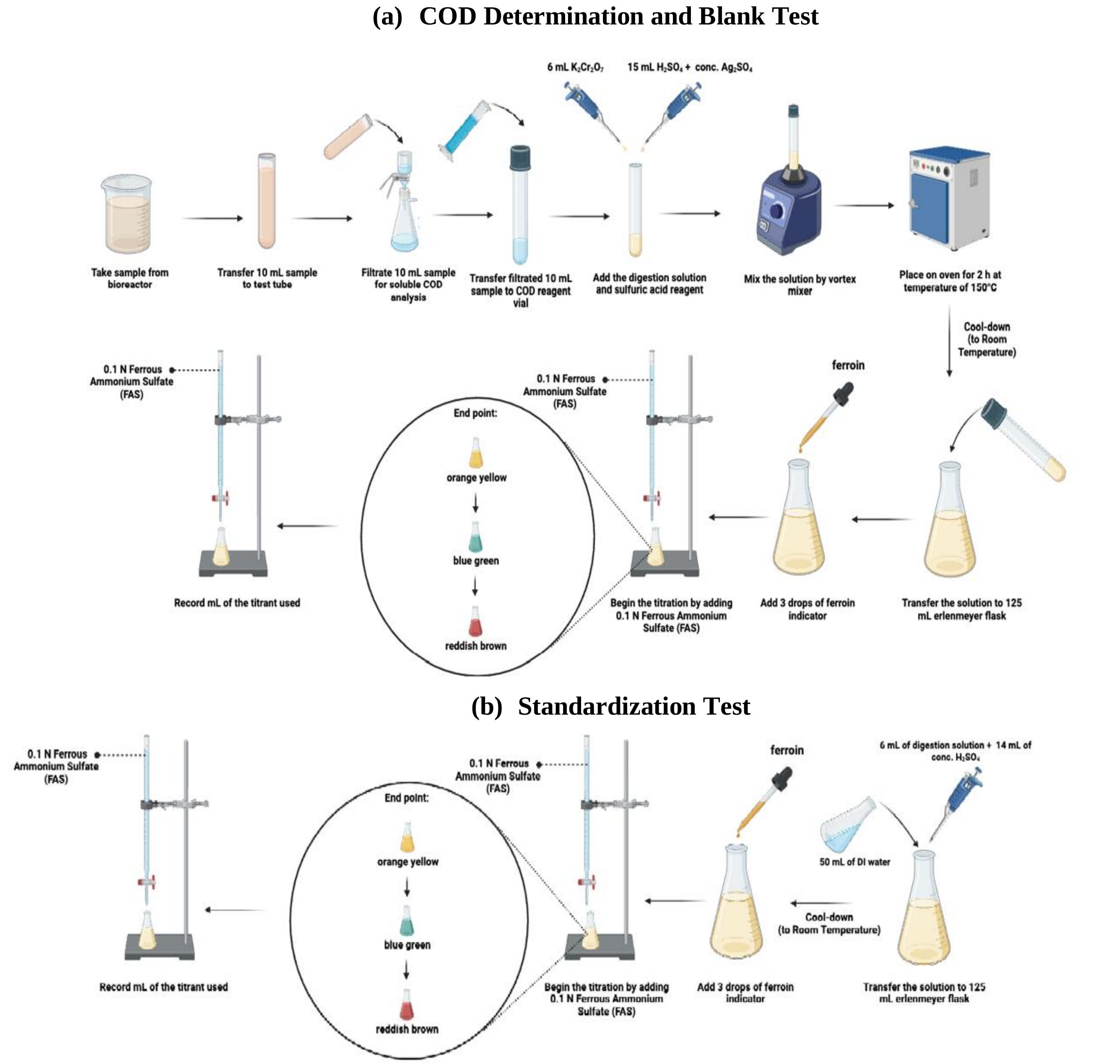


Figure 2. COD determination by closed reflux titrimetric method: (a) COD determination and blank test and (b) standardization test

Microbial Dynamics Analysis

For microbial analysis, samples were stained using wet staining technique and observed under light microscope equipped with digital camera connected to computer. The qualitative examination was conducted to identify presence and abundance of different microbial morphologies including bacterial flocs, flagellates, free-swimming and stalked ciliates.

