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Biological Treatment of Synthetic Oilfield-Produced Water in Activated Sludge Using a Consortium of Endogenous Bacteria Isolated from A Tropical Area

Edwan Kardena*, Syarif Hidayat, Silvia Nora and Qomarudin Helmy

Faculty of Civil and Environmental Engineering, Bandung Institute of Technology, Bandung 40132, Indonesia

Abstract

The aim of this study was to investigate the biological treatment of synthetic oilfield-produced water in activated sludge in an attempt to remove the organic compounds using endogenous bacteria; we also hope to determine the biokinetic coefficients. The activated sludge was operated with various hydraulic retention times (HRT=20 hours, 12 hours, 8 hours), solid retention times (SRT=25 days, 20 days, 15 days, 10 days), and substrate concentrations (500 mg L^{-1} to 1,100 mg L^{-1}). The endogenous bacterial strains, which were isolated from existing wastewater treatment facilities, were identified as *Pseudomonas* sp., *Enterobacter* sp., *Bacillus* sp1., and *Bacillus* sp2. It was observed that the highest COD removals were obtained in reactors A (80.7%) and B (82.4%), which had high SRTs (25 days and 20 days) and HRT (20 hours). At shorter SRTs (15 days and 20 days), the concentration of the COD effluent did not comply with the Indonesian regulations for oilfield-produced water quality standards, which means that these SRTs were not recommended as appropriate operational conditions. Furthermore, the results showed that the yield (Y), decay coefficient (kd), maximum specific growth rate (k), and saturation constant (K_s) were 0.533 mg MLVSS mg^{-1} COD, 0.167 day^{-1}, 0.985 day^{-1}, and 255.46 mg COD L^{-1}, respectively. These biokinetic coefficients (obtained from the Y and K_s values) indicated that although the strains of bacteria can grow well in the reactor, they had low affinities to the substrate, which caused the concentration of the COD effluent to be relatively high.

Keywords: Petrochemical wastewater; Petroleum hydrocarbon; Oil-degrading bacteria; Biokinetic coefficients; Endogenous bacteria

Introduction

Oilfield-produced water is a byproduct of drilling activities that occurs when extracted oil and gas are carried out; it includes formation water, injection water, and chemicals that are used for the drilling and/or separation of water and oil. The major compounds of these waters include dissolved and dispersed oil compounds, dissolved formation minerals, production chemical compounds, and production solids [1]. In the oil and gas industry, the oilfield-produced water makes up more than 80% of the total volume of waste that is generated; it is becoming a major problem because the amount of oilfield-produced water typically increases from year to year and it represents an environmental hazard [2,3].

Options for oilfield-produced water management include discharging the waste into water bodies or using a well re-injection process for oil-enhanced recovery and other beneficial re-uses. Nowadays, many countries have implemented stringent regulations for discharging oilfield-produced water, especially with regards to organic compounds. For example, the Indonesian government has changed the regulations for oilfield-produced water quality standards from site-based regulations to terminal-based regulations. As a consequence, every offshore oil exploration that purifies crude oil onshore must pay attention to the organic compound parameters; these were not considered in the previous standard [4]. In another case, the European standard sets a very low allowable concentration of the total petroleum hydrocarbon (<5.0 mg L^{-1}) for treated oilfield-produced water from onshore petroleum activities. Several physico-chemical treatments, including coagulation and flocculation [5], reverse osmosis [6], and membrane nanofiltration [7], have been applied for the removal of organic compounds from oilfield-produced water. However, most of these technologies are energy-consuming and only suitable for *in situ* re-use. Additionally, they have several problems related to their operation, maintenance, and secondary waste stream [8]. Thus, to comply with water body quality standards and avoid generating secondary waste byproducts, a more effective and efficient technology should be used to treat this wastewater.

In the last few years, many researchers have investigated biological treatments in attempts to treat organic compounds/oil-contaminated wastewater. These methods are appealing because they are cost effective, environmentally friendly, and they have high removal efficiencies. However, since oilfield-produced water contains toxic substances that can inhibit bacterial activity, the application of appropriate bacteria is important. Several strains of bacteria, including *Pseudomonas, Marinobacter, Halomonas, Aeromonas, Bacillus, Ochrobactrum, Achromobacter,* and *Rhodococcus*, have shown good performance in the removal of organic compounds in oil-contaminated wastewater [9-12]. It has been reported that the performances of these bacteria depend on the concentration of petroleum hydrocarbon [2], the salinity/total dissolved solid (TDS) concentration [9], the nutrient composition [13], and other environmental conditions. Alternatively, the solid retention time (SRT) [14] and hydraulic retention time (SRT) [15] are also considered to be critical operating parameters that affect the performance of biological treatment processes. In most cases, the SRT and HRT influence the removal efficiency of organic compounds, the structure and physical characteristics of sludge, the composition, and the activity of bacteria.

