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Morphological Identification of Microbes in Activated Sludge: Effects of Aeration and Sludge Age

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ABSTRACT: Aeration and sludge age are critical parameters in designing, operating, and controlling biological wastewater treatment plants to produce high-quality effluent. Several factors affect the types of microbes in the activated sludge process, including environmental factors and plant design. The main objective of this study was to investigate the effects of aeration and sludge age on the types of microorganisms present in active sludge. The microbial diversity will be analyzed morphologically by visual inspection and light microscopy. The microbes in the activated sludge process were analyzed at different aeration flows and sludge ages to determine their types. The findings from this research revealed that low aeration of 2L/min -4L/min and SRT of > 8 days favor microbes in degrading organic matter with higher removal efficiency. The common microbes found included *Bacillus, coccus, coccobacillus, streptococcus, Staphylococcus, Streptobacillus,* and *Vibrio*

KEYWORDS: Activated sludge, Activated sludge process, Aeration, Microbe, Sludge retention time

I. INTRODUCTION

The activated sludge process (ASP) is a common biotechnology implemented worldwide for municipal and industrial wastewater treatment. Microbes in activated sludge (AS) are the basic functional units, as they transform biodegradable pollutants and produce qualified water that is safe for humans and the environment (Peces et al., 2022). Among the problems encountered in ASP are excess sludge, which seems to increase the plant cost (ALHARBI, 2016), and incompetent knowledge about microbial community in water and wastewater. To produce effective effluent with high quality standards and low cost, knowledge about the types of microbes present in the process and the factors affecting them must be well understood. Two pivotal operational factors, aeration and sludge age, significantly influence the composition and function of the microbial community. Therefore, this study undertakes a comprehensive investigation into the "Effect of aeration and sludge age on the types of microbes in activated sludge. This knowledge helps optimize the requirements that favor microbes (as a basic functional unit) to survive and work effectively and efficiently; hence, effluent with low sludge can be produced.

The microbial community is an important aspect to determine in the biotechnology of AS. (Ye et al., 2020), with the help of machine learning, we were able to present 2045 metagenome-assembled genomes (MAGs) of bacteria and archaea from 114 global municipal wastewater samples of AS. 21 out of 2045 MGAs were discovered to be three phyla of archaea, including *Halobacteria, Micrarchaeota, and Nanoarchaeota*. The remaining MAGs were found to be phyla of bacteria. The bacteria phyla with large numbers of 508, 409, 178, 164, 161, 122, 114, and 96 MAGs were *Proteobacteria, Bacteroidota, Patescibacteria, myxococcota, Actinobacteriota, Planctomycetia*, *Chloroflexota,* and *Acidobacteriota, respectively, while Proteobacteria* and *Bacteroidota* seemed to be dominant (Gao et al., 2016). The study of (Begmatov et al., 2022) revealed that the microbial community in activated sludge is dominated by *Proteobacteria, Bacteroidia,* and *Actinobacteriodata*.

Aeration in the ASP provides oxygen that dissolves in wastewater as dissolved oxygen (DO) in the oxidation ditch of the wastewater treatment plant. DO is utilized by microorganisms to convert organic pollutants into CO2 and biomass (Yaparatne et al., 2022); hence, aeration facilitates biological oxidation and biosynthesis (Samer, 2015). The amount of DO affects the efficiency of bacteria in AS. Some microbes survive and grow well at high DO concentrations, whereas others grow well at low DO concentrations. An adequate DO concentration is 2 mg/L (Lizette de Leon Gallegos & Sc geboren in Monterrey, 2018). Fan et al. (2017) revealed that low DO concentrations inhibit the growth of nitrifying bacteria, which may result in poor denitrification.

Sludge age or sludge retention time (SRT) is a parameter in biological wastewater treatment that represents the average time spent by microorganisms in degrading pollutants in the reactor. This parameter is useful for selecting microbial populations

(Penteado et al., 2016). Previously, it was observed that long SRT makes slow-growing microbes thrive and increases diversity; this situation provides enough time for microbes to contact and digest the limited substrate, hence the effluent with little sludge produced (Almasi et al., 2016). The dominance of microbes depends on the sludge age. (Chen et al., 2021), his study of the anaerobic acidification of waste revealed that at a young sludge age, the phylum Proteobacteria of the genus *Candidatus Competibacter* was dominant, which later slightly decreased as the sludge age increased from 0 to 5 days, 10 to 30 days, and 30 to 40 days with >30%, 20~30%, and 20~30% respectively whereby *AAP99* was <5% at a young age of sludge of 5 to 20 days.

The primary objective of this study was to investigate the effects of aeration, concentration, and sludge age on the types of microbes in activated sludge. This investigation will help optimize the effective aeration level and sludge age for the types of microbes in activated sludge to produce a qualified wastewater effluent.

II. MATERIAL AND METHOD

A. *Experimental setup of the ASP*

Both wastewater and sludge samples were collected from the Keputih septage treatment plant in Surabaya, Indonesia. The experiments were conducted on a laboratory scale using batch reactors. The reactor was made of a 5-L plastic container and contained a 2-L mixture of wastewater and sludge. The experimental variables consisted of two levels of initial BOD initial (2071 and 4142 mg/LO_2); three levels of aeration flowrate (2,4 and 6 L/min), and three levels of SRT (3, 8, and 12 days), as illustrated in **Fig. 1**.

Chemical analysis

The BOD was analyzed using the Winkler method, gravimetric method, and DO was analyzed using the iodometric method. Mixed liquid suspended solid (MLSS) and mixed liquid volatile suspended solid (MLVSS) were analyzed using the gravimetric method. The sludge volume index was determined by measuring the volume (mL) of activated sludge settling after 30 min in a cylinder divided by MLSS. All parameters were analyzed based on Indonesia's wastewater quality standard under the regulation of the Ministry of Environment and Forestry No. 68 of 2016.

Microbial analysis

At the end of each selected SRT, the sludge is collected to analyze and finally identify the microbes. Microbial analysis was performed using the pour plate method and bright field microscopy. The pour plate method involved spreading the sample to tryptic soy agar. The Tryptic Soy Agar (TSA) media is helpful in counting the number of growing colonies after incubating for 24 h and analyzing the morphology of the colonies. The bright-field microscopy was implemented for the gram staining test and to determine the morphology of the cells.

