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Study of acclimatization and kinetics of pollutant degradation by mixed culture microorganisms in restaurant wastewater

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Abstract *With the development of the restaurant industry, a series of environmental problems emerged, including high concentrations of oily wastewater. As a developing country, Indonesia has a large amount of effluent produced that is not processed but is discharged directly to the available pool. In order to handle restaurant wastewater, physical and chemical approaches can be combined with biological degradation (biodegradation). This biodegradation can be increased by bacterial acclimatization, namely by the addition of microorganisms that have been developed that can efficiently degrade pollutants in a contaminated environment. Stages of research were carried out by propagation of bacteria (Seeding), then the acclimatization process was carried out to adapt bacteria to waste, biodegradation process, and calculate the growth rate curve of microorganisms. It was found that the half-saturation constant of mixed culture microorganisms to treat restaurant waste was 41,336, with a maximum growth rate of 0.124 per day.* [STUDY OF ACCLIMATIZATION AND KINETICS OF POLLUTANT DEGRADATION BY MIXED CULTURE MICROORGANISMS IN RESTAURANT WASTEWATER] ***(J. Math. Nat. Sci., 1(2): 61 - 66, 2021)***

Keywords: *Restaurant wastewater, Acclimatization, Kinetics, Mixed culture microorganism*

Introduction

With the development of the restaurant industry, a series of environmental problems have emerged, including food waste and high concentrations of oily wastewater (Gao et al., 2019). As a developing country, Indonesia has a large amount of effluent produced that is not treated discharged directly into the general pool, which causes serious environmental pollution.

Restaurant wastewater is generated from the use of large amounts of animal and vegetable oils during cooking. Oils and fats generally have high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values (Gao et al., 2019). Oily restaurant wastewater contains fat, oil, and suspended materials on a large scale, resulting in a foul odor and blockage of drains (Brooksbank et al., 2007). These wastewater clogs can potentially cause regional flooding in some areas.

Physical and chemical approaches can be combined with biological degradation (biodegradation) to handle restaurant wastewater. The biodegradation process utilizes the ability of microorganisms to decompose hydrocarbons, in this case, oils and fats, as a carbon source (Nzila et al., 2017). This biodegradation can be enhanced by bacterial acclimatization, namely by adding microorganisms developed previously that can efficiently degrade pollutants in a contaminated environment (Nzila et al., 2017). This biological approach is considered environmentally friendly and cost-effective, thus enabling the treatment of large quantities of wastewater.

Oils and fats are triglycerides known as fatty acid esters bonded to a glycerol molecule (three fatty acids to one glycerol molecule). In order to degrade oils and fats, bacteria with extracellular lipase are needed. Bacteria with specific or mixed cultures have been shown to aerobically degrade oils and fats under thermophilic conditions (Wang et al., 2022). In this study, mixed culture from the septic tank of residents' houses in Bandung was isolated and acclimatized. The overall objective of this research is to see the COD removal ability and the growth rate of mixed culture in restaurant wastewater.

Materials and Methods

In this study used Padang restaurant wastewater obtained on Jalan Tubagus Ismail, Bandung City. Materials such as in Table 1.

a. Restaurant Waste Characterization. The restaurant waste characterization test was carried out by testing the wastewater quality parameters, namely pH, temperature, dissolved oxygen (DO), COD, TSS and VSS.

b. Seeding. Seeding is done to multiply the bacterial culture used in the biological treatment process. The seeding process was carried out under batch conditions and nutritional and environmental conditioning suitable for aerobic growth (Wang et al., 2015). The stages of the seeding process are as follows:

1. The parent culture of bacteria from the septic tank as much as 2 L is inserted into the reactor and added with distilled water until it reaches 5 liters
2. Source of biodegradable carbon, namely glucose added up to COD 1000 mg/L
3. The supporting nutrients are added to the reactor, namely nitrate and phosphate with a ratio of COD: NO3: PO4 = 120: (3 or 5): 1
4. Bacterial growth was measured from the TSS parameter every day. Seeding is discontinued when TSS is more than 4000 mg/L.

c. Acclimatization. The process of acclimatization is carried out to adapt the bacteria to the waste to be processed. Environmental parameters such as pH, DO, and the temperature was measured every day during the acclimatization process.

COD and TSS measurements were also carried out every day to determine the extent to which the acclimatization process was stopped and continued to the next stage. The initial COD was set at 1000 mg/L. Each acclimatization stage was stopped when the addition of TSS and reduction of COD reached a stagnant value (stationary in the curve). Due to the batch process, the aerator is stopped, the sludge is allowed to settle before the effluent is removed, and a new carbon source is added (next stage) (Fito and Alemu, 2019). Acclimatization is carried out in 3 stages, namely:

i. Acclimatization 1: is done by making a mixture of sugar and waste as a carbon source with a ratio of 75: 25.
ii. Acclimatization 2: is done by making a mixture of sugar and waste as a carbon source with a ratio of 50: 50.
iii. Acclimatization 3: Made a mixture of sugar and waste as a carbon source with a ratio of 25: 75.

d. Biodegradation. After acclimatization 3, the biodegradation process was carried out by introducing 100% of the waste into the reactor by diluting up to 5 L with a composition of 0.98 L of restaurant waste.