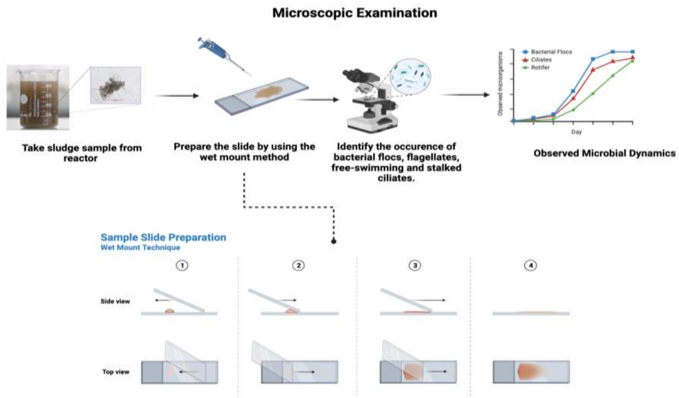


Figure 3. Microscopic examination of sludge

RESULT AND DISCUSSION

COD Removal Efficiency

The COD removal efficiency during the 12 days of activated sludge treatment of wastewater by utilizing the aerobic degradation experiment are presented in Table 1 and Figure 4. On day 0, the initial COD concentration of the synthetic wastewater was 79.52 mg/L. The COD decreased over time during 24-hour monitoring period on day 0, with a removal efficiency reaching 78.57% after 24 hours. The initial rapid COD reduction indicated the acclimation of the activated sludge microbial community to utilization of the synthetic substrates. The maximum COD reduction rate of 73.2% occurred between 6 to 9 hours, indicating the exponential growth phase of microbial community during initial start-up and acclimatization.

Table 1. COD removal on day 0 and 12

Day	Time (hrs)	COD (mg/L)	COD removal (%)
0	0	79.52	0.00
	3	68.16	14.29
	6	56.8	28.57
	9	21.3	73.21
	24	17.04	78.57
12	0	83.6	-5.13
	3	45.6	42.66
	6	68.4	13.98
	9	76	4.43
	24	91.2	-14.69

The COD removal profile fits a typical microbial growth curve with an initial lag phase from 0-3 hours as microbes adapt to the new substrate or environment (Mirbolooki *et al*., 2017; Shi *et al*., 2021). This is followed by logarithmic growth between 3-9 hours as populations rapidly utilize available organic matter, reflected in the high COD elimination rate. A declining growth rate occurs after 9 hours as substrates become limited, yielding lower COD removal by 24 hours as shown at day 12.