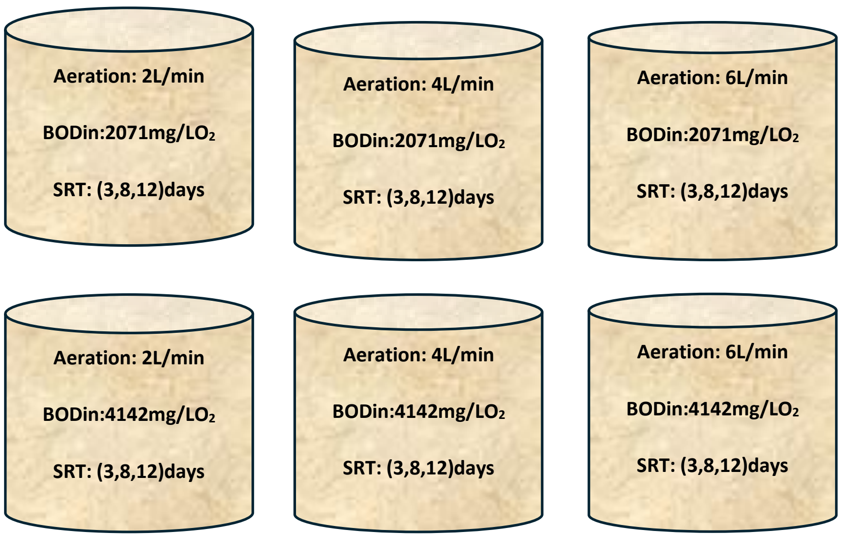


Fig 1. Activated sludge process optimized for various aeration flows (2L/min,4L/min, and 6L/min), initial BOD (4142mg/LO_2 and 2071mg/LO_2), and SRT (3-days, 8-days and 12 days)

III. RESULTS AND DISCUSSION

A. Effect of optimized conditions on the ASP

In this work, the effects of implemented aeration, initial concentration, and SRT on ASP were investigated through DO, effluent BOD, TSS, MLVSS, SVI, BOD removal efficiency, and microbial analysis.

Dissolved Oxygen

The variation in the DO concentration is presented in **Fig. 2, which** generally shows an increase in the DO concentration with increasing SRT. During the first cycle of 3 days of SRT, the DO differed among all nine reactors. ASP with low aeration and concentration (2L/min, 2071mg/LO_2) had the highest DO concentration of 9.58 mg/L O_2 while the ASP with the same aeration but high concentration (2L/min, 4142mg/LO_2) possessed the lowest DO concentration of 0.27 mg/LO_2. The difference in concentration leads to different DO concentrations. For the treatment cycle of 8 days SRT, the ASP (4L/min, 2071mg/LO_2) showed the highest DO concentration of 4.74 mg/L O_2, while (2L/min, 4142mg/LO_2) showed a low DO concentration of 3.27 mg/LO_2. At this age, the DO concentration ranged from 3 to 6 mg/L O_2. At 12 days SRT, ASP (2L/min, 4142mg/LO_2), (2L/min, 2071mg/LO_2), and (4L/min,2071mg/LO_2) exhibited a high DO concentration of ≥7mg/LO_2. While ASP (4L/min, 4142mg/LO_2) and (6L/min, 2071mg/LO_2) exhibited relatively lower concentrations of 4.26 mg/LO_2 and 5.36 mg/LO_2, respectively. The previous study by Carlsson (2009) emphasized that the high air flow rate is insufficient oxygen transfer, which reduces the DO concentration as observed in ASP of (4L/min,4142mg/LO_2) and (6L/min,2071mg/LO_2). They were observed to have low DO concentrations in all SRTs because they possessed a high aeration flow rate of 6 L/min.

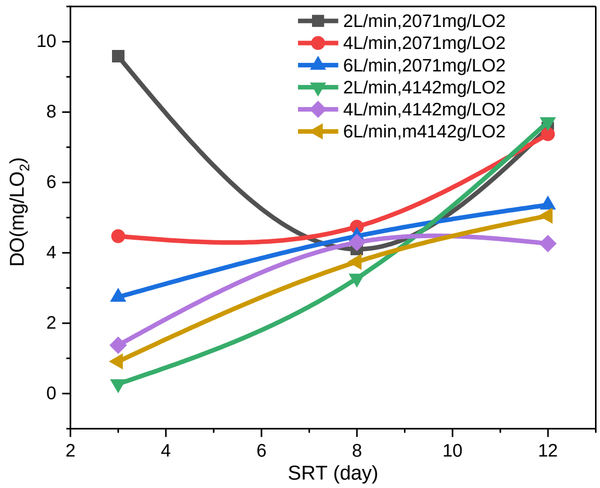


Fig 2. Variation in DO concentration at 3, 8, and 12 days of SRT

Mixed liquor volatile suspended solid (MLVSS)

MLVSS is the number of organic or volatile suspended solids that serves as an indicator of the number of microorganisms present in wastewater. Provide insights into the health biological activities of microbes in ASP. The high concentration of MLVSS in a treatment plant indicates that the low concentration of it emphasizes insufficient microbial biomass to degrade organic matter, resulting in effluent with higher organic loading and poor treatment efficiency. From **Fig 3**, which shows the variation of MLVSS, the ASP of high aeration and concentration (6L/min, 4241mg/LO_2) was observed to have the highest concentration values of 17072 mg/L, 7844 mg/L, and 11723 mg/L of MLVSS to 3-days, 8-days, and 12-day SRT compared to all optimizations. For ASP with low aeration and concentration (2L/min, 2071mg/LO_2), medium aeration and low concentration (4L/min, 2071mg/LO_2), and low aeration with high concentration (2L/min, 4241mg/LO_2), the MLVSS concentration increased gradually with time due to extended aeration and retention time, as mentioned previously by Almeida-Naranjo et al., (2017). On the other hand, ASP exhibit low aeration with high concentration (2L/min, 2071mg/LO_2), low aeration and high concentration (2L/min, 4142mg/LO_2), and high aeration and high concentration (6L/min, 4142mg/LO_2), and they tend to decrease their MLVSS from 3 days to 8 days but rise moderately at 12-day SRT.

