



Journal Homepage: - www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/3254
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/3254>



RESEARCH ARTICLE

BIODEGRADATION OF ORGANIC MATERIAL FROM PRODUCE WATER USING CONSORTIUM MICROORGANISM WITH STEP AERATION PROCESSING

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Manuscript Info

Manuscript History

Received: 8 December 2016
 Final Accepted: 12 January 2017
 Published: February 2017

Key words:-

biodegradation, consortium
 microorganism, step aeration

Abstract

Produce water is an effluent discharge from oil processing, which have an physical-chemical characteristic is a high organic pollutant as an Chemical Oxygen Demand (COD), ammonium, sulfida, phenol, oil content and high temperature. Sometimes the pollutant also metal content as a mercury which an toxic substance that can be an inhibitor for biological processing. Five strain microorganisms i.e. *Pseudomonas*, *Staphylococcus aureus*, *Thiobacillus ferrooxidans*, *Bacillus* sp, and *Alcaligenes* to be acclimated using produce water as a carbon source from real waste on batch system for 30 days. The condition in the batch system is a biomass concentration 10% (v/v), waste concentration 100%, CNP ratio 100:10:1, incubation time 4 days at room temperature (28°C). Growth rate microorganism was checked for make sure that microorganism could be growth, and the kinetic constant obtained is specific growth rate (μ) : 0,037/h, maximum growth rate (μ_{max}): 0.45/day organic removal coefficient (k) : 207.8 mg/l COD/day, and half velocity constant (Ks) : 35 mg COD/l. The operation continued to the biological reactor with step aeration processing after batch system. The flow rate in to continuous reactor is 0,90 l/h with detention time 2 (two) days and return sludge (r) : 0.5, dissolved oxygen > 2 mg/l and sampling periodic interval 0, 2 and 4 days. The result show that removal efficiency for organic matter as a COD is 87.1%, ammonia 95.88 %, sulfide 99.2% and phenol 99.7%

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Introduction:-

Water is very often found together with petroleum in the reservoirs where the water, as a consequence of higher density than oil, lays in vast layers below the hydrocarbons in the porous reservoir media. This water, which occurs naturally in the reservoir, is commonly known as formation water (Dorea et al., 2007). After oil and gas production has been occurring for a time, the formation water will reach the production wells and water production will initiate. The well water-cuts will normally increase throughout the whole oil and gas field lifetime, such that when the oil production from the field is shut down, the oil content can be as low as a couple of percent with ninety eight percent

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water (Wang et al., 2007). To maintain the hydraulic pressure in the petroleum reservoir, which is reduced as soon as production is started, seawater is commonly pumped into the reservoir water layer below the hydrocarbons. This pressure maintenance due to water injection causes high extensions in recoverable hydrocarbons but simultaneously contributes to increased water production. During petroleum production, vast volumes of liquids have to be managed each day. Deferred production causes high economical losses and therefore continuous operations are always strived for. The capacity, reliability and performance of the produced water management system is often critical for continuous oil production particularly in mature oil field where the water production can greatly exceed the oil production (Liu et al., 2010). The water production system needs to be designed to receive continuously increasing quantities of water as oil production continues (Riviere and Garland, 1994). The most common practice in use in Indonesia for management of produced water is treatment in gravity based separation equipment and discharge to sea/water body (Sponza and Gok, 2010). This research is carried out to see if the biological process can be a good candidate to treat the produced water to meet the standards or regulation requirement.

Materials and Method:-

Microorganism:-

Isolation and characterization of bacteria isolated from several site of oil contaminated soil was conducted in order to find the bacteria that have an ability to metabolize produced water as sole carbon source. Cultures of bacteria were adapted with produced water as sole carbon source. Bacteria that able to degrade or use produced water as sole carbon were isolated and then used as inoculums in this research. Consortium bacteria containing five different bacteria which is *Pseudomonas* sp, *Staphylococcus aureus*, *Thiobacillus ferrooxidans*, *Bacillus* sp, and *Alcaligenes* to be acclimated using produced water as a carbon source from real waste on batch system for 30 days.

Kinetics study of produced water biodegradation:-

To prepare the inoculums, mixed bacterial isolates were grown in 1-liter Erlenmeyer flasks containing 500 ml of the minimal medium (CNP ratio as Glucose:(NH₄)₂SO₄:K₂HPO₄ of 100:10:1) as sole carbon and energy source. Isolates were grown to mid-log phase (app. 10⁶ CFU/ml) and used as the inoculums (2%) for the biodegradation of produced water in the reactor. The growth conditions in the reactor were as follows: Room temperature, agitation at 110 rpm, pH 7.0 (adjusted with 1 N HCl-NaOH). The growth of microorganism was measured every certain time.