Although several studies have been carried out investigating the treatment of oilfield-produced water with activated sludge, the values of biokinetic coefficients are not widely available. Therefore, in order

***Corresponding author:** Edwan Kardena, Faculty of Civil and Environmental Engineering, Bandung Institute of Technology, Bandung 40132, Indonesia, Tel: +62 22 2500935; E-mail: kardena@pusat.itb.ac.id

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to determine the biokinetic coefficients and evaluate the performance of the activated sludge, the degradation of the organic compounds in synthetic oilfield-produced water by endogenous bacteria was investigated in this study. The endogenous bacteria, which were isolated from existing oilfield-produced water treatment facilities, were used as organic-degrading bacteria. The activated sludge was operated with different HRTs, SRTs, and initial concentrations of organic compounds. Furthermore, biochemical tests were conducted to identify the selected bacteria.

Materials and Methods

Characteristics of oilfield-produced water

Samples of oilfield-produced water were taken from an Indonesian oilfield located in Balikpapan; these were used to perform screening and kinetic growth tests for the bacteria. In the other hand, the synthetic oilfield-produced water was used throughout our study to determine the capability of strains of bacteria to degrade organic compounds. The synthetic oilfield-produced water was prepared by mixing crude oil (1.5 mL L^{-1}) and salt water (real sea water and tap water) in a container, as described in the literature [12,16]. The C/N/P ratio of the medium was adjusted to approximately 100/5/1 by adding appropriate concentrations of $(NH_4)_2SO_4$ and KH_2PO_4 to the synthetic oilfield-produced water. This synthetic oilfield-produced water was used throughout our study to determine the capability of isolated strains of bacteria to degrade organic compounds. The characteristics of the real and synthetic oilfield-produced water samples are listed in Table 1.

Growth medium

A standard basal salts (SBS) medium was used as the bacterial growth medium; this contained K_2HPO_4 (1.5 g), KH_2PO_4 (0.5 g), $MgSO_4$ (0.2 g), $(NH_4)_2SO_4$ (0.5 g), yeast extract (0.5 g), and 1 mL of a trace-element solution in 1 L of distilled water. The pH of this medium was adjusted to 7.0. The real oilfield-produced water was added as a carbon source at 2.0% (v v^{-1}). The SBS solid medium was prepared by adding 5 g L^{-1} of bacto agar. All media and solutions were sterilized with an autoclave at 121°C for 20 min.

Isolation and identification of bacteria

The samples for inoculum were isolated from the existing units of oilfield-produced water treatment facilities; these included the dissolved air flotation (DAF) unit (strain-D), sediment (strain-S), inlet (strain-I), and outlet (strain-O). Cultures of the strains of bacteria were obtained by inoculating 5.0 mL of each sample into a 500 mL SBS liquid medium. These were then incubated at 30°C and stirred at 120 rpm for six days, 10 mL of the previous enrichment cultures were inoculated into fresh liquid SBS with 2.0% (v v^{-1}) of real oilfield-produced water and cultivated under the same conditions. After four cycles of enrichment, 1.0 mL of each culture was serially diluted (10^{-1} to 10^{-7}), spread on the surface of the SBS solid medium, and incubated at 30°C for three days, 500 µL of sterile, real oilfield-produced water was spread on the surface of the plates as the sole source of carbon. The predominance of a single colony was picked out and inoculated in a fresh SBS liquid medium supplemented with 2.0% of oilfield-produced water as the carbon source; this was subsequently incubated for two days. The colony and cell morphology of the selected bacteria were determined using macroscopic, microscopic, and motility tests. The bacterial strains were identified by classical methods, as outlined by Cowan and Steele's manual for the identification of medical bacteria [17] and Bergey's manual on systematic bacteriology [18].