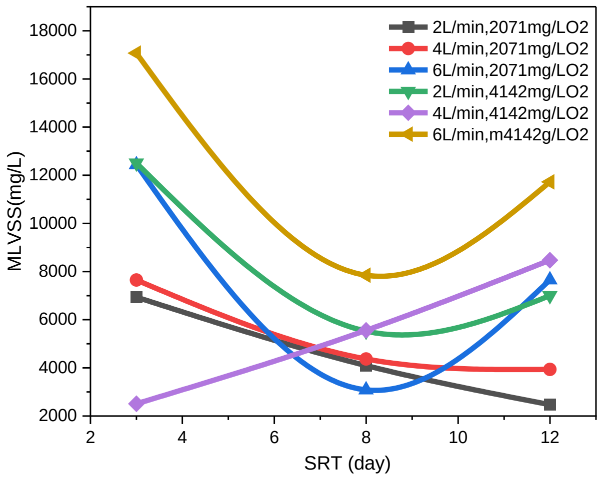


Fig 3. Variation in MLVSS at 3, 8, and 12 days of SRT

Sludge volume index (SVI)

The SVI is a crucial parameter for the sludge-settling characteristics of ASP (Tchobanoglous et al., 2003). The higher SVI indicates a poor settling property, whereas the lower SVI indicates a good settling property. **Fig. 4 shows the variations** in SVI at various SRTs. This work revealed that at the early age of 3-days, ASP (2L/min, 2071mg/LO_2) and (2L/min,4241mg/LO_2) possessed the highest SVI value of 196 ml/g and 300 ml/g indicating a poor settling character while at middle age of 8-days (2L/min,4241mg/LO_2) possessed highest SVI value of 139.91 ml/g followed by (6L/min, 2071mg/LO_2) with 130.42ml/g, R1(2L/min,4241mg/LO_2) with 125ml/g and (2L/min, 2071mg/LO_2) having 113.86ml/g. At a retention time of 12 days, all reactors excluding R4 possessed the value of SVI ≤ 100ml/g. A previous study (Valter Tandoi et al., 2017) indicated that <100 ml/g is a good sludge settling, 100-150 is moderate while >150 is poor, indicating bulking problems. This study revealed that at the early age of 3-day SRT, the ASP (2L/min,2071mg/LO_2), and (2L/min,4241mg/LO_2), and (2L/min,2071mg/LO_2) at 12-day SRT possessed poor settling characteristics due to bulking.

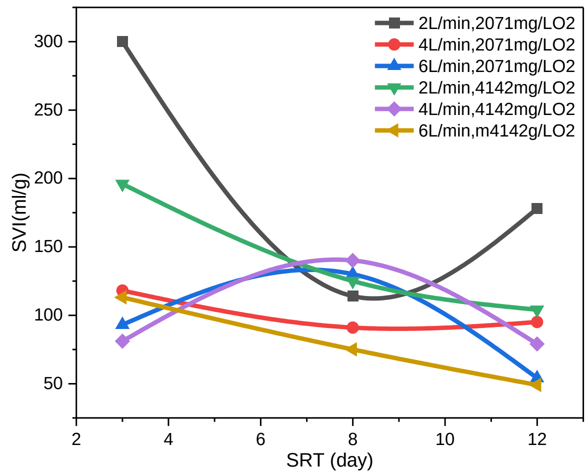


Fig. 4: Variations in SVI

Total suspended solid (TSS)

This parameter is critical for analyzing the concentration of suspended solids in water, wastewater, and other liquids. There are many suspended solids, including decaying matter, industrial waste, sewage, and silt. According to (Wirabumi et al., 2021), a high TSS concentration indicates high turbidity in water, whereas a low TSS concentration indicates that the water contains few suspended particles and is cleaner. **Fig. 5** shows the variation in TSS with SRT.

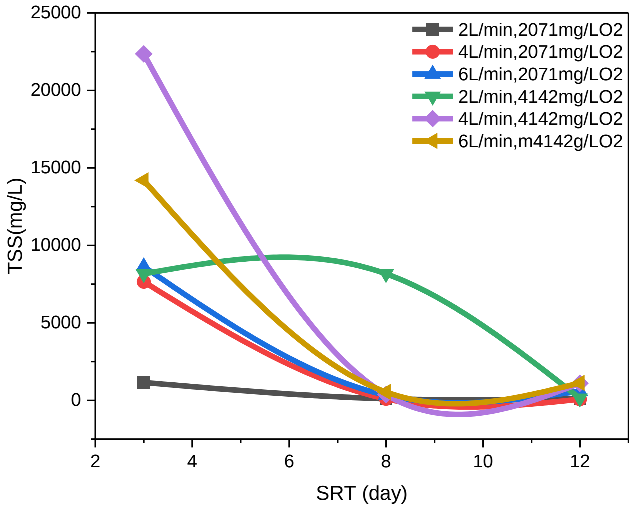


Fig 5. Variation in TSS at various SRTs

TSS was observed to decrease with time for all reactors. At an early age of 3 days, SRT R2(4L/min, 4142mg/LO_2) and (2L/min, 2071mg/LO_2) were observed to have higher TSS values of 22352 mg/L and 1148 mg/L, respectively. From this study, it was observed that the TSS concentration decreased with increasing SRT.

BOD removal efficiency

The BOD removal efficiency in this study varied due to differences in the optimization setup. **Figure 6 shows** the variation in the BOD removal efficiency.

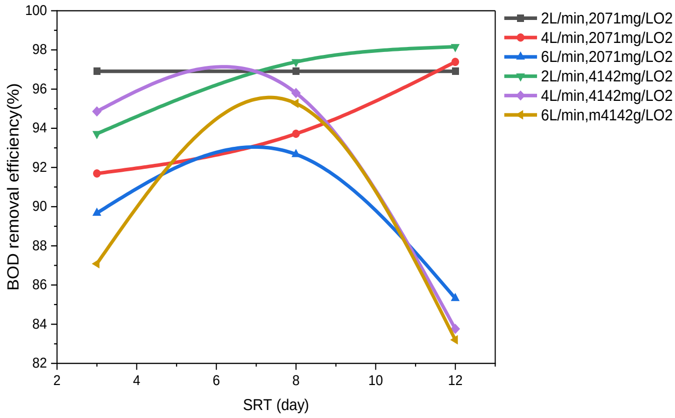


Fig 6. Variations in the BOD removal efficiency for all reactors after 3, 8, and 12 days of SRT

During the early age of 3-day SRT, the removal efficiencies of (2L/min, 4241mg/LO_2), (4L/min, 4241mg/LO_2), (6L/min, 4241mg/LO_2), (2L/min, 2071mg/LO_2), (4L/min, 2071mg/LO_2) (6L/min, 4241mg/LO_2) were 94%, 95%, 87%, 97%, 92%, and 90%, respectively. An ASP of (2L/min, 2071mg/LO_2) was observed to have the highest removal efficiency due to the large number of microbes and DO concentration.

At the middle age of 8 days SRT, the removal efficiencies of (2L/min, 4241mg/LO_2), (4L/min, 4241mg/LO_2), (6L/min, 4241mg/LO_2), (2L/min, 2071mg/LO_2), (4L/min, 2071mg/LO_2), and (6L/min, 2071mg/LO_2) were 97%, 96%, 95%, 97%, 94%, and 93%, respectively, where (2L/min, 4241mg/LO_2) and (4L/min, 4241mg/LO_2) were observed to have the highest removal efficiencies for all reactors while (6L/min, 2071mg/LO_2) was found to have the lowest BOD removal efficiency.

