International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-8, Issue-4, April 2022
ISSN: 2395-3470
www.ijseas.com

Kinetics of biosurfactant activity produced by *Bacillus Amyloliquifaciens.*

Shaikh Shagufta Mujiburrehman [1] And P V Dharani [2]

Department of microbiology.

SHETH P T Arts And Science College, Affiliated To Shri Govind Guru University, Godhra, Gujarat India

EMAIL: sshagufta279@gmail.com.

prasannaaiyer30809@gmail.com

Abstract.

Use of hydrocarbons is being increased in different fields for various purpose which can pollute surrounding environment including fauna and flora of aquatic system. Certain microbes can degrade hydrocarbons by using it as substrate by producing compound known as biosurfactant. Present study aims to know the effect and activity of biosurfactant produced by *Bacillus amyloliquifaciens*.Prameter studied here are hydrocarbon degradation activity of purified biosurfactant on (Petrol, Diesel, Servo engine oil, Lubricant oil, paraffin oil, Glycerol, vegetable oil and kerosene) and emulsification activity of biosurfactant at different pH, salt concentration and temperature.

Keywords: Kinetics, Hydrocarbons, Temperature, Biosurfactant.

Introduction.

Utilization of engine oil in automobiles is leading reason for hazardous effect on soil .When used engine oil is not discarded properly it can lead to harmful effects on aquatic ecosystem (Akintunde WO, 2015).Currently oil spillage is regarded as third most important source of pollutant in sea areas (Wang, Liu, Guo, Lv, & Li, 2018).To protect aquatic ecosystem treatment of engine oil is required ,Although different physical ,chemical and biological methods are being used to remove hydrocarbon from contaminated areas in which biological approach also known as bioremediation is being used over physical and chemical method because it is less cost effective and ecofriendly (Arora, 2018) (Azubuike, Chikere, & Okpokwasili, 2016). Bioremediation exerts slow rate on degrading hydrocarbons (Patowary, Patowary, Kalita, & Deka, 2016).Different types of chemicals are also being used to treat such oil spillage one of operation is oil recovery (VD, 1989) in waterbodies oil spillage can lead to release of contaminants (Sujata SJ, 2016) (Moghimi H, 2017).Certain genus of *Bacillus* is able to degrade hydrocarbon present in engine oil by producing biosurfactant which are of different chemical composition and molecular weight and can enhance the availability of hydrophobic organic compound in engine oil (Marchut-Mikolajczyk O, 2018). Yalaoui-Guellal et al.2020, characterized biosurfactant structurally for their role in remediation of oil spills. Link between biosurfactant and hydrocarbon degradation was determined by Khan et al. 2017.In this study main purpose is kinetics study of purified biosurfactant produced by *Bacillus amyloliquifacians* (gram negative).Biosurfactant from different bacterial species have different role on hydrocarbons. Additional importance of biosurfactant is that it also efficient extreme temperature, pH and salt concentration this study aim to know the effectiveness of purified biosurfactant at different temperature, pH and salinity.

Materials and methodology.

Inoculum.

Bacillus amyloliquifaciens was isolated from waste sample (water drained after boiling corn supplemented with ghee) and identified based on morphological, microscopic biochemical characteristics and 16srRNA sequencing .Isolated bacterial species was screened for ability to produce biosurfactant by performing emulsification assay, agar tolerance assay, hemolytic assay, drop collapse assay, oil spread assay. Growth of inoculum was performed by addition of loop full of bacterial culture into 5ml Nutrient broth (Shagufta SM, 2021)

Biosurfactant production.

250 ml Erlenmeyer flask containing 100 ml of media of following composition:1% w/v carbon source(potato peels),1% nitrogen source(ammonium chloride),0.2% ions (disodium hydrogen phosphate) and pH maintained at 8 were inoculated with inoculum of *Bacillus amyloliquifaciens*(Incubated at 37°C for 48 hours). After inoculating culture fermentation was incubated at room temperature for 20 days.

