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Screening of the bio-decolorization ability of synthetic dyes and the degradation of hydrocarbon bacteria *Serratia marcescens* MBC1

Achmad Arifiyanto[1*], Berliana Damayanti[1], Meishy Handerlin Putri[1], Cindy Lukita Ratih Riyanto[1]
[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung
Jl. Prof. Dr. Ir. Sumantri Brojonegoro No. 1 Gedong Meneng, Kota Bandar Lampung, Lampung, Indonesia. 35141
*Email: achmad.arifiyanto@fmipa.unila.ac.id

ABSTRACT. Humans are inseparable from using hydrocarbons, including fossil fuels, dyes, and oleochemical products. This has frequently resulted in a high possibility of waste spills polluting the environment. A biological approach using microbes was used to facilitate many of these countermeasures. This study aimed to determine the ability of the bacterium *Serratia marcescens* MBC 1 isolate as a biodegradation agent for various hydrocarbons and a bio-decolorizing agent for synthetic dyes. The bacterial growth media used were solid and tryptic soy broth. Methylene blue, congo red, methyl red, methyl orange, and crystal violet were synthetic dyes at a concentration per million. Within 7 days, several synthetic dyes tested showed decolorization. Methylene blue has the fastest decolorization time, taking just 24 h. The lipase index method was used to assess the propensity of hydrocarbons to degrade qualitatively. Kerosene had the highest lipolytic index at 6.31, followed by used cooking oil at 5.48 index, used lubricant at 5.37 and diesel at 3.63 index. Quantitative and comprehensive in-depth testing of the potential achievements of this initial test will be used in solving environmental problems that may occur further, especially related to the impact of the use of synthetic dyes.

Keywords: bacterial growth media; bio-decolorization; bioremediation; *Serratia marcescens*; synthetic dyes

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INTRODUCTION

The textile industry has evolved into a multinational enterprise enhance the water solubility of hydrophobic substances (Gazzola *et al*., 2020). Despite its undeniable value, this industry was among the most polluting in the world, using vast quantities of fuels and chemicals (Perera, 2017). The textile industry's most prominent environmental impact was its discharge of untreated waste into bodies of water. Aside from the various losses incurred by synthetic dyes, using petroleum products in automobiles and manufacturing contributed to environmental pollution (Yadav *et al*., 2020). Explosions, tank spills, and handling of waste petroleum products were also possible sources of hydrocarbon pollution. In addition, increased petroleum levels in the soil resulted in a decline in soil quality, making the ground unusable (Koshlaf & Ball, 2017).

Serratia marcescens strain was found to be capable of decolorizing Reactive orange, Reactive blue-19, Ranocid Quick blue (RFB), and Procion Brilliant blue-HGR (PBB-HGR) by up to 90% in several tests (Gondaliya & Parikh, 2013). This microbe was classified as an oil-degrading microbe (Almansoory *et al*., 2017). They secreted the enzyme lipase, which hydrolyzed fatty bonds to produce fatty acids and glycerol. *S. marcescens* was one of the bacteria that could degrade hydrocarbons. This bacteria could generate lipase enzymes at a concentration of up to 29.39/mL after a 16 h incubation period (Fatimah *et al*., 2019).

S. marcescens MBC1 bacteria were successfully isolated from *Drosophila melanogaster* in our previous studies. These bacteria are proven resistant to some heavy metals, are antioxidants, and have several extracellular enzymes (Arifiyanto *et al*., 2021; Putri *et al*., 2021; Variani *et al*., 2021; Damayanti *et al*., 2022; Lestari *et al*., 2022;). However, there was no information available regarding the potential ability to reduce the contamination of synthetic textile dyes and the power of lipases on hydrocarbons. Therefore, this study intended to gather preliminary data by screening the potential of the *S. marcescens* MBC 1 isolate as a hydrocarbon biodegradation agent and synthetic dye bio-

decolorization. Hydrocarbon wastes that are often produced in abundance include used oil, diesel and kerosene spills, as well as cooking oil used for household and industrial frying. Not limited to this waste, the use of synthetic dyes is also a challenge because they are not easily degraded without human intervention.

