

<< Bioremediation of Dairy Waste Water using Lipid Degrading Microbial

Isolates >>

<< S.M.P.B.Geethachapa¹, K.P.M.Ruwanara¹, Y.M.Kulasinghe¹, T.U.Ariyadasa^{1*}>>

¹Department of Chemical and Process Engineering, University of Moratuwa, Sri Lanka.

<<smpbimsara@gmail.com,mihiri.ruwanara@gmail.com,
yashoramanduli@gmail.com,thilini@uom.com>>

¹* Author for Correspondence:

Email address : thilini@uom.com or (tuariyadasa@gmail.com)
Department of Chemical and Process Engineering, University of Moratuwa, Moratuwa,
Sri Lanka.

Human establishment on earth and the technical advancement followed by, has created water as a resource in the world. Waste water has brought severe environmental and social impacts. Study is an investigate the water pollution caused by lipids in the domestic and industrial sewage which influenced flora and fauna and to come up with appropriate remedies. The immiscible nature of lipids with water is difficult to be degraded and removed via conventional treatment systems. The interest towards lipid water treatment by microbial isolates has been rejuvenated due to the high operational and maintenance associated with conventional treatment systems.

Study was attempted to isolate microorganisms capable of degrading lipids efficiently from diverse environments. Hundred strains were screened and six strains were lipolytic

positive. Among the lipolytic positive strains, three strains (S1, S3 and S5) exhibited the cell growth $OD_{600}=1$ after 48 hours of cultivation. The optimum temperature was found to be 30 °C for maximum during 48 hours of culturing. Subsequently the lipid degradation competency of each strain was investigated at 30 °C for about 24 hours where, the S1 yielded the highest lipid degradation efficiency ($39.02\pm 0.01\%$). S1 was accustomed for investigating the lipase activity (2.605 ± 0.01) and the lipid degradation ($39.02\pm 0.01\%$) against cell growth. S1, S3 and S5 were identified in accordance with morphologic biochemical testing and 16S rRNA sequencing as *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Acinetobacter pittii* respectively.

water drainage columns. Clogging of drainpipes, corrosion of sewer pipes resulted due to the accumulation of FOGs and environmental pollution occurred creating unpleasant odor.

Therefore, it is all-important to act on proper treatments to remove lipids from wastewater. physicochemical waste water treatment methods that have been studied so far are sedimentation, degasification, screening, aeration, ozonation, coagulation, neutralization, chlorination, ion exchange and adsorption. Certain boundaries such as high cost, partial separation, occurrence of secondary pollutants and solid large quantities and usage of chemicals associated with physicochemical strategies have replaced biological approaches as the promising candidates towards lipid contained waste water treatment.

2. Introduction and research problem/issue

Lipid, depicted as fat, oil, grease (FOGs) and long chain fatty acids, is a significant constituent in do industrial sewage which creates heavy-handed environmental befouling. Squeeze off of lipid contained w to the ecosystem by food processing and dairy industries, restaurants, mega kitchens and inadvertent o greatest in degree. Special attention is deserved by the dairy industry due to the presence of high lipid c load which oversteps the levels of hazardous, concerned for domestic waste water.

Lipids by definition are known as hydrophobic compounds immiscible with water, form oil droplets an on the surface of aqueous systems. Therefore, build-up of a barrier impermeable for oxygen to diffuse water, overriding to the destruction of aquatic lives due to insufficient oxygen for respiration. Due to h matter load, eutrophication is mor e ubiquitous in lakes spells rising algae blooms, which can foremo dissolved oxygen levels. Clusters of oil droplets in the waste water effluents obstruct

Keywords: Bacteria, Bioremediation, Dairy effluents, Lipid-degradation, , Waste water

At the very beginning of the research, samples were collected from three different ecosystems. More than six species were screened and the six strains that showed the lipolytic activity were isolated. Three exhibited cell growth rate above $OD_{600} = 1$ during 48 hours of cultivation period were selected for experiments. S1, S3 and S5 were isolated from soil, spoiled milk and dairy industrial sludge respectively degrading bacterium in this study. Isolated strains were identified and characterized via microscopic observations under microscope, biochemical testing and 16S rRNA sequence analysis. Luria Bertani (LB) was utilized for the isolation. Appropriate standard culture mediums were used for the culturing. Cell growth of each isolate at different temperatures for about 48 hours was determined with the aid of a spectrophotometer. After that the lipid degradation rate at the optimum temperature for about 24 hours for each species, was determined. The strain that showed the highest lipid degradation rate, was used for the determination of lipid degradation efficiency with cell growth and lipase activity.

4. Results and findings

Among the screened microorganism population, six bacterial strains were positive for

lipolytic activity. Within this selective community, only three strains mentioned as S1, S3 and S5 showed cell growth rate above $OD_{600}=1$. The optimum growth rate of each species specified above was achieved. Hence 30 °C was considered as the optimum temperature required for the maximum growth rate of lipid bacteria. Lipid degradation rate was highest in S1 at 30 °C after 24 h of cultivation.