Parameters	Unit	Oilfield-produced water			
		Site-1	Site-2	Site-3	Synthetic
COD	mg L^{-1}	1,863	1,048	504.8	1,120
pH	mg L^{-1}	7.38	7.42	7.65	6.85
Oil and grease	mg L^{-1}	30.2	18.5	10.2	-
TDS	mg L^{-1}	8,800	6,125	5,125	11,000

Table 1: Characteristic of real and synthetic of oilfield produced water.

Biodegradation potential

In order to assess the biodegradation potential of organic compounds by the selected bacteria, the bacteria were tested with three different types of real oilfield-produced water (Site-1, Site-2, and Site-3). The degradation potential was based on the specific growth rate (µ) and chemical oxygen demand (COD) removal efficiency. To make a growth curve, 2.0 mL samples were taken and the optical density (OD) of the media was measured every 1.0 h to 2.0 h using a spectrophotometer. The absorbance was measured at 600 nm against a blank sample (2.0 mL of the produced water). The growth curve was obtained by plotting the OD versus the observation time (t). By using the general equation of the 1st order kinetics reaction, we can obtain the specific growth rate of bacteria (µ) in the exponential phase. The initial and final CODs were measured using standard methods (DR-2800, Hach Company).

Bioreactor design/operation and determination of the biokinetic coefficients

The schematic diagram of the experimental set-up for the activated sludge system is shown in Figure 1. The aeration tank and clarifier were made of acrylic glass with 7.0 L and 10.0 L working volumes, respectively. In order to maintain the dissolved oxygen (DO) in the system (DO>4.0 mg L^{-1}), air was supplied to the aeration tank by a blower through the diffused aerator. The synthetic oilfield-produced water was fed continuously into the bioreactor as the sole carbon source for the microbial population growth. Peristaltic pumps were used to maintain the desired hydraulic retention time (HRT) and solid retention time (SRT) in the system.

The system operation was divided into two stages: (1) an acclimation period and (2) a treatment period. During the acclimation period, the activated sludge was first operated with the batch conditions for approximately 20 days. During this period, the activated sludge was fed with a COD concentration of 1040 mg L^{-1} to 1170 mg L^{-1}. The treatment period was carried out in four different reactors (reactors A, B, C, and D) with SRTs of 25 days, 20 days, 15 days, and 10 days and HRTs of 8 h, 12 h, and 20 h for each SRT, respectively (with 100% return sludge). The average initial concentration for each HRT was 1000 mg L^{-1}, 700 mg L^{-1}, and 500 mg L^{-1}. The performance of the activated sludge was monitored by analyzing the COD and biomass in the system.

The biokinetic coefficients were determined according to the following equations [19]:

$$\frac{1}{\grave{e}_c} = YU - k_d = Y\frac{S_o - S_e}{\grave{e}_h X} - k_d \quad , \tag{1}$$

$$\frac{1}{U} = \left(\frac{K_s}{k}\right)\left(\frac{1}{S_o}\right) + \frac{1}{k} \quad . \tag{2}$$

Here, θ_c is the solid retention time (day), Y is the biomass yield (mg MLVSS mg^{-1} COD), U is the substrate utilization rate (mg COD mg^{-1} MLVSS day^{-1}), k_d is the endogenous decay coefficient (day^{-1}), S_o is the influent substrate concentration (mg COD L^{-1}), S is the effluent substrate concentration (mg COD L^{-1}), X is the biomass concentration

(mg MLVSS L^{-1}),θ_h is the hydraulic retention time (d), K_s is the half-velocity constant (mg COD mg^{-1} MLVSS), and k is the maximum rate of substrate utilization (mg COD mg^{-1} MLVSS).

Results and Discussion

Isolation and identification of bacteria

Four bacterial strains were isolated from existing oilfield-produced water treatment facilities by enriching the cultures and performing dilution plate separation. The isolated bacterial strains were characterized by their colony, cell morphology, and biochemical characteristics (Table 2). Based on the data obtained from the colony morphology tests, selected bacteria were observed to be circular in shape with curled edges. They were also unpigmented or pigmented with shiny-yellow and cream colors. The cell morphologies of the bacteria were in the form of rods in chains, spore-forming or non-spore-forming, and motile with flagella. Two strains were Gram-positive and two strains were Gram-negative.