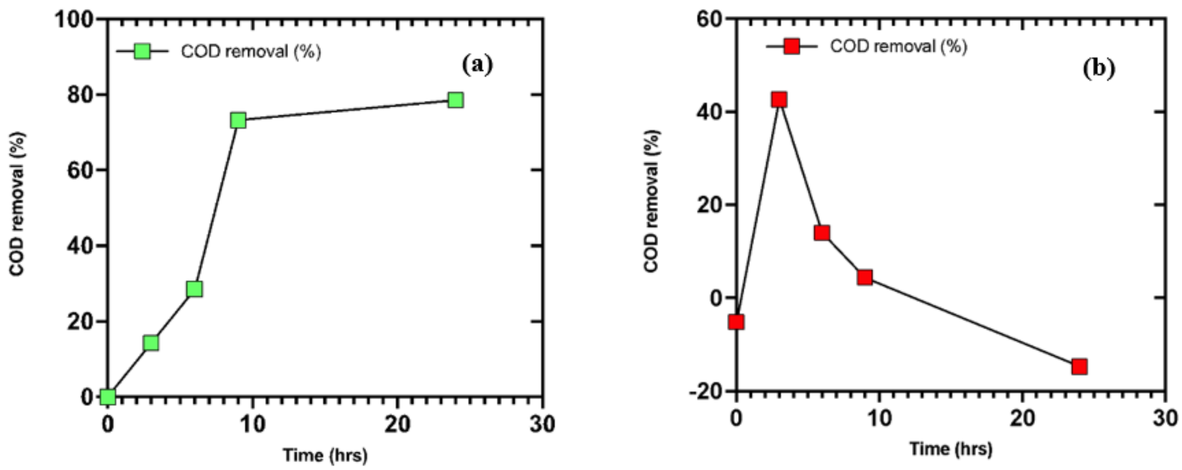


Figure 4. COD removal efficiency (a) on day 0 (b) on day 12

In contrast, on day 12 the COD removal pattern was highly erratic with no consistent trend across time interval points. The removal efficiency was firstly markedly lower at 0 hours (-14.69%) compared to day 0, suggesting a prolonged lag phase and slower initial microbial activity. The removal rate further dropped to -14.69% at 24 hours. The irregular COD removal kinetics and extended lag phases indicates process instability and loss of acclimation by day 12 likely due to shifts in microbial community structure and catabolic functions. According to (Kawai *et al*., 2016; Lee *et al*., 2019), lags in COD removal after re-inoculation of activated sludge reactors reflect declining metabolic versatility and substrate affinities in the microbial community over time.

The overall removal declined from 38.93% to day 0 to 8.25 % on day 12. Reflecting a 30.67% difference or 78.8% reduction in treatment performance. This progressive loss of COD removal capacity overtime aligns with previous temporal studies of activated sludge systems.

Potential mechanisms for the declining COD removal kinetics and efficiency include accumulation of recalcitrant organic matter, nutrient limitations leading to catabolic repression, or unfavorable ecological shifts in microbial community structure. Adding supplemental micronutrients (e.g., nitrogen, phosphorus, metals) or switching to a sequencing batch reactor configuration could potentially improve long term performance.

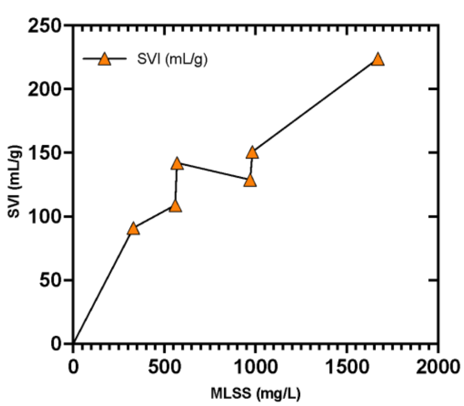
Relationship Between Operational Parameters and COD Removal

To further understand factors influencing COD removal trends, operational key parameters were measured on each day of reactor operation as shown in Table 2. The pH remained stable around neutral ranging from 6.99 to 7.34 across all days. A moderate increase in mixed liquor suspended solids (MLSS) concentration was observed from 330 mg/L initially to 970 mg/L by day 7. However, the MLSS sharply dropped to just 670 mg/L by day 12 indicating significant washout of biomass.

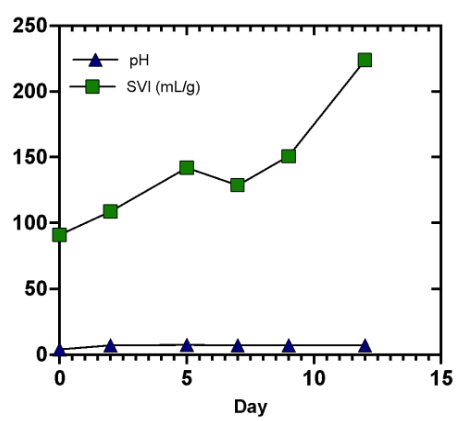
Table 2. Characteristics of activated sludge operational parameters

Day	Parameters					
	pH	F/M ratio	SV_{30} (mL/L)	MLSS (mg/L)	SVI (mL/g)	COD (mg/L)
0	4.01	0.24	30	330	90.91	79.52
2	6.99	0.095	61	560	108.93	53.2
5	7.34	0.080	81	570	142.11	45.6
7	6.99	0.055	125	970	128.87	53.2
9	7.02	0.0024	150	9520	15.76	-22.8
12	7	0.24	150	670	223.88	83.6