MATERIALS AND METHODS

Rejuvenation of bacterial isolates. *Serratia marcescens* strain MBC 1 was inoculated on Tryptic soy agar (TSA) using the streak plate method, and it was then cultured for 24-72 h at room temperature. As a starter, a pure isolate that had been aged for 24 h was used, and the density was measured up to 10^8 cells/mL (Variani *et al*., 2021).

Hydrocarbon biodegradation test. *S. marcescens* isolate MBC 1 strain was inoculated with the point method on TSA media with added 1% hydrocarbons (used cooking oil, used lubcricant, diesel, and kerosene), 0.04% methyl red, and 1.5% Tween 80, and incubated at room temperature for 24 h. This step was repeated three times. A clear zone was observed after 24 h, and the diameter was measured. The lipolytic index was determined after obtaining a clear area. The lipolytic index was determined using the following formula (Lim *et al*., 1987):

$$\text{Lipolytic index} = \frac{\text{clear zone diameter} - \text{the diameter of the bacterial colony}}{\text{diameter of the bacterial colony}}$$

Textile dye bio-decolorization test. A total of 1 mL of biomass with a density of 10^8 cells/mL was inoculated into 9 ml of tryptone water which had been added with 25 ppm synthetic dye. The media was incubated at room temperature for seven days, and observed the color change that occurred every day (Karim *et al*., 2018). Methylene blue, congo red, methyl red, methyl orange, and crystal violet were synthetic dyes.

Data analysis. The data collected were analyzed by using descriptive quantitative method on the isolates with the highest lipolytic index. Analysis of multivariate analysis of variance was carried out with one-way ANOVA using SPSS ver. 25 at 95% confidence level, and significance $P<0.05$.

RESULTS AND DISCUSSION

Strain and hydrocarbon biodegradation. On Tryptone soy agar media, *Serratia marcescens* strain MBC1 bacteria released pale pink pigment in the first 24 h, which turned magenta as the colony grew older. When bacteria are stained with Gram stain and examined under a microscope, they appear as short rods (Fig. 1).

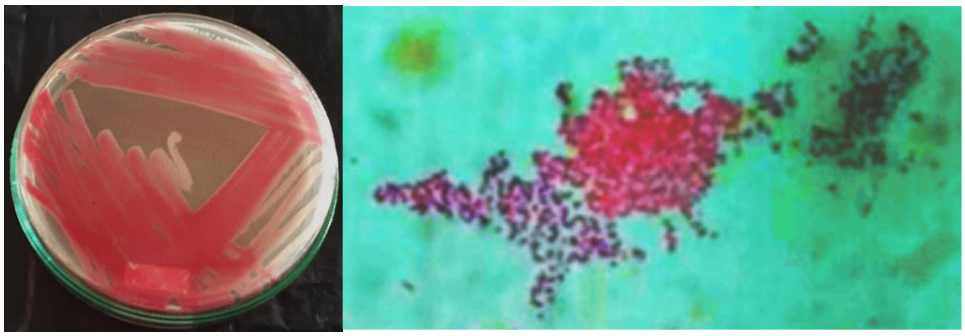


Fig. 1. Colony of *Serratia marcescens* strain MBC1: a. on TSA media; b. on Gram stain

S. marcescens bacteria were Gram-negative bacteria with full colony margins, a slimy texture, and an umbonate elevation. Bacteria were also motile because they have peritrichous flagella around their bodies, allowing them to move around (Abdullah *et al*., 2017). The observed characters are presented in Table 1.

Table 1. Colony characteristics of *Serratia marcescens* MBC1 strain

Colony character	Observed characters
Colony color	Reds
Margin	Entire
Colony texture	Mucoid
Elevation	Umbonate

The bacteria were cultured using the point method in Nutrient Agar media, which was given a mixture of 1% hydrocarbons in the form of used oil, diesel, used cooking oil, and kerosene, as well as methyl red and tween-80. These bacteria grew in hydrocarbon-containing media and broke down long-chain hydrocarbons into simpler compounds, as demonstrated by a transparent