At the old age of 12-SRT, the highest removal efficiency was observed in (2L/min, 4241mg/LO_2) (98%, followed by (4L/min, 2071mg/LO_2), (2L/min, 2071mg/LO_2), (6L/min, 2071mg/LO_2), (4L/min, 4241mg/LO_2), and (6L/min, 4241mg/LO_2), with efficiency values of 97.39%, 96.9%, 85.32%, 83.77%, and 83.19%, respectively.

Based on the observations, (2L/min, 4241mg/LO_2), (4L/min, 4241mg/LO_2), and (4L/min, 2071mg/LO_2) were found to have good trends in removal efficiency from the early age of 3-days to old age of 12 days SRT. (2L/min, 4241mg/LO_2) and (4L/min, 4241mg/LO_2) have the same aeration flow rate of 2L/min, but they differ in influent BOD concentrations. This study revealed that low aeration flow to diluted wastewater is good for ASP performance.

B. *Effect of optimization on microbes*

Microbes are small organisms that cannot be seen with a necked eye. The basic principle of microbes in ASP is to degrade organic matter to produce carbon dioxide (CO_2), and new cells. In this study, microbial analysis was performed morphologically using the pour petri method and a bright light microscope, reflecting colony isolation, colony counting, colony morphology, gram staining test staining, and microscopic visualization (Costa et al., 2013). Colony counting was performed using a colony counter, and microbial identification was performed based on previous studies (Bergey & Holt, 1994). At 3-day SRT, ASPs with low aeration and concentration (2L/min, 2071mg/LO_2) were investigated to have large numbers of microbes, as observed in Table I. The results of the colony counting, morphology, and Gram staining tests are presented in Tables (I, II, and III) for 3 days, 8 days, and 12-day SRT respectively. Table 4 presents common cell shapes, stains, arrangement, and identifications in this study. Images of the colonies and Gram staining are presented in Appendix 1.

Table I. Colony Numbers, Morphological Characteristics, and Gram Staining Tests of Colonies in the Activated Sludge Process at 3-Days-SRT

ASP	Number of colonies	Colony isolated	Colony characteristics					Gram staining + or
			Colony size (cm)	Color	Margin	Elevation	Form	
2L/min, 4142mg/LO_2	104×10^3	A	1.3	White	Entire	raised	Circular	+
	22500	B	1.2	Cream	Undulate	Umbonate	Irregular	+
4L/min, 4142mg/LO_2	19×10^3	A	1.2	White	Lobate	Raised	irregular	+
	67×10^3	B	1	Cream	Undulate	Raised	irregular	+
6L/min, 4142mg/LO_2	81×10^8	A	0.8	Yellow	Undulate	Umbonate	irregular	+
	81×10^8	B	0.8	Light yellow	Entire	Raised	circular	+
2L/min, 2071mg/LO_2	83×10^{10}	A	0.5	Cream	Entire	Raised	circular	-
	83×10^{10}	B	0.5	Cream	Entire	Raised	circular	+
4L/min, 2071mg/LO_2	84×10^3	A	1.2	Light yellow	Entire	Umbonate	circular	+
	84×10^3	B	1	Light yellow	Undulate	Umbonate	irregular	+
6L/min, 2071mg/LO_2	64×10^3	A	1.4	Cream	Entire	Umbonate	circular	+
	3×10^4	B	0.9	Light yellow	Entire	Umbonate	circular	+

Table II. Colony Numbers, Morphological Characteristics, and Gram Staining Tests of Colonies in the Activated Sludge Process at 8 Days of SRT

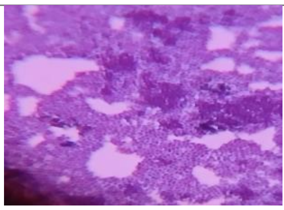
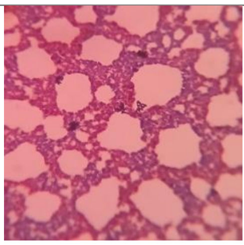
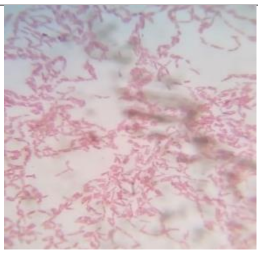
ASP	Number of colonies	Colony isolated	Colony characteristics					Gram staining + or
			Colony size (cm)	Color	Margin	Elevation	Form	
2L/min, 4142mg/LO_2	10^6	A	2	Cream	Lobate	Crateriform	Irregular	+
	65×10^5	B	1.7	White	Undulate	raised	Irregular	+
4L/min, 4142mg/LO_2	32×10^5	A	1.2	White	Undulate	Flat	irregular	+
	32×10^5	B	0.9	White	Entire	Raised	Circular	+
6L/min, 4142mg/LO_2	155×10^5	A	1.5	White	Undulate	Crateriform	irregular	+
	10^8	B	1.2	cream	Entire	Flat	circular	+
2L/min, 2071mg/LO_2	53×10^5	A	1.5	White	Filiform	Flat	Filamentous	-
	53×10^5	B	0.5	White	Entire	crateriform	circular	+
4L/min, 2071mg/LO_2	65×10^4	A	1.3	Cream	Entire	crateriform	circular	+
	65×10^4	B	0.7	yellow	Undulate	Raised	Circular	-

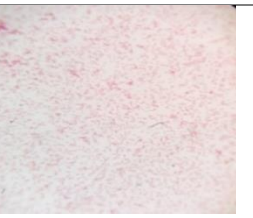
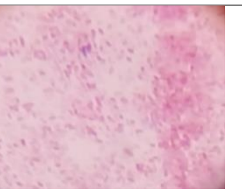
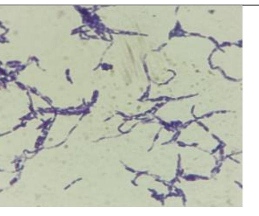
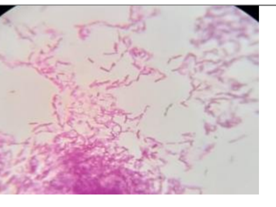
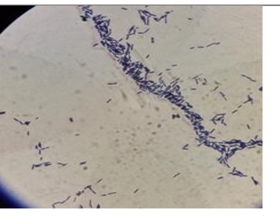
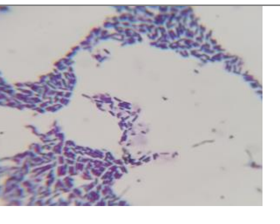
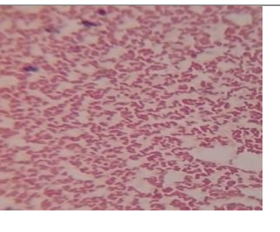
6L/min,	73×10^4	A	0.6	Cream	Entire	Convex	circular	-
2071mg/LO$_2$	35×10^5	B	1	White	undulate	Raised	Irregular	+