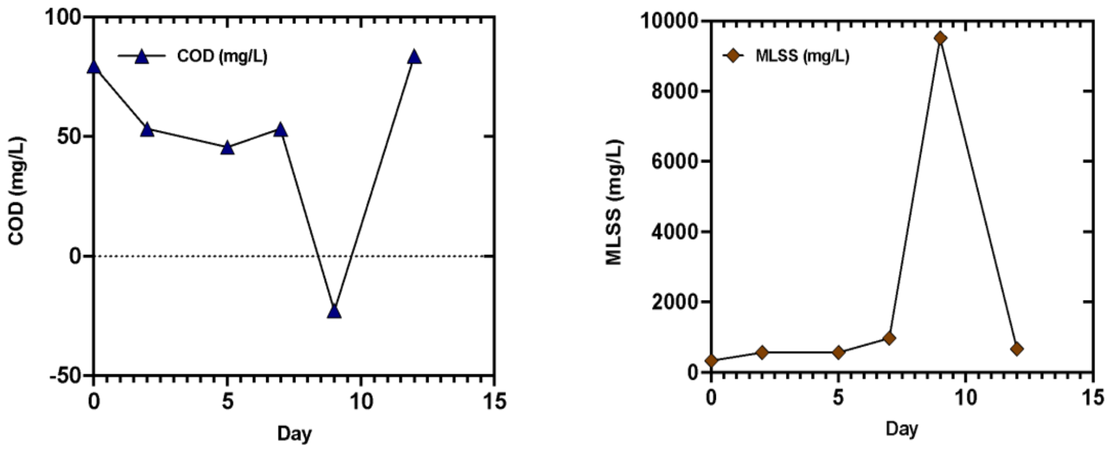
The sludge volume index (SVI) followed an opposing trend to the MLSS as shown in Figure 4, increasing from 90.91 mL/g on day 0 to 223.88 mL/g on day 12. The elevated SVI signifies poorer sludge settleability and compaction. According to (Bitton, 2005; Liu *et al*., 2016; Mesquita *et al*., 2011; Zhang *et al*., 2019), SVI values above 150 mL/g are categorized as bulking sludge whereas a well-settling sludge has an SVI below 80 mL/g. The high SVI correlates with the low MLSS observed on day 12, as poor settling leads to loss of solids through effluent discharge (Maltos *et al*., 2020; Metcalf & Eddy, 2003; Tchobanoglous *et al*., 2014).



(a)



(b)

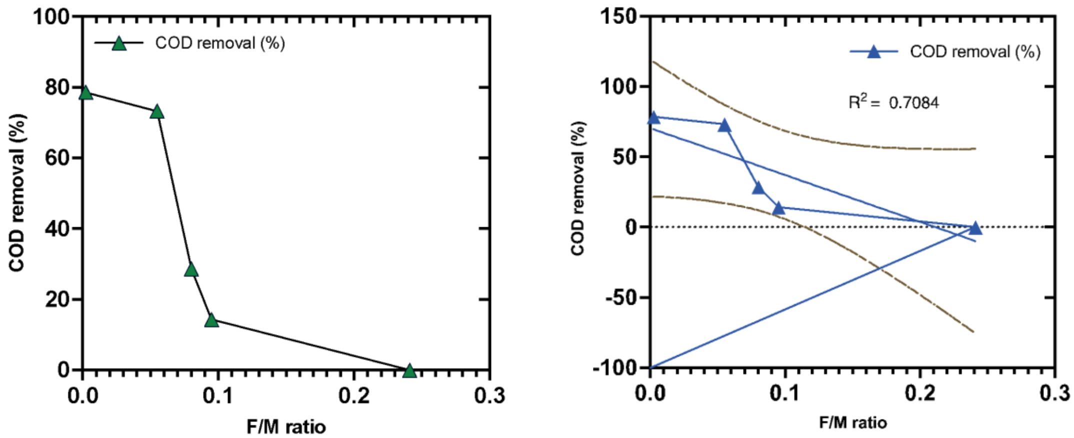


(c) (d)