Emulsification index.

Emulsifying activity of biosurfactant was determined by emulsification index. For this assay 2ml of cell free supernatant was added to 2 ml servo engine oil in test tube and vertex at high speed for 2 minute (Chandran P, 2010) .Left in standing position for 24 hours. Emulsification index was calculated by following formula.

Emulsification index EI24% = Height of emulsified layer /Total Height of liquid column × 100.

Extraction and recovery of biosurfactant.

Acid precipitation and solvent recovery techniques were performed to recover biosurfactant from cell free supernatant (Bezza, 2010).For recovery broth was centrifuged at 5000rpm for 20 minutes followed by acidifying cell free supernatant (pH 2.0) with 10N HCl and incubated 4°C overnight. After incubation Precipitates were collected by centrifugation at 5000rpm for 15 minutes. Precipitates collected after centrifugation were treated with solvent mixture of chloroform and methanol in the ratio of 2:1.Biosurfactant was recovered after evaporating solvents. Recovered biosurfactant dissolved in distilled water to carry out kinetics study.

Kinetics of purified biosurfactant.

Hydrocarbons degradation activity.

Activity of the biosurfactant were studied with different hydrocarbons like
Petrol, Diesel, Servo engine oil, Lubricant oil, paraffin oil, Glycerol, vegetable oil and kerosene .Emulsification index of biosurfactant was performed with each hydrocarbons. Emulsification activity of biosurfactant were noted as E1% and E24%. (Marchut-Mikolajczyk O, 2018), (O. A. Agwu, 2012).

Study on activity of biosurfactant on lubricant oil at different salt concentration and pH.

Salt concentration of biosurfactant studied were 5%, 10%, 15%, 20%, 25%, and 30% with sodium chloride salt. pH maintained at 1,2,4,6,8,10 and 12 (Vaishya, 2017) using sodium hydroxide and hydrochloric acid. Emulsification activity of biosurfactant at different salt concentration were noted as E1% and E24%.

Activity of biosurfactant at different temperature.

To know the best temperature at which purified biosurfactant gives highest activity 2ml of purified biosurfactant was taken in 4 test tubes then 2 ml of lubricant oil was added in each test tube, mixed at high speed in vortex mixer for 2 minutes after mixing tube were incubated at different temperature one tube at 37°C (in incubator), one at 29°C (room temperature), one at -18°C (freezer), one at -4°C (refrigerator) for 24 hours. After incubation emulsification activity of biosurfactant at different temperature were noted as E1% and E24%.

Result.

Biosurfactant produced by isolated bacteria were able to emulsify range of hydrocarbons ,here highest emulsification activity observed with Lubricant oil, servo engine oil, diesel and vegetable oil (Table 1, Fig 1).Activity of biosurfactant observed at range of salt concentration at 5%, 10%.20%, 25% but best activity observed with emulsification index of 66% at 15% salt concentration (Table 2,fig 2).Activity of biosurfactant at different pH range (1-12) observed in which purified biosurfactant able to emulsify lubricant oil at different pH ,maximum emulsification observed in the pH range of 6-8 whereas minimum activity observed at pH 1-4 (Table 3,Fig 3). Biosurfactant gives the emulsification activity at most of tested temperature except at -18°C, efficient activity of biosurfactant observed at 29 °C (Table 4, Fig 4).

Table -1. Hydrocarbons degradation activity.

Hydrocarbon	E1%	E24%
Petrol	0%	0
Diesel	0%	31%
Servo engine oil	16%	58%
Lubricant oil	14%	62%
paraffin oil	1%	0%
Glycerol	0%	0%
vegetable oil	0%	22%
kerosene	0%	1%

Table-2.Activity of biosurfactant at different salt concentration.