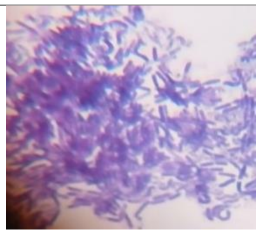
Table III. Colony Numbers, Morphological Characteristics, and Gram Staining Tests of Colonies in the Activated Sludge Process at 12-Days-SRT

ASP	Number of colonies	Colony isolated	Colony characteristics					Gram staining + or
			Colony size (cm)	Color	Margin	Elevation	Form	
2L/min,	55×10^4	A	2	White	Filiform	Flat	Filamentous	-
4241mg/LO$_2$	55×10^4	B	0.7	White	Undulate	Crateriform	Irregular	-
4L/min,	5×10^6	A	0.5	Cream	Entire	Convex	Circular	-
4241mg/LO$_2$	5x10^6	B	0.4	Cream	Entire	Convex	Circular	-
6L/min,	94×10^4	A	0.4	Cream	Entire	Raised	Circular	-
4241mg/LO$_2$	94×10^4	B	0.4	Yellow	Entire	Convex	Circular	-
2L/min,	75×10^4	A	1.4	White	Entire	Flat	Irregular	-
2071mg/LO$_2$	75×10^4	B	0.5	Orange	Entire	Raised	Irregular	-
4L/min,	96×10^4	A	1.3	White	Lobate	Flat	Irregular	-
2071mg/LO$_2$	96×10^4	B	0.5	Cream	Entire	Convex	Circular	-
6L/min,	63×10^5	A	0.5	Cream	Entire	Convex	Circular	+
2071mg/LO$_2$	31×10^6	B	0.4	Cream	Entire	Convex	Circular	+

Table IV. Common Cell Morphology and Identification

Cell shape and Gram staining	Cell arrangement	Genus Identification	Image
Cocci (+): circular	Irregular cluster	Staphylococci	
Cocci (-): circular	Irregular cluster	Staphylococci	
Cocci (-): circular	Chain	Streptococci	

Cell shape and Gram staining	Cell arrangement	Genus Identification	Image
Cocci (-): circular	Scattered	Cocci	
Cocci: circular	Double	Diplococci	
Bacillus (+): rod	Chained	Streptobacillus	
Bacillus (-): rod	Scattered	Bacillus	
Bacillus (+): pared rod	Scattered	Diplobacillus	
Bacillus (+): rod	Clustered	Bacillus	
Oval	Single	Coccobacillus	

Cell shape and Gram staining	Cell arrangement	Genus Identification	Image
Curved rod	Single	Vibrio	

Identification of microbes via Gram staining analysis

The identification of microbes in this study was based on the morphology of the cells under Gram staining analysis from a previous study (Bergey & Holt, 1994). Staining analysis involves a Gram staining test and analysis of the cell shape and some features for identifying microorganisms. Previously, Rosanna Hartline (2024) reported that gram-negative cells appear pink, whereas gram-positive cells stain purple. Gram staining helped identify the cell shape and arrangement and finally the genus of the microbes, as described in Bergey's manual of systematic bacteriology. A bright light microscope was used under the magnification of x100/1.25 oil to observe gram staining, cell shape, and cell arrangement and finally identify the microbes. Table IV shows common cell morphological characteristics observed in this study. The result of the identified microbes is presented in **Table V**.

Table V. Identification of Microbes at 3-day, 8-day, and 12-day SRT

Reactor	Colony	3 days	8 days	12 days
2L/min, 4142mg/LO_2	A	*Cocci*	*Streptobacillus*	*Bacillus*
	B	*Vibrio*	*Coccobacillus*	*Bacillus*
4L/min, 4142mg/LO_2	A	*Bacillus*	*Palisade*	*Bacillus*
	B	*Bacillus*	*Bacillus*	*Bacillus*
6L/min, 4142mg/LO_2	A	*Coccobacillus*	*Bacillus*	*Streptococcus*
	B	*Coccobacillus*	*Bacillus*	*Bacillus*
2L/min, 2071mg/LO_2	A	*Staphylococci*	*Cocci*	*Coccobacillus*
	B	*Diplococci*	*Coccobacillus*	*Bacillus*
4L/min, 2071mg/LO_2	A	*Coccobacillus*	*Coccobacillus*	*Bacillus*
	B	*Bacillus*	*Coccobacillus*	*Bacillus*
4L/min, 2071mg/LO_2	A	*Cocci*	*Cocci*	*Coccus*
	B	*Bacillus*	*Bacillus*	*Coccus*

IV. CONCLUSIONS

Based on this work, it is concluded that the best aeration flow to ensure efficient microbes work is 2–4 L/min - 4L/min as observed in (2L/min, 2071mg/LO_2), (4L/min, 4142mg/LO_2), (2L/min, 4142mg/LO_2), and (4L/min, 2061mg/LO_2); these aeration flow rates provide the optimum dissolved oxygen sufficient for microbes in activated sludge to degrade biological pollutants. On the other hand, a sludge age of ≥ 8 days SRT appears to be the optimum age for microbes to work efficiently. Proper optimization influences microbe growth and organic matter degradation. The optimized conditions in (2L/min, 2071mg/LO_2) favored *cocci* and *vibrio* at the early age of 3-day SRT, (2L/min, 4142mg/LO_2) favored *streptobacillus* and *coccobacillus* at middle age of 8-day SRT, and (2L/min, 2071mg/LO_2) favored *cocci* and *coccobacillus* at 8-day SRT to work efficiently.