The environmental conditions were kept the same as the conditions during acclimatization. This experiment aims to determine the rate of biodegradation kinetics and the efficiency of waste removal. The calculation of the removal efficiency is:

$$\%R = ((Co - Ce)/Co) \times 100\% \quad (1)$$

Table 1. Materials used in the study.

No	Materials	Information
1	Sugar (sucrose)	Acclimatization process
2	Sludge from cultured septic tank cultures	Acclimatization process
3	NO_3	Acclimatization process
4	PO_4	Acclimatization process

e. Growth kinetics. In batch conditions, the growth rate of bacterial cells was analyzed using the following formula:

$r_g=\mu X$ (2)

$dX/dt=r_g$ (3)

$dX/dt=\mu X$ (4)

Where (r_g) growth rate of bacteria, mg/L.day (μ) specific growth rate, 1/day (X) mass of microorganisms, mg/L.

Table 2. Sample analysis method.

Parameter	Method
Temprature	APHA-2550-2012
pH	APHA-4500-H+-B-2012
DO	APHA-4500 O G-2012
COD	APHA-5220-B-2012
MLVSS	Gravimetri
Ammonium	APHA-4500-NH3-F & B-2012
Fosfat	ammonium molybdate–spektrofotometri

Results

A. Seeding. At the seeding stage, bacteria are propagated to treat restaurant wastewater. The propagation of microorganisms aims to grow the biomass of microorganisms in order to be able to treat waste in greater concentrations. Within six days, biomass with a concentration of 2200 mg/L was produced, divided into two reactors, each with 1100 mg/L. Figure 1 shows that the concentration ofsubstrate was quite low on day 3. The decrease in COD is balanced with the increase in the number of microorganisms reaching a concentration of 4932 mg/l, which means that the next step is acclimatization.

B. Acclimatization. Visually the formation of activated sludge is characterized by a suspension that initially turns brown to black accompanied by increased turbidity (Friedrich et al., 2015). MLVSS is a parameter where the sign that the biomass component has been formed.

The COD concentration in the acclimatization stage 1 decreased, while the MLVSS concentration increased, but after the 3rd day, it decreased. The decrease in COD concentration was caused by bacteria's decomposition of a mixture of glucose and restaurant waste (complex organic matter). Bacteria use both substances as a source of food/substrate. The decrease in COD parameters represents biodegradable and non-biodegradable organic matter contained in restaurant waste that has been exhausted.

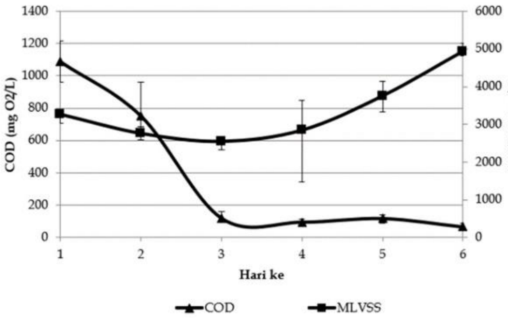

Figure 1. Substrate conditions and microorganisms in the seeding period.

Discussion

The MLVSS condition of the precipitated batch reactor bacteria showed that the values were in the range of 900 mg/L. The MLVSS concentration was close to the existing MLVSS concentration after the acclimatization process 1 ended. The difference between the MLVSS value on the last day of acclimatization 1 ended, and the beginning of acclimatization two was caused by bacterial deposition before entering the acclimatization stage 2. When the bacteria settled in approximately 1 hour, there was a process of breeding and compaction (Friedrich et al., 2015).

The COD concentration decreased gradually from day 1 to day 4 of the acclimatization process 3, followed by a significant increase in MLVSS on days 1 to 3. However, it showed a decrease in MLVSS on days 3 to 4. This decrease was possible due to the shock loading of the system—activated sludge. In phenol degradation, it has been proven that phenol has a significant inhibitory effect on the growth of microorganisms at higher concentrations (Zeng et al., 2016). Therefore, acclimatization of mixed cultures isolated from sewage treatment plants was carried out to grow in the presence of phenol as the sole carbon source up to the maximum concentration (Saravanan et al., 2008).

Environmental conditions, namely pH, temperature, and dissolved oxygen, have an important role in developing microorganisms. The decrease in environmental conditions was detected due to the decreased DO condition due to the decreased function of the aerator at that stage (Firra et al., 2016). Based on this, the aerator repair in the reactor was carried out. The aerator provides oxygen supply as an electron acceptor in the degradation process; disruption of oxygen supply can be indicated by a decrease in MLVSS in a reactor.