Salt concentration (%).	E1%	E24%
0	13%	26%
5	00%	14%
10	02%	16%

15	10%	66%
20	00%	28%
25	01%	15%
30	02%	10%

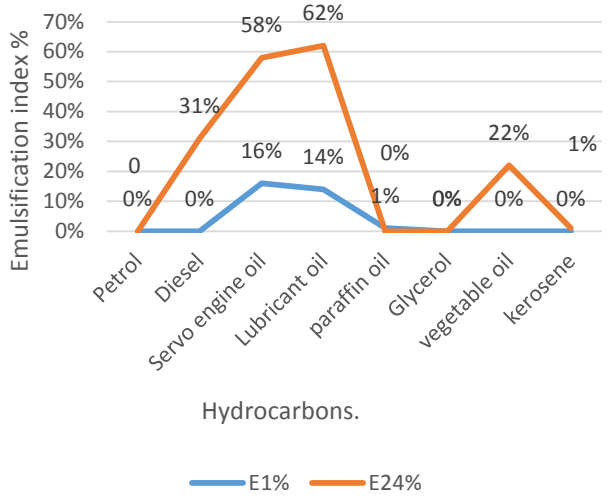
Table-3.Activity of biosurfactant at different pH.

pH	E1%	E24%
1	7.5 %	25%
2	7.5%	25%
4	44%	37%
6	57%	51%
8	57%	50%
10	50%	33%
12	50%	33%

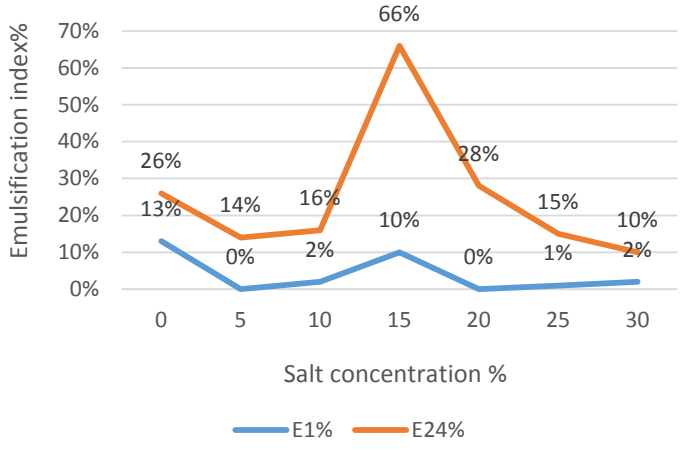
Table-4.Activity of biosurfactant at different temperature.

Temperature	E1%	E24%
37°C	65%	63%
29°C	85%	66%
-18°C	00%	00%
-4°C	26%	85%

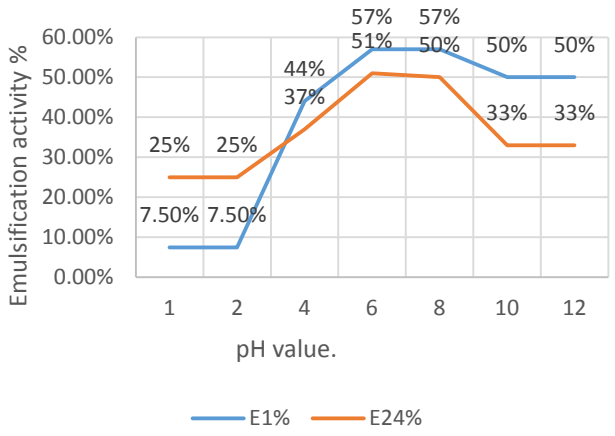
Fig,1.Hydrocarbon degradation activity.