Figure 5. Operational parameters of synthetic wastewater (a) relationship between SVI and MLSS (b) pH and SVI overtime (day) (c) COD overtime (day) (d) MLSS overtime (day)

As shown in Figure 5, the increased SVI likely contributed to the deteriorating COD removal efficiency by day 12. Higher SVI values have been associated with lower COD and BOD elimination in activated sludge processes (Bitton, 2005; Canals *et al*., 2023; Metcalf & Eddy, 2003).

According to (Canals *et al*., 2023; Kan *et al*., 2021), high SVI can directly reduce COD removal by increasing effluent suspended solids. Poor settling also decreases solid retention time and contact between microbes and wastewater constituents. SVI values above 150 mL/g result in clarifier solids loading rates that impair separation. Reduced sludge compaction further limits COD removal by decreasing biomass density and activity (Bitton, 2005). Based on these relationships, the elevated SVI coupled with lower MLSS by day 12 likely created conditions unfavorable for COD reduction. Improving sludge settleability through operational strategies or chemical addition could potentially stabilize COD removal performance long-term (Bitton, 2005; Lee *et al*., 2019; H. Lin *et al*., 2023).



(a) (b)

Figure 6. Effect of F/M ratio to COD (mg/L) (a) Effect of F/M ratio on COD removal (b) Linear regression analysis of the effect of F/M ratio on COD removal

The food to microorganism (F/M) ratio is another vital operational parameter that stimuluses the COD removal efficiency. The F/M ratio is defined as the amount of organic load applied per unit of biomass. The organic load is measured based on COD while the biomass is measured in terms of MLSS. In this study, the F/M ratio was calculated daily based on the initial COD amount of synthetic wastewater and the MLSS concentration in the activated sludge reactor. The F/M ratio ranged from 0.0024-0.24 per day as shown in Table 2. Based on literature review, there is an optimal range of F/M ratio to achieve maximum COD removal efficiency. Some studies have shown that the optimal F/M ratio of 0.38 can yield the COD removal efficiency of about 96% (Mirbagheri *et al*., 2014) and in its same range of F/M ratio of 0.3 the excellent COD removal was investigated (Hafez *et al*., 2012).

On day 0, the applied F/M ratio was 0.24, at this F/M ratio, the initial COD concentration of the synthetic wastewater was 79.52 mg/L. During the 24-hour monitoring period, the COD concentration decreased to 17.04 mg/L at hour 24, reflecting a COD removal efficiency of 78.57%. The rapid COD decline in the early stage indicated that the microbial community could well adapt to the new substrates at an F/M ratio of 0.24. This F/M ratio provided sufficient organic load for microbial growth and activity to efficiently degrade COD. On day 12, the same F/M ratio of 0.24 g COD/g MLSS/day was applied. However, the COD removal pattern became highly erratic with no consistent trends across time intervals. The removal efficiency even dropped to -14.69% at hour 24.

Although the same F/M ratio of 0.24 was used, the results on day 12 showed that the microbial community lost its acclimation and catabolic activity abilities. This was likely due to natural changes in microbial community structure over the 12 days of experiment. Therefore, it can be concluded that when the F/M ratio of 0.24 gave the best results on the first day, it failed to maintain system stability over 12 consecutive days. Spontaneous changes in microbial community structure could affect system performance even at a constant F/M ratio as the positive correlation shown in Figure 6 by linear regression analysis. Maintaining stable F/M alone is not sufficient to ensure continuous COD removal performance long-term (Bitton, 2005; Metcalf & Eddy, 2003).

Observed Microbial Dynamics

Along with the operational parameters, microscopic analysis provided insights into the shifting microbial community structure over the 12-day study as shown in Table 3. While bacterial flocs were consistently present throughout the experiment, flagellates, free-swimming ciliates, stalked ciliates, and rotifers proliferated from absent or negligible initially to abundant populations by day 12.