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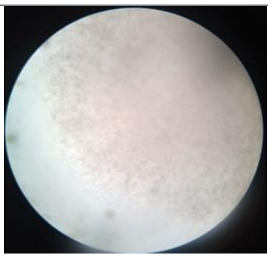
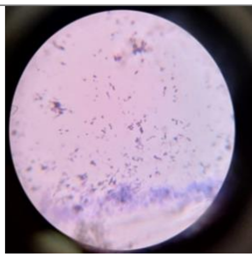
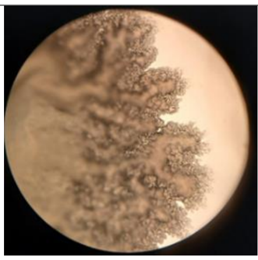
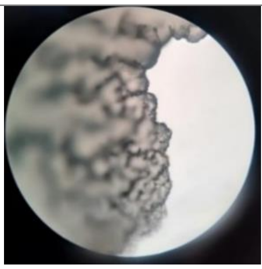
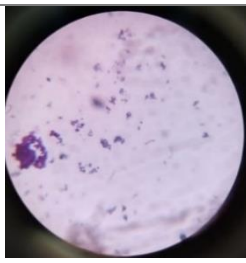
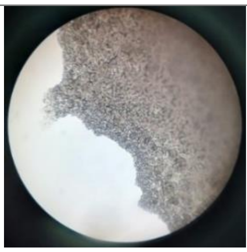
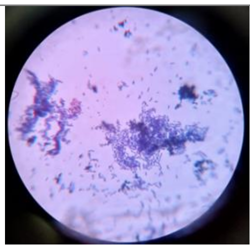
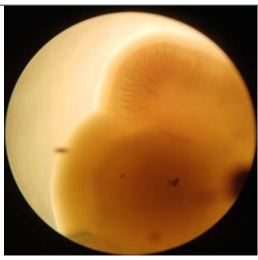
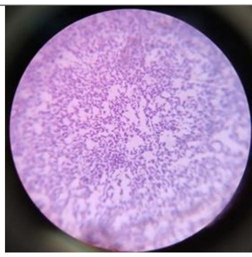
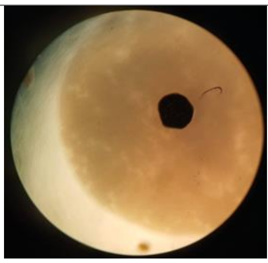
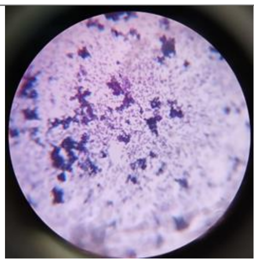
APPENDIX 1

Table 1A. Image of the colony and Gram staining under a bright light microscope at 3 days of SRT

Reactor	Colony	Colony image x10	Gram staining x100	Gram test (-/+), cell shape, and arrangement	Cell identification
2L/min, 4142mg/LO_2	A			Gram (+) Circular Scattered	Cocci
	B			Gram (+) Curved rod sing cell	Vibrio
4L/min, 4142mg/LO_2	A			Gram (+) Rod shape Scattered	Bacillus
	B			Gram (+) Rod shape Scattered	Bacillus
6L/min, 4142mg/LO_2	A			Gram (+) Oval Scattered	Coccobacillus
	B			Gram (+) Oval Scattered	Coccobacillus

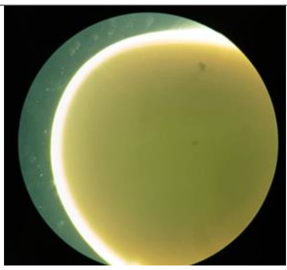
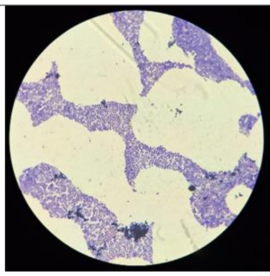
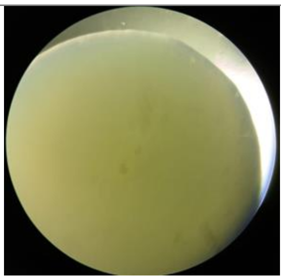
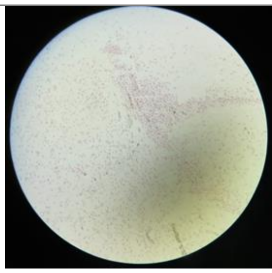
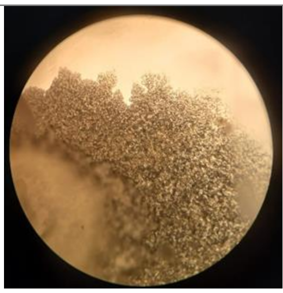
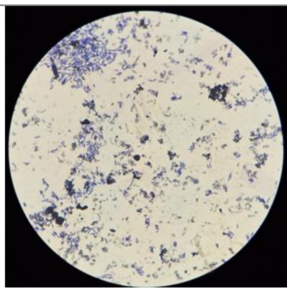
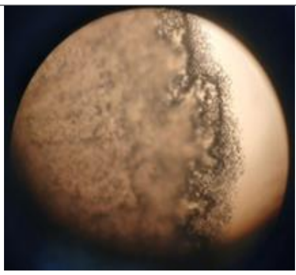
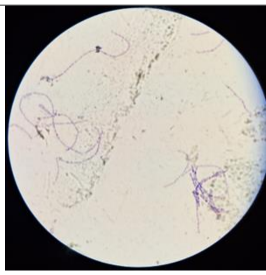
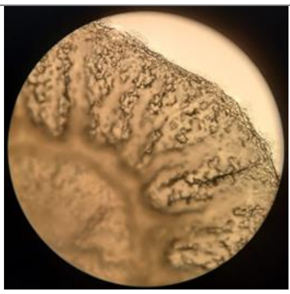
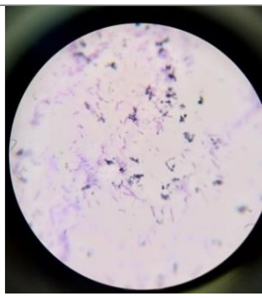
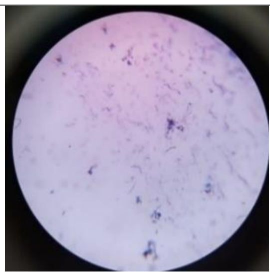
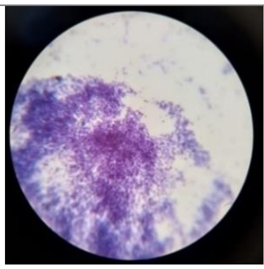
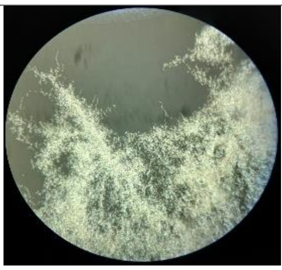
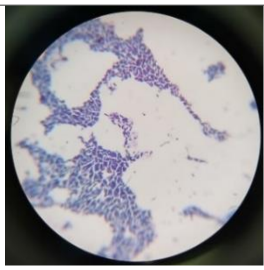
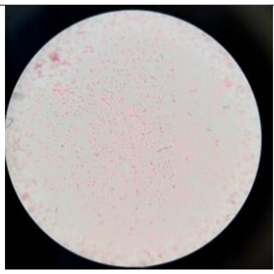
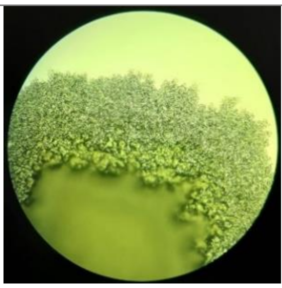
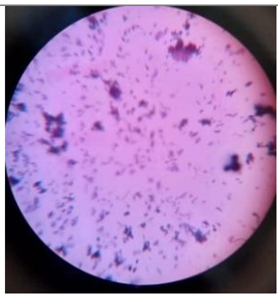
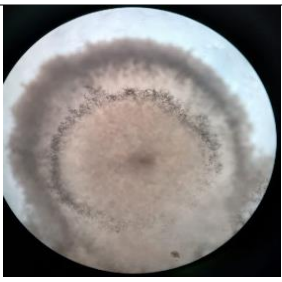
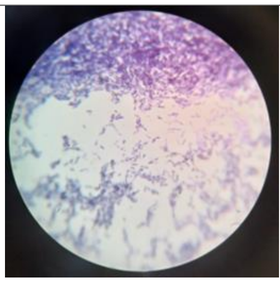
Reactor	Colony	Colony image x10	Gram staining x100	Gram test (-/+), cell shape, and arrangement	Cell identification
2L/min, 2071mg/LO$_2$	A			Gram (+) Circular. Grouped cells	Staphylococci
	B -			Gram (-) Circular Pared cells	Diplococci
4L/min, 2071mg/LO$_2$	A +			Gram (+) Oval shape Clustered	Coccobacillus
	B +			Gram (+) Rod shape Scattered	Bacillus
6L/min, 2071mg/LO$_2$	A			Gram (+) Circular Chained	Cocci
	B			Gram (+) Rod shape Scattered	Bacillus