According to the data obtained from biochemical testing, the selected bacteria were closely related to *Pseudomonas* sp., *Enterobacter* sp., *Bacillus* sp., and *Bacillus* sp. for strain-D, strain-S, strain-O, and strain-I, respectively. Bacteria of the genera *Pseudomonas*, *Enterobacter*, and *Bacillus* have been widely reported as bacteria that can degrade the organic compounds in petroleum oil and gas wastes, including oilfield-produced water [9,20], soil contaminated with hydrocarbons [21-24], and wastewater contaminated with hydrocarbons [10]. A recent study reported that the genus *Enterobacter* also has the ability to degrade the biopolymers that are used in oil-recovery processes [25]. Furthermore, it is well known that these strains can synthesize bioemulsifiers, which can enhance the bioavailability of hydrocarbon as a carbon source [26,27]. Strains that have genes involved in bioemulsifier synthesis and regulation are versatile for the degradation, emulsification, and metabolizing of hydrocarbons [28].

Biodegradation potential of organic compounds by a bacterial strain

To determine the biodegradation potential, mixed cultures of the selected bacteria were tested in three different types of real oilfield-produced water (water from Site-1, Site-2, and Site-3). The COD and TDS concentrations of these oilfield-produced water samples varied

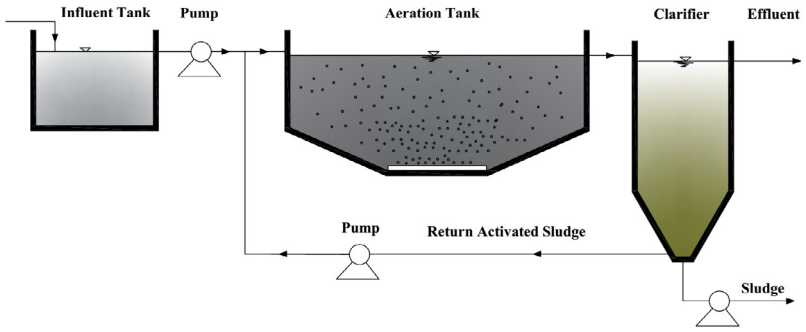

Figure 1: Schematic diagram of experimental set-up for the activated sludge.

Characteristics	Tests	Results			
		Strain-D	Strain-S	Strain-O	Strain-I
Colony and cell morphology	Macroscopic	Circular, Entire, Convex, Pigmented, Shiny yellow (fluorescent), Translucent	Circular, Entire, Convex, Unpigmented, Transparent	Circular, Curled, Raised, Unpigmented, Opaque	Circular, Entire, Convex, Pigmented, Cream, Opaque
	Microscopic	Rod, Gram (-), Non-endospore forming	Rod, Gram (-), Non-endospore forming	Rod, Gram (+), Endospore forming	Rod, Gram (+), Endospore forming
	Motility	Motile	Motile	Motile	Motile
Biochemical	Starch hydrolysis	(-)	(-)	(+)	(-)
	Fat hydrolysis	(-)	(-)	(-)	(-)
	Casein hydrolysis	(+)	(-)	(+)	(-)
	Gelatin hydrolysis	(+)	(-)	(+)	(-)
	Glucose fermentation	(+)	(+)	(+)	(-)
	Sucrose fermentation	(-)	(-)	(-)	(-)
	Lactose fermentation	(-)	(-)	(-)	(-)
	H_2S production	(-)	(-)	(-)	(-)
	Indol production	(+)	(+)	(-)	(-)
	Urease production	(+)	(+)	(+)	(+)
	Catalase production	(+)	(+)	(+)	(+)
	Metil red	(+)	(+)	(+)	(-)
	Voges-Proskauer	(-)	(-)	(-)	(-)
	Triple sugar iron	(+)	(+)	(+)	(-)
	Simmon's citrate agar	(+)	(+)	(-)	(-)
	Nitrate reduction	(+)	(+)	(+)	(-)
Conclusion*		***Pseudomonas sp.***	***Enterobacter sp.***	***Bacillus sp1.***	***Bacillus sp2.***

**Bergey's Manual of Systematic Bacteriology*

Table 2: Colony and cell morphology and biochemical characteristics of selected bacterial strain.