Produced Water Biodegradation Assay:-

To determine the performance of bacteria in degrading produced water, a biodegradation assay developed and set up as follows:

- Continues baffled reactor equipped with diffused aerator.
- The flow rate adjusted to 0,90 l/h with detention time of 2 (two) days and return sludge (r) : 0,5, dissolved oxygen > 2 mg/l.
- Organic and biomass concentration were observed on certain time.

Result and Discussion:-

Growth kinetics determination:-

The aim of growth kinetic experiments is to determine the ability of isolated microorganisms in the degradation of produced water. The characteristics of produced water used in this experiment are as depicted in **Table 1**.

Table 1:- Characteristics of Produced Water

No	Parameters	Unit	Value
1	pH	-	7.470
2	BOD	mg/l	368
3	COD	mg/l	829.84
4	Nitrate	mg/l	0.8167
5	Nitrite	mg/l	0.023
6	Ammonia	mg/l	66.04
7	Total Oil	mg/l	37
8	Phenol	mg/l	2.56
9	Ortho Phosphate	mg/l	<0.002

The kinetics parameters to be determined are as follows:-

- Specific growth rate, μ , 1/time
- Decay coefficient, k_d , 1/time
- Organic removal coefficient, k , mg/l.day
- Half saturation constant, K_s , mg/L

The growth of a microbial culture is a complex phenomenon composed of a number of simultaneously occurring events. The relative magnitudes of the respective rates determine what the net effect is upon the culture. The primary events are the utilization of substrate and the growth of organisms. These two events are closely related because it is only through the utilization of substrate that energy and carbon are made available for cell growth. The growth rate is referred to as a specific rate because it defines the rate of cell growth in terms of the concentration of cell present. To find the relation of cell growth and substrate utilization, bacterial consortia were grown on produced water with variation in substrate concentration which is 10%, 20%, 40%, 60%, 80% and 100% of produced water. Growth curve of bacterial consortia were linearized at exponential phase resulting regression line which slope is its specific growth rate.

Figure 1:- show the time-course of a liquid culture of the bacterial consortium using produce water as sole carbon source.

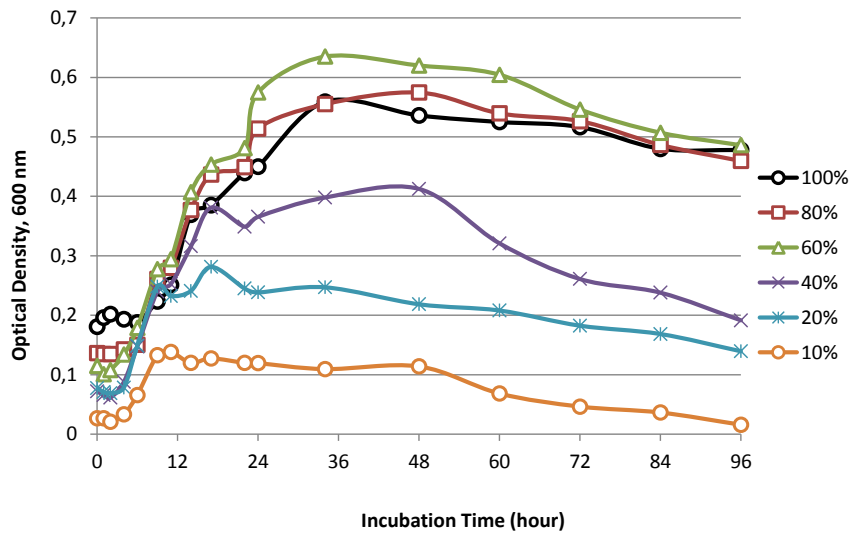


Figure 1:- Time course of mixed culture grown on various concentration of produced water as sole carbon source.

Growth curve of bacterial consortia were linearized at exponential phase resulting regression line which slope is its specific growth rate. Specific growth rate of bacterial consortia were shown in **Table 2**. The value of the kinetics parameters are very dependent upon the organism and substrate employed. If a given species of organism is grown on each of several substrates under fixed environmental conditions the value of kinetics parameters observed will depend upon the substrate. Likewise, if several pure cultures are fed the same substrate under identical environmental conditions, the value of kinetics parameters will depend upon the species of organisms.