Strain 1 was identified using morphological and biochemical tests and 16S rRNA sequence analysis. It was a short rod-shaped, gram-negative bacteria that was oxidative positive and catalase positive. It was a negative and aerobic gamma proteobacteria belonging to the family Pseudomonadaceae containing 1 described species. The 16S rRNA sequence of S1 showed high similarity (more than 99%) with that of *Pseudomonas*. On the basis of the characteristics and the 16S rRNA sequence, strain 1 was identified as *Pseudomonas aeruginosa* species. The members of the genus demonstrate a great deal of metabolic diversity and consequently are able to colonize a wide range of niches.

Strain 3 was a short rod-shaped, gram-positive, endospore negative bacteria that was oxidative negative and catalase positive. It was a facultatively anaerobic bacteria belonging to the family Enterobacteriaceae. Several of these bacteria are pathogenic and cause opportunistic infections in immune compromised (usually human) hosts.

The present investigations indicated that the *Pseudomonas aeruginosa* owned the highest lipid degra over *Acinetobacter pitii* and *Enterobacter cloacae*. Therefore findings reveal *Pseudomonas* will be the among all three isolates, towards dairy waste

water treatment. After 48 hours period of culturing at 30°C was proved to be the optimum temperat maximum cell growth of each bacteria used in the research.

6. References (Selected)

The 16S rRNA sequence of S2 showed high similarity (more than 99%) with that of the genus En On the basis of the characteristics and the 16S rRNA sequence, strain 3 was identified as the *Enterobac* species. Strain 5 was a short rod-shaped, gram positive, endospore negative, oxidative negative and catala bacteria. It was a strictly aerobic non-motile bacterium. The 16S rRNA sequence of S5 showed high (more than 99%) with that of the genus *Acinetobacter*. On the basis of the characteristics and the sequence, strain 5 was identified as the *Acinetobacter pitii* species which plays a significant role in the c and infection of patients to hospitals. Their predominant role is as agents of nosocomial pneumonia.

5. Conclusions, implications and significance

-
1. Affairs, R. (2006). Environmental Impacts of Food Production and Consumption A research report for the Department for Environment , Food and Rural Affairs by Manchester Business School, (December).
 2. Australian Dairy Industry Represented by Australian Dairy Industry Council Inc . and Dairy Australia to The Agricultural Competitiveness Issues Paper. (2014), (April).
 3. El-bestawy, E., El-masry, M. H., & El-adl, N. E. (2005). The potentiality of free Gram-negative bacteria removing oil and grease from contaminated industrial effluents, 815–822. <https://doi.org/10.1007/s11274-004-2239-4>
 4. Matsumiya, Y., Wakita, D., Kimura, A., Sanpa, S., & Kubo, M. (2007). Isolation and Characterization Degrading Bacterium and Its Application to Lipid-Containing Wastewater Treatment, *103*(4), 325–330 <https://doi.org/10.1263/jbb.103.325>
 5. Mcgarvey, J. A., Miller, W. G., Zhang, R., Ma, Y., & Mitloehner, F. (2007). Bacterial Population Dynamics in Dairy Waste during Aerobic and Anaerobic Treatment and Subsequent Storage *□*, *73*(1), 193–202. <https://doi.org/10.1128/AEM.01422-06>
 6. Porwal, H. J., Mane, A. V., & Velhal, S. G. (2015). Biodegradation of dairy effluent by using microbe obtained from activated sludge. *Water Resources and Industry*, *9*, 1–15 . <https://doi.org/10.1016/j.wri.2014.11.007>
 7. Matsumiya, Y., Wakita, D., Kimura, A., Sanpa, S., & Kubo, M. (2007). Isolation and characterization of degrading bacterium and its application to lipid-containing wastewater treatment. *Journal of Bioscience Engineering*, *103*(4), 325–30. <https://doi.org/10.1263/jbb.103.325>
 8. Mazzucotelli, C. A., Ponce, A. G., Kotlar, C. E., & Moreira, M. del R. (2013). Isolation and characterization of bacterial strains with a hydrolytic profile with potential use in bioconversion of agroindustrial by-product waste. *Food Science and Technology (Campinas)*, *33*(2), 295–303. <https://doi.org/10.1590/S0101-20612013005000>
 9. Mongkolthanaruk, W., & Dharmsthiti, S. (2002). Biodegradation of lipid-rich wastewater by bacterial consortium, *50*, 101–105.
 10. Shivsharan, V. S., Wani, M. P., & Kulkarni, S. W. (2013). “ Isolation of Microorganism from Dairy Waste for Activated Sludge Treatment .” *International Journal Of Computational Engineering Research (Ijceronline.com)* *161–167*.
-

*Corresponding Author, Tel:+94 071 3118461

E-mail Address:thilini@uom.com