from 505 mg L^{-1} to 1,863 mg L^{-1} and 5,125 mg L^{-1} to 8,800 mg L^{-1}, respectively. Recent research states that the concentrations of organic compounds and TDSs in oilfield-produced water can be affected by the biodegradation capability of bacteria [9]. They can inhibit the bacterial production of enzymes and cause cell plasmolysis, leading to low organic removal efficiencies. In this study, the strains of bacteria seem to adapt well in the oilfield-produced water because there is no indication of any inhibitory effects during the degradation test. The curve of growth shows that the strains of bacteria can grow very well in the oilfield-produced water (Figure 2). The strains of bacteria tend to grow and reach the exponential phase after 5 h of incubation, which indicates that the strains of bacteria can consume organic compounds in the oilfield-produced water as a carbon source as soon as they come into contact with the wastewater. It was reported that endogenous bacteria can grow and adapt well to the target contaminants and/or to extreme conditions due to their environmental suitability [11].

The value of the specific growth rate (μ) and COD removal after four days of incubation for each site were 5.42 day^{-1}, 5.23 day^{-1}, and 4.37 day^{-1} and 81.1%, 77.3%, and 64.3%, respectively (Table 3). The specific growth rate (μ) and COD removal of the strains of bacteria tended to

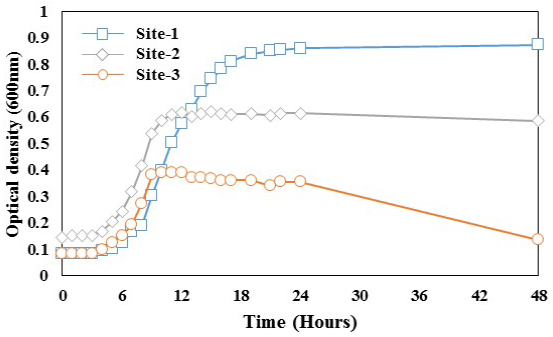

Figure 2: Growth of selected bacteria in the three different types of real oilfield-produced water.

Parameters	Unit	Oilfield-produced water		
		Site-1	Site-2	Site-3
Specific growth rate (k)	day^{-1}	5.42	5.23	4.37
COD removal	%	81.1	77.3	64.3

Table 3: Specific growth rate (μ) and COD removal of selected bacteria in different types of oilfield-produced water.

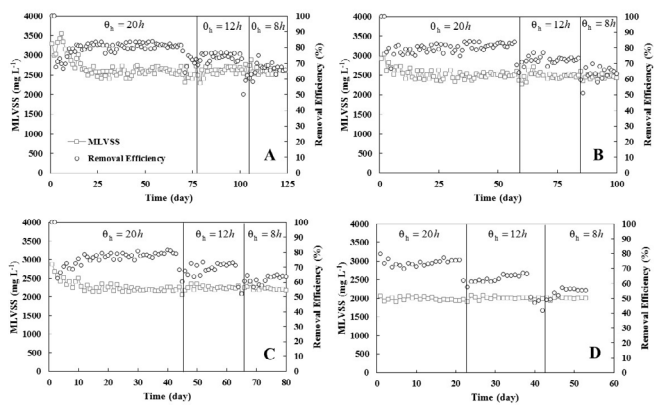

Figure 3: Profile of COD removal and MLVSS concentration of activated sludge system for oilfield-produced water treatment at (A) 25 days, (B) 20 days, (C) 15 days, and (D) days of SRT and different HRT (20 h, 12 h, 8 h).

No.	SRT (day)	HRT (hours)	MLVSS (mg L^{-1})	Se (mg L^{-1})
1	25	20	2586	194
2		12	2653	179
3		8	2637	172
4	20	20	2504	180
5		12	2483	190
6		8	2467	180
7	15	20	2192	208
8		12	2241	199
9		8	2199	191
10	10	20	1950	265
11		12	2000	260
12		8	2000	250

Table 4: The concentration of MLVSS and COD effluent at the treatment period.

increase as the initial COD concentration in the oilfield-produced water increased. It was assumed that the activity of the strains of bacteria in the oilfield-produced water was higher with higher COD concentrations than that with lower concentrations due to the high availability of the substrate. The μ value achieved in this study was slightly greater than or less than those reported by Tellez et al. [29] and Talaie et al. [30]. It is well known that the μ value depends on the type and composition of bacteria as well as on the characteristics of wastewater. In terms of the COD removal, even with an extended incubation time of six days, there was no significant change; the concentration of the final effluent was still high (181 mg L^{-1} to 358 mg L^{-1}). This suggested that the oilfield-produced water had a lack of nutrients (nitrogen and phosphate). It has been reported that an appropriate composition of nutrients is an important factor in the degradation of organic compounds, especially for hydrocarbon degradation [12,13]. A sufficient amount of nutrients in the wastewater can stimulate the bacteria activity, leading to high removal of the targeted contaminants.