Table 3. Microbial community observed

Day	Observed microorganisms					
	Bacterial flocs	**Flagellates**	**Free-swimming ciliates**	**Stalked ciliates**	**Rotifer**	**Other things**
0	Yes	N/A	Yes	N/A	N/A	Yes
2	Yes	N/A	Yes		N/A	Yes
5	Yes	N/A	Yes		N/A	Yes
7	Yes	Yes	Yes		Yes	N/A
9	Yes	Yes	Yes		Yes	Yes
12	Yes	Yes	Yes		Yes	Yes

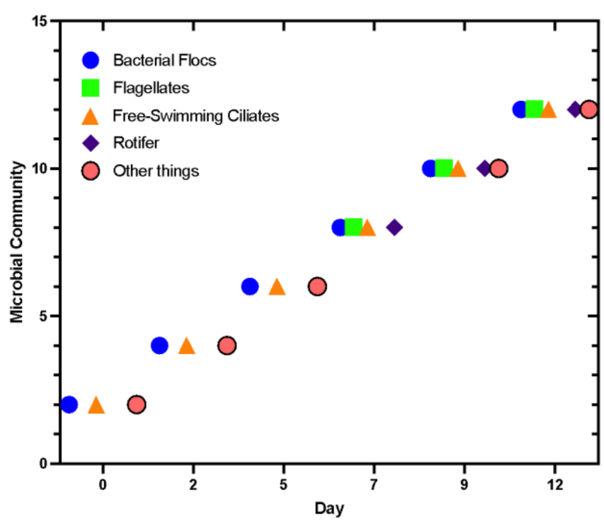


Figure 7. Microbial dynamics in activated sludge treatment of synthetic wastewater

These results as shown in Figure 7 agree with previous observations that protozoa and metazoa become increasingly predominant over time in activated sludge systems (Araújo dos Santos *et al*., 2014; Bitton, 2005; Kuśnierz *et al*., 2022). According to (Madoni, 2011), protozoan grazer populations progressively rise toward climax communities in mature activated sludge.

The proliferation of bacterivorous protozoa and rotifers corresponds with the reduced COD removal performance, as they can prey on floc-forming bacteria responsible for organic matter biodegradation. Investigations from Bitton (2005) and Siqueira-Castro *et al*. (2016) revealed that activated sludge reactors with abundant flagellates and ciliates showed capability of good COD elimination compared to those dominated by bacterial biomass.

Predation pressure can directly reduce the number and metabolic activity of bacterial populations. Protozoan grazing also selects for filamentous and viscous bacterial phenotypes more resistant to predation but less effective for COD removal. By preferentially consuming highly active floc-forming bacteria, bacterivorous protozoa indirectly lower treatment efficiency (Bitton, 2005; Janiak *et al*., 2023; Madoni, 2011).

Strategies to control excessive protozoan growth include optimizing F/M ratio, sludge age, and DO levels to favor floc-forming bacteria (Bitton, 2005; Metcalf & Eddy, 2003). Future studies should apply high-throughput sequencing to better characterize these ecological shifts and interactions affecting COD removal dynamics (Cydzik-Kwiatkowska & Zielińska, 2016; Vincent *et al*., 2018).

Inclusively, the declining COD removal kinetics coupled with temporal changes in operational parameters and microbial community structure underscores the importance of stability for optimizing activated sludge performance. A balanced mixed community with high functional redundancy appears critical to maintain treatment resilience and consistency long-term performance of treatment (Li *et al*., 2020).