Table 2:- Specific growth rate (μ) of bacterial consortia at difference substrate concentration

No	Produced Water (%)	μ (1/hour)
1	10	0.0118
2	20	0.0141
3	40	0.0163
4	60	0.0178
5	80	0.0171
6	100	0.0123

Based on **Table 2** above showed that specific growth rate of bacterial consortia tend to increase as substrate concentration increased. The highest specific growth rate occurs in bacterial culture that grown on 60% produced water which is 0.0178 hour^{-1} . Once data are available relating to μ and substrate concentration, it can be used to estimate the kinetics parameter which is the half saturation constant (K_s) and the maximum specific growth rate (μ_m). The most common transformation is obtained by taking the reciprocal of both side of the Monod equation, known as the Lineweaver-Burk equation. A plot of $1/\mu$ as a function of $1/C_s$ (**Figure 2**) give a linear regression were slope or gradient is K_s/μ_m therefore the intercept on X is $(-1/K_s)$ and to the Y is $1/\mu_m$ (Schulz, 1994).

From linear regression yielding the half saturation constant K_s value of 35 mg/l and maximum growth rate value of 0.45 per day. It means that if μ_m condition have achieved, increasing in substrate concentration specific would not take effect on growth rate of viable biomass anymore

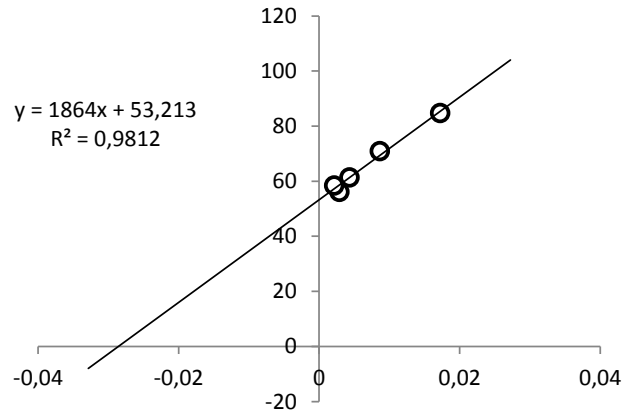


Figure 2:- A plot of $1/\mu$ as a function of $1/C_s$ to determine the kinetics parameters K_s value showed the affinity of biomass to substrate and therefore a concentration of substrate at half of its maximum growth rate. Biomass which high affinity to substrate have a low K_s value, that mean growth rate will not effected until substrate concentration decrease to a very low value. As opposite if biomass which low affinity to substrate have a high K_s value, its mean growth rate will be effected by the remain substrate concentration which still high. If the substrate concentration is below the K_s value thus the growth of biomass will very slow. That why the addition of substrate in the reactor at least equal to or much higher than its K_s value.

Produced Water Biodegradation Assay:-

To determine the performance of both isolated bacteria in degrading produced water and bioavailability of produced water, a biodegradation assay was established as described in methods. A continuous step aeration reactor with flow rate of 0,90 l/h, detention time 2 (two) days, return sludge (r) : 0.5 and dissolved oxygen to be maintain $> 2 \text{ mg/l}$ as depicted in **Figure 3**.



Figure 3:- A continuous step aeration reactor used for biodegradation assay of produced water

Table 3:- Organic (COD) removal efficiency

Hari	Inlet mg/l	Outlet mg/l	Efisiensi (%)
0	827	114	86.2
2	827	110.5	86.6
4	827	107	87.1

Figure 4. Time course of COD degradation and bacterial growth by bacterial consortia

Figure 5. Pollutant removal efficiency

Conclusion:-

Referring to the Indonesian Ministry of Environmental (Decree no. 4/2007), it was mandatory to manage a safe treatment of these wastes (produced water) and also disposed off in an environmentally friendly manner. It was regulated that the final concentration of COD, ammonia, sulfide and phenol must less than 200; 5; 0.5 and 2 mg/l respectively. Based on the data presented in this paper indicate that the bacterial consortia have a quiet low K_s value (35 mg/L). Its mean that bacterial consortia that used in this study were effective to degrade the produce water to a final low concentration. COD, ammonia, sulfide and phenol removal efficiency in the continuous step aeration reactor was 87.1;95.88;99.2 and 99.7%, respectively.

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