Bioreactor performance and biokinetic determination

The reactor was acclimated for 20 days using batch conditions with an initial COD concentration of 1.040 mg L^{-1} to 1.170 mg L^{-1}. During the first three days of the acclimation period, the average COD removal was 38.7%. This value increased to 83.4% after 10 days (data not shown). The COD concentration of the effluent remained below 162 mg L^{-1} during the remainder of the acclimation period. The acclimation period becomes important as the bacteria undergo phase adaptation in a new environment on the substrate. These results indicated that the selected bacteria were acclimatized such that they could use the organic compounds in the synthetic oilfield-produced water as a carbon source [10].

The performance of the activated sludge in the treatment period can be seen in Table 4 and Figure 3. It was observed that, at the beginning of each phase, the concentration of MLVSS decreased. However, the system reached a steady-state condition after a few days and became more adapted to the new environment as a function of time. In the steady-state condition, the highest MLVSS concentration was observed in reactor A (approximately 2.586 mg L^{-1} to 2.653 mg L^{-1}) while the lowest concentration was found in reactor D (approximately 1.950 mg L^{-1} to 2.000 mg L^{-1}). The average MLVSS concentrations of reactors A, B, C, and D (SRT: 25 days, 20 days, 15 days, and 10 days) were relatively constant but decreased with reduced SRTs (from 25 days to 10 days). This means that, in these experiments, the reduction of the HRT (from 20 to 8 h) and initial COD concentration (from 1000 mg L^{-1} to 500 mg L^{-1}) did not affect the MLVSS concentration in the reactor. These results indicated that the MLVSS concentration in the reactor was controlled by the SRT. A similar trend was observed by Tellez et al. [2] who reported the ability of an activated sludge to remove petroleum

hydrocarbons from oilfield-produced water. It was clear that a shorter SRT increases the sludge waste volume, which leads to a decrease in the MLVSS concentration in the reactor.

As observed in Figure 3, the COD removal was decreased at the beginning of each phase but then recovered after a few days. In these experiments, reducing the HRT and initial COD concentration caused a slight increase in the organic loading rate (OLR). Alternatively, increasing the OLR in the bioreactor caused the microorganisms to be faced with a sudden increase in the organic loading, which limited their metabolism and decreased the reactor performance [10,31-33]. However, with respect to time, the microorganisms recovered and adapted to the changed loading conditions and eventually reached steady-state biodegradation [33]. Under steady-state conditions, the concentrations of the COD effluent for each HRT (20, 12, 8 h) in reactor A (194 mg L^{-1}, 179 mg L^{-1}, 172 mg L^{-1}), B (180 mg L^{-1}, 190 mg L^{-1}, 180 mg L^{-1}), C (208 mg L^{-1}, 199 mg L^{-1}, 191 mg L^{-1}), and D (265 mg L^{-1}, 260 mg L^{-1} and 250 mg L^{-1}) were slightly decreased as the HRT and initial COD concentration decreased. However, the concentrations of the COD effluent increased as the SRT decreased. These results suggested that the highest COD removal can be obtained with longer HRTs and SRTs. Longer HRTs increase the contact time between the target contaminant and the biomass [32], while longer SRTs cause the MLVSS to be better adapted to the target contaminant [33], leading to improved reactor performance. The highest COD removal was obtained in reactors A and B when an HRT of 20 h was applied; this produced value close to 80.7% and 82.4%, respectively. Furthermore, in reactors C and D (SRTs of 15 days and 10 days), the residual COD in the effluent is still high and does not comply with the Indonesian regulations for oilfield-produced water quality standards. This was consistent with the previous research of Tellez et al. [2], which reported that an SRT of 20 days is the minimum retention time required to degrade the organic compounds contained in oilfield-produced water.