Table 1B. Image of the colony and Gram staining under a bright light microscope at 8 days of SRT

Reactor	Colony	Colony image	Gram staining	Gram test (-/+), cell shape, and arrangement	Cell identification
2L/min, 4142mg/LO$_2$	A			Gram (+) Rod shape	Streptobacillus
	B			Gram (+) Oval Scattered	Coccobacillus
4L/min, 4142mg/LO$_2$	A			Gram (+) Rod shape Linear arrangement	Palisades
	B			Gram (-) Rod shape Scattered	Bacillus
6L/min, 4142mg/LO$_2$	A			Gram (+) Rod shape Scattered	Bacillus
	B			Gram (+) Rod shape Scattered	Bacillus

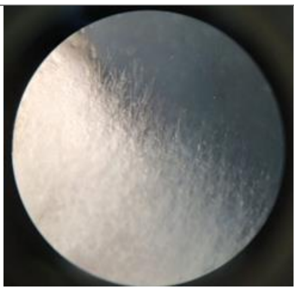
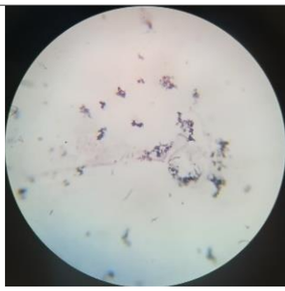
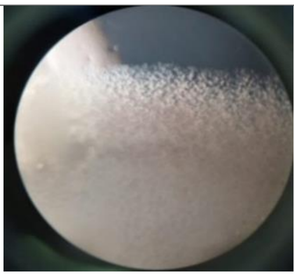
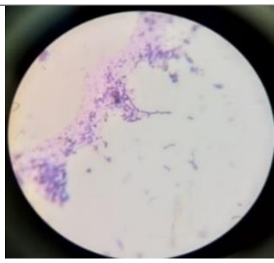
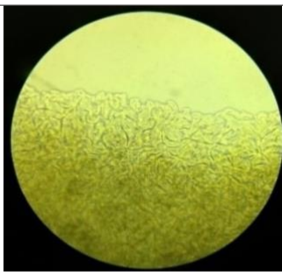
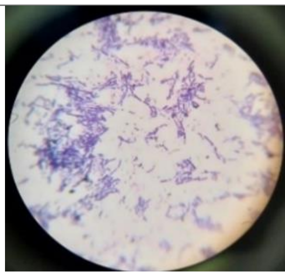
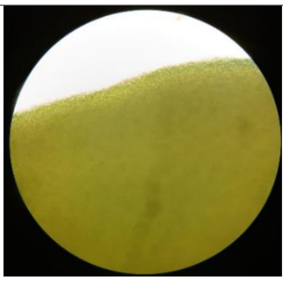
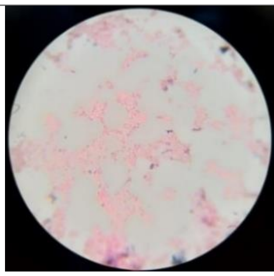
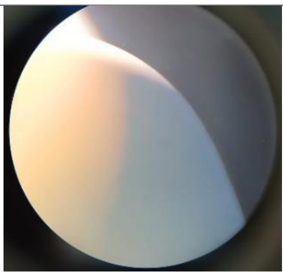
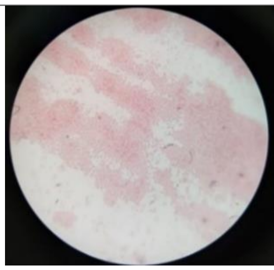
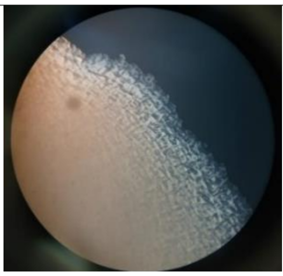
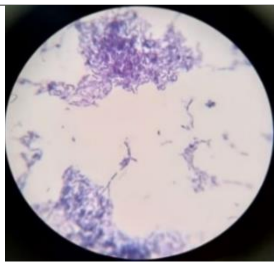
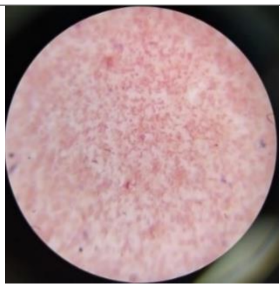
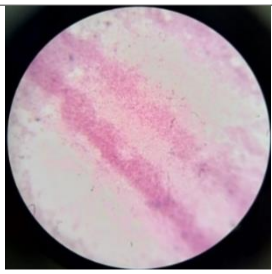
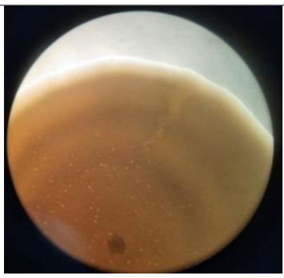
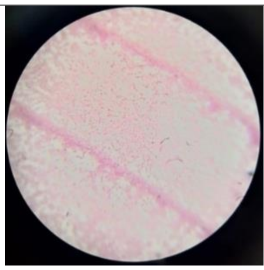
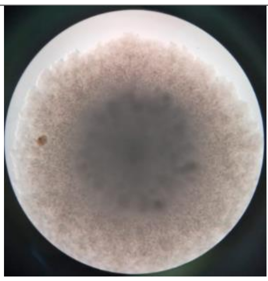
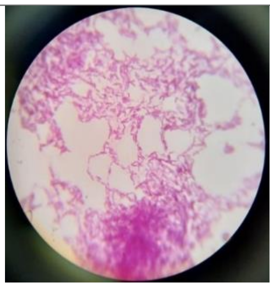
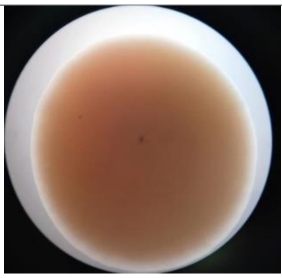
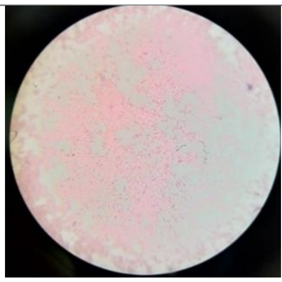
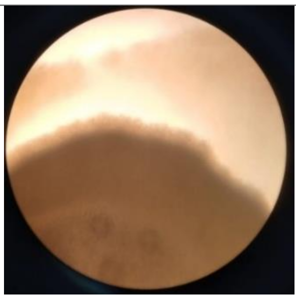
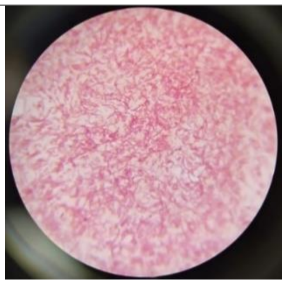
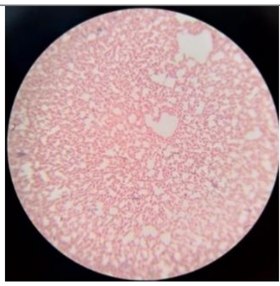
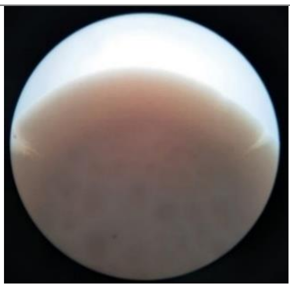
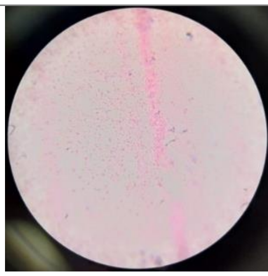
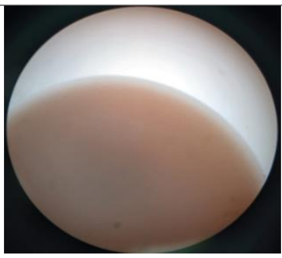