Table 4. Comparison of COD removal efficiency based on the technique used and operational parameters

References	Method used	Operational parameters/conditions	COD removal efficiency
(Thi Tuyet Nhi *et al*., 2022)	Biocord-integrated fixed-film activated sludge (Biocord-IFAS)	COD, Hydraulic retention time (HRT)	82.9% and 94.2%
(Mirbolooki *et al*., 2017)	Sequencing Batch Reactor (SBR)	TDS, MLSS, MLVSS, and Absorption	80.71%
(Mukhtar *et al*., 2019)	Activated Sludge in combination with Activated carbon addition	COD, pH, SVI, F/M ratio, Sludge age and MLSS	75% during the acclimatization stage of the study (without AC) 89.97% (with AC)
(Cheibub *et al*., 2014)	Coupled coagulation–AOP process	pH and COD	75%
(Ghosh *et al*., 2014)	Sequential bioreactor using fungi and bacteria	COD	76.9%
(Jagaba, Kutty, Noor, *et al*., 2022)	Activated Sludge (Extended aeration and the addition of activated carbon medium)	pH, COD, Settleability, SVI, MLSS, and MLVSS	98.11%
Present work	Activated Sludge by aerobic degradation (extended aeration)	COD, pH, MLSS, SVI, and F/M ratio	78.57% during the acclimatization stage of the study

The present exertion of study as shown in Table 4 employed an activated sludge system with controlled operational parameters of MLSS, SVI, and F/M ratio to achieve 78.57% COD removal during early-stage treatment of synthetic wastewater. This initial performance exceeds the 75% removal reported by (Mukhtar *et al*., 2019) during acclimatization but falls short of the >90% reductions attained in normal activated sludge and with appendage of activated carbon (Jagaba, Kutty, Noor, *et al*., 2022). The lower efficiency likely owes to the short 12-day duration and use of synthetic rather than real wastewater. Additionally, COD elimination declined markedly to just 4% by day 12 as the balanced microbial community degraded. This contrasts the stable 82.9% and 94.2% removal by using an integrated fixed-film configuration to maintain biomass (Thi Tuyet Nhi *et al*., 2022). Overall, the present work highlights the importance of sustained community structure, diversity, and functional redundancy in activated sludge for consistent biological COD treatment.

Table 4 comprehensively delineates various treatment methods that have been investigated for their effectiveness in reducing COD levels in industrial wastewater. A wide range of technologies are referenced, including activated sludge, sequencing batch reactors, batch reactors, and more innovative approaches such as coupled coagulation–AOP process and employment of fungi, bacteria, and the chemical (AC) coupled with activated sludge. The studies last 10 years from 2014 to 2022, providing valuable insights into the evolution of wastewater treatment capabilities over time. Precisely defined operational parameters are stated clearly, allowing for like-for-like performance comparisons between different process configurations.

Prominently, the achievement of high COD reductions above 90% with technologies such as extended aeration and the addition of activated carbon medium, Biocord-IFAS, four-stage SBRs, chemical activation of dye sludge and biofilm processes like moving bed biofilm reactors (Jagaba, Kutty, Noor *et al*., 2022; Khalidi-Idrissi *et al*., 2023; Meena *et al*., 2022; Thi Tuyet Nhi *et al*., 2022). Many conventional activated sludge plants also realized removals in the 75-85% band. Overall, the comparative survey in Table 4 builds a compelling case for a diversity of biological, physical, and advanced oxidation techniques proving their efficacy at remediating COD contamination at an industrial scale. The thorough compilation of peer-reviewed literature creates an authoritative reference for evaluating state-of-the-art municipal and industrial wastewater treatment.

CONCLUSION

This research explains various influential deductions regarding to the activated sludge treatment process. Notably, the study observed a precipitous decline in COD removal efficiency from 79% on day 0 to a meager 4% by the 12^{th} day of operation. Concurrent with this deterioration in treatment performance was a rise in sludge volume index and fall in mixed liquor suspended solids over the assessment period, indicating a clear correlation with poorer effluent quality outcomes. The proliferation of flagellates, ciliates and rotifers by the final evaluation juncture likely suppressed floc-forming bacteria via grazing pressures. It appears sustaining a balanced mixed microbial consortium with adequate bacterial numbers is vital for continual COD extraction. Moreover, the investigation provides insight into the linkages between operational metrics, microbial interactions, and variability in treatment effectiveness. While constrained by a brief timeframe and use of synthetic wastewater, the findings underscore the importance of community stability and functional redundancy for resilient process function. Further correlational research exploring key organisms and their treatment functions could help optimize system performance.

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