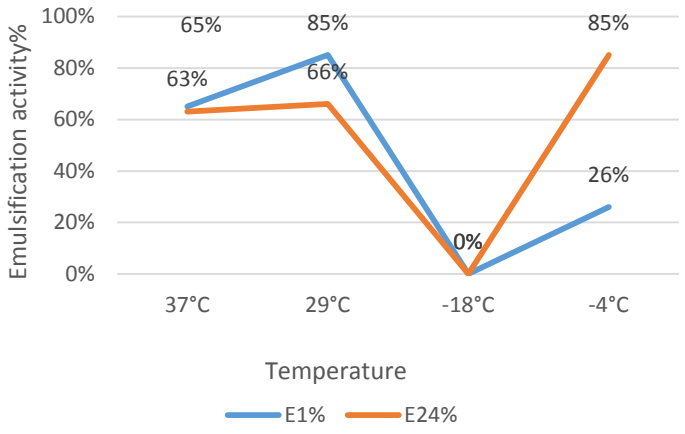
Fig,2.Activity of biosurfactant at different salt concentration.



Fig,3.Activity of biosurfactant at different pH



Fig,4.Activity of biosurfactant at different temperature.



Discussion.

In this study biosurfatant produced by *Bacillus amyloliquifaciens* exhibits potential activity to degrade hydrocarbons like lubricant oil (E24%-62%) engine oil (E24%-58%) and diesel (E24%-31%),in the study performed by (Sandia Primeia, 2020) on bacterial consortia(Pseudomonadaceae, Xanthomonadacea, and Moxarelaceae, Alcaligenes) producing biosurfactant showed degradation activity on total petroleum hydrocarbons with emulsification activity of 10 to 50%,comparatively biosurfactant produced by *Bacillus amyloliquifaciens* exhibits more good activity against petroleum containing hydrocabons. Produced biosurfactant also tolerates salinity in the range of 5to 30% concentration in which maximum activity obtained at 15% salt concentration. Sarrubo et al. (2006) and Ilori et al. (2005) noted stability and activity at 5 to 10 % suggesting limited application of such biosurfactant in marine environment. Produced biosurfactsnt emulsify lubricant oil in the pH range of 1 to 12 maximum at pH 6-7 indicating its activity at range of acidic and basic condition same studies also observed with biosurfactant produced by *Rhodococcus sp* showing activity at pH 6.2 to 7.2 (ABU, 1991).Whereas bioemulsifier activity of *Bacillus cereus* reported by (Cooper and Goldenberg, 1987) gets reduced at pH above 7.Observation for activity at different temperature indicates activity of biosurfactant at temperature range -4°C to 37°C.

Conclusion.

Results observed indicates activity of biosurfactant against hydrocarbons, stability at extreme pH, temperature and tolerance in salinity suggest our biosurfactant could be exploited in oil recovery, removal of oil contaminant ,treatment of oil spillage.

Reference.

Akintunde WO, Olugbenga OA, Olufemi OO. Some adverse effects of used engine oil (common waste pollutant) on reproduction of male sprague dawley rats. Maced J Med Sci. 2015;3:46–51. https ://doi.org/10.3889/oamjm s.2015.035.

Wang, C.; Liu, X.; Guo, J.; Lv, Y.; Li, Y. Biodegradation of marine oil spill residues using aboriginal bacterial consortium based on Penglai 19-3 oil spill accident. Ecotoxicol. Environ. Saf. 2018, 159, 20–27. [CrossRef] [PubMed]

Rafael VD. Environmental impact of used motor oil. Sci Total Environ.1989;79:1–23. https ://doi.org/10.1016/0048-9697(89)90049 -1.

Arora, N.K. Bioremediation: A green approach for restoration of polluted ecosystems. Environ. Sustain. 2018, 1, 305–307. [CrossRef] 6.

Azubuike, C.C.; Chikere, C.B.; Okpokwasili, G.C. Bioremediation techniques–classification based on site of application: Principles, advantages, limitations and prospects. World J. Microbiol. Biotechnol. 2016, 32. [CrossRef]

Patowary, K.; Patowary, R.; Kalita, M.C.; Deka, S. Development of an efficient bacterial consortium for the potential remediation of hydrocarbons from contaminated sites. Front. Microbiol. 2016, 7. [CrossRef]

Sujata SJ, Sanket JJ, Geetha SJ. Lipopeptide production by *Bacillus subtilis* R1 and its possible applications. Braz J Microbiol. 2016;47:955–64. https ://doi.org/10.13005 /msri/07013 3.