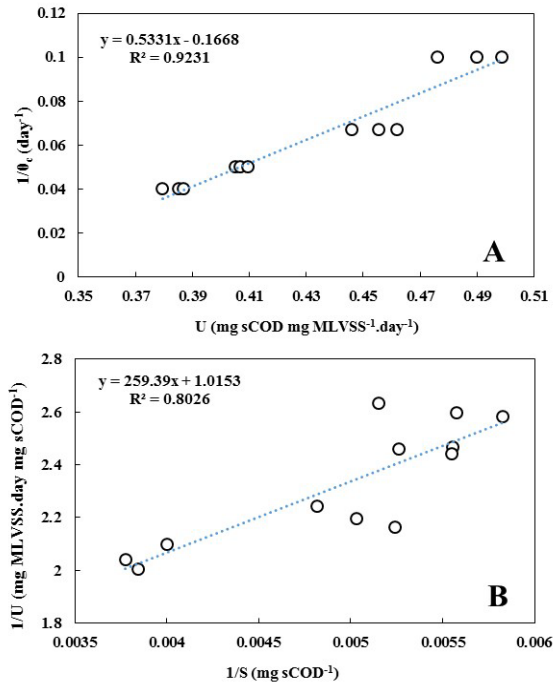

Figure 4: Determination of (A) Y and k_d and (B) Ks and k values of activated sludge system.

The biokinetic coefficients were determined by Equations (1) and (2). These equations describe the growth of biomass, substrate utilization rates, and mean cell residence time in the activated sludge processes. A plot of 1/θc as a function of the substrate consumption rate U (Figure 4A) provides the linear regression as the slope and the gradient is the yield. Therefore, the x-intercept is kd. Additionally, a plot of 1/U as a function of 1/S (Figure 4B) gives the linear regression as the slope and the gradient is Ks/k. Therefore, the x-intercept is kd and the y-intercept is 1/k. The values of the yield (Y), decay coefficient (k_d), maximum specific growth rate (k), and saturation constant (K_s) were 0.533 mg MLVSS mg^{-1} COD, 0.167 day^{-1}, 0.985 day^{-1}, and 255.46 mg COD L^{-1}, respectively. The Y, k_d, and K_s values obtained from these experiments were higher than those of the design criteria specified by Tchobanoglous et al. [19]. The Y and k_d values represent the sludge production and the biomass lost due to endogenous respiration during the sludge processes, respectively. Therefore, a high Y value indicates a higher sludge production, while a high k_d value causes a reduced net sludge production [34]. In the process design of a wastewater treatment facility, the Y and k_d values play an important role in determining the volume of the reactor of the sludge handling facilities. Meanwhile, the k value affects the total volume of the reactor; a smaller k value leads to a greater reactor volume. The K_s value shows the affinity of the biomass to the substrate; therefore, a concentration of substrate at half of its maximum growth rate. The high K_s value (255.46 mg COD L^{-1}) obtained in this experiment indicated that the strains of bacteria have a low affinity for the substrate, which means that the growth rate of the strains of bacteria will be affected by the residuals on the substrate, which are still high.

Conclusion

Activated sludge with endogenous bacteria was used to treat synthetic oilfield-produced water under different hydraulic retention times (HRT=20 h, 12 h, 8 h), solid retention times (SRT=25 days, 20 days, 15 days, 10 days), and initial substrate concentrations (500 mg L^{-1} to 1,100 mg L^{-1}). Through biochemical testing, the endogenous bacteria, which were isolated from existing wastewater treatment facilities, were identified as *Pseudomonas* sp., *Enterobacter* sp., *Bacillus* sp1., and *Bacillus* sp2. It was observed that the MLVSS concentration was controlled by the SRT, while the COD removal depended on the HRT and SRT. Therefore, due to the high of MLVSS concentration and the long contact time, high removal efficiencies (80.7% and 82.4%) were obtained with a long HRT (20 hours) and SRT (20 days and 25 days). Meanwhile, at SRTs of 15 days and 10 days, the concentration of the COD effluent did not comply with the Indonesian oilfield-produced water quality standards. This indicates that these SRTs are not suitable for operational treatment conditions. The values of the biokinetic coefficients obtained from these experiments were 0.533 mg MLVSS mg^{-1} COD, 0.167 day^{-1}, 0.985 day^{-1}, and 255.46 mg COD L^{-1} for the yield (Y), decay coefficient (kd), maximum specific growth rate (k), and saturation constant (Ks), respectively. These results suggested that although the strains of bacteria can grow well in the reactor (as indicated by the high Y value), their low affinity for the substrate causes the COD concentration of the effluent to be relatively high (as indicated by the high Ks value).

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