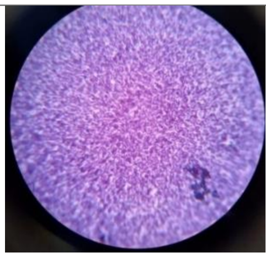
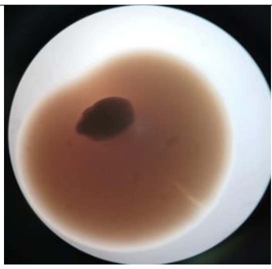
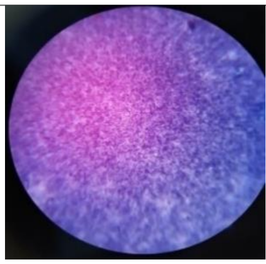
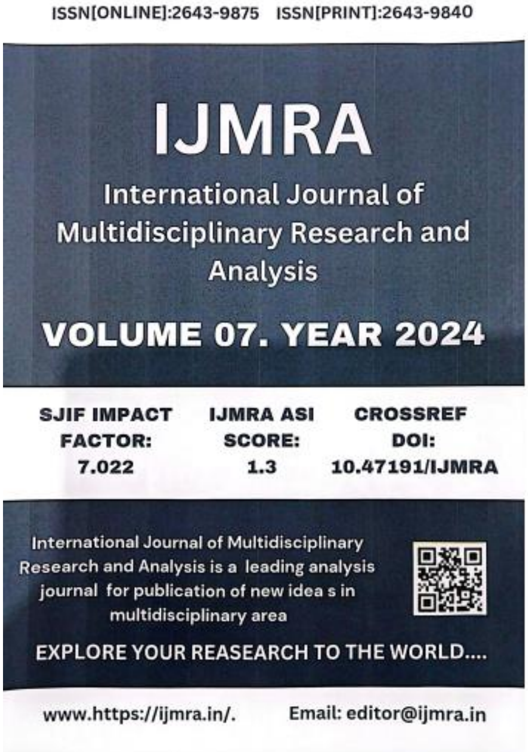
Reactor	Colony	Colony image	Gram staining	Gram test (-/+), cell shape, and arrangement	Cell identification
2L/min, 2071mg/LO_2	A			Gram (+) Circular Scattered	Cocci
	B			Gram (+) Oval shape Clustered	Coccobacillus
4L/min, 2071mg/LO_2	A			Gram (+) Oval shape Clustered	Coccobacillus
	B			Gram (+) Oval shape Clustered	Coccobacillus
6L/min, 2071mg/LO_2	A			Gram (-) Circular Scattered	Cocci
	B			Gram (+) Rod shape Scattered	Bacillus

Table 1C. Image of the colony and Gram staining under a bright light microscope at 12 days of SRT

Reactor	Colony	Colony image	Gram staining	Gram test (-/+), cell shape, and arrangement	Cell identification
2L/min, 4142mg/LO2	A			Gram (-) Rod shape Scattered	Bacillus
	B			Gram (-) Rod shape Clustered	Bacillus
4L/min, 4142mg/LO2	A			Gram (-) Rod shape Scattered	Bacillus
	B			Gram (-) Rod shape Scattered	Bacillus
6L/min, 4142mg/LO2	A			Gram (-) Rod shape Chained cells	Streptobacillus
	B			Gram (-) Rod shape Scattered	Bacillus

Reactor	Colony	Colony image	Gram staining	Gram test (-/+), cell shape, and arrangement	Cell identification
2L/min, 2071mg/LO_2	A			Gram (-) Oval shape Clustered	Coccobacollus
	B			Gram (-) Oval shape Clustered	Bacillus
4L/min, 2071mg/LO_2	A			Gram (-) Rod shape Clustered	Bacillus
	B			Gram (-) Rod shape Scattered	Bacillus
6L/min, 2071mg/LO_2	A			Gram (+) Circular Scattered	Cocci
	B			Gram (+) Circular Scattered	Cocci

Form



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