Moghimi H, Heidary Tabar R, Hamedi J. Assessing the biodegradation of polycyclic aromatic hydrocarbons and laccase production by new fungus *Trematophoma* sp. UTMC 5003. World J Microbiol Biotechnol.

Marchut-Mikolajczyk O, Drożdżyński P, Pietrzyk D, et al. Biosurfactant production and hydrocarbon degradation activity of endophytic bacteria isolated from *Chelidonium majus* L. Microb Cell Fact. 2018;17:171. https ://doi.org/10.1186/s1293 4-018-1017-5.

O. A. Agwu, M. O. Ilori, S. A. Adebusoye & O. O. Amund (2012): A Comparative Study of Biosurfactant Synthesis by Pseudomonas aeruginosa Isolated from Clinical and Environmental Samples, Petroleum Science and Technology, 30:5, 503-51

Kanhaiya Kumar Singh and R. C. Vaishya , A Study on Kinetics of Bio-surfactant Produced by Bacterial Strains Isolated from Municipal Wastewater, *International Journal of Civil Engineering and Technology*, 8(4), 2017, pp. 014–021.
http://iaeme.com/Home/issue/IJCIET?Volume=8&Issue=4

Yalaoui-Guellal, D., Fella-Temzi, S., Djafri-Dib, S., Brahmi, F., Banat, I.M., Madani, K., 2020. Biodegradation potential of crude petroleum by hydrocarbonoclastic bacteria isolated from Soummam wadi sediment and chemical-biological proprieties of their biosurfactants. J. Pet. Sci. Eng. 184, 106554.

Khan, A.H.A., Tanveer, S., Alia, S., Anees, M., Sultan, A., Iqbal, M., Yousaf, S., 2017. Role of nutrients in bacterial biosurfactant production and effect of biosurfactant production on petroleum hydrocarbon biodegradation. Ecol. Eng. 104, 158–164.

Shagufta SM, Dharani PV. Screening and Isolation of Bacteria Producing Biosurfactants from Waste. *J Pure Appl Microbiol.* 2022;16(1):567-577. doi: 10.22207/JPAM.16.1.55

Chandran, P., Das, N., 2010. Biosurfactant production and diesel oil degradation by yeast species Trichosporon asahii isolated from petroleum hydrocarbon contaminated soil.Int. J. Eng. Sci. Technol. 2, 6942–6953.

Bezza, F.A., Chirwa, E.M.N., 2015. Production and applications of lipopeptide biosurfactant for bioremediation and oil recovery by Bacillus subtilis CN2. Biochem. Eng. J. 101, 168–178.

Primeia S, Inoue C, Chien M-F. Potential of Biosurfactants' Production on Degrading Heavy Oil by Bacterial Consortia Obtained from Tsunami-Induced Oil-Spilled Beach Areas in Miyagi, Japan. *Journal of Marine Science and Engineering*. 2020; 8(8):577. https://doi.org/10.3390/jmse8080577

Sarrubo, L. A., Moura de Luna, J., and Maria de Campos-Takaki, G. (2006). Production and stability studies of the bioemulsifiers obtained from a new strain of Candida glabrata UCP 1002. Elect. J. Biotechnol. 9:1–7

Ilori, M. O., Amobi, A. C., and Odocha, A. C. (2005). Factors affecting biosurfactant production by oil degrading Aeromonas spp. isolated from a tropical environment. Chemosphere 61:985–992.

ABU, Ruwaida, Banat M, Haditirto, Salem, Kadri'Isolation of biosurfactantproducing bacteria product evaluationandcharacterization' Acta Biotech, 11(4): 315-24, 1991.

Cooper, D. G., and Goldenberg, B. G. (1987). Surface active agents from two Bacillus species. Appl. Environ. Microbiol. 53:224–229.