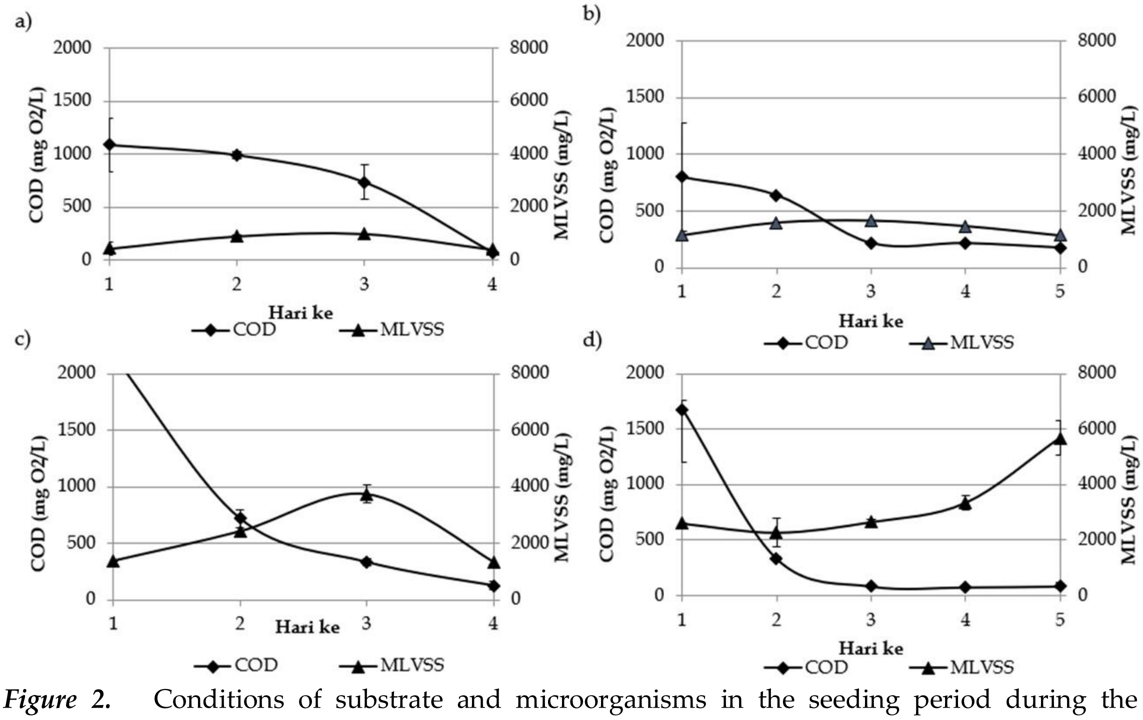

Figure 2. Conditions of substrate and microorganisms in the seeding period during the acclimatization and running period (a) acclimatization stage 1, (b) acclimatization stage 2, (c) acclimatization stage 3, (a) running reactor.

Table 3. Specific Growth Value based on the Relationship ln (X/Xo) to Time.

Variation	S	1/S	μ	1/μ
1	100	0.01	0.2243	4.458315
2	200	0.005	0.1516	6.596306
3	300	0.003333	0.1249	8.006405
4	400	0.0025	0.1318	7.587253
5	500	0.002	0.165	6.060606

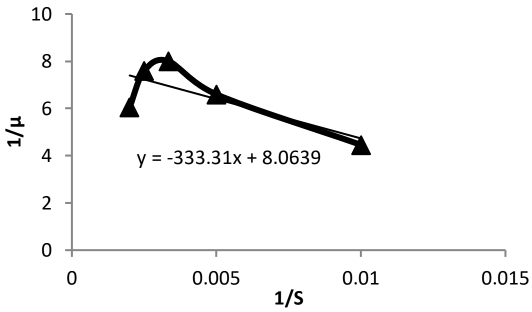

Figure 3. Graph of the Relationship of 1/μ to the value of 1/S.

MLVSS at five different concentrations and different periods was then used to calculate the specific growth rate and the value of the half-saturation constant. The values obtained from the plotting above are the data obtained to calculate the maximum specific growth rate (μmax) and Ks (half-saturation constant) of the mixed culture of microorganisms in the system (Lim & Vadivelu, 2019). Figure 4.11 is a graph of MLVSS growth against a time of the five variations of COD concentration. From these data, the plotting is carried out to find the value of the half-saturation constant by using the slope of the five data. Slope can be interpreted so that 1/μ results will be obtained as a reference for the relationship with the substrate to obtain a saturation half constant. Kinetic Model of Microorganism Growth Rate

The microorganism growth rate curve is done by designing five reactors containing microorganisms ready to treat wastewater with varying concentrations of COD. The five COD concentrations were respectively 100, 200, 300, 400, 500 in mg/L units. Within 5 hours, the MLVSS increase was measured every hour. The increase in the average

Based on the plotting of the 1/μ and 1/S relationships, the linear regression values obtained are:

y = -333.31x + 8.0639

For this reason, the Ks value can be calculated as follows:

1/μmaks = 8.0639

Ks/μmaks = 333.31

μmaks = 1/8.0639 = 0.124 /day

Ks = 333.31 x 0.124 = 41.3336

Conclusion

Based on the calculation, it was found that the half-saturation constant of mixed culture microorganisms to treat restaurant waste was 41,336 with a maximum growth rate of 0.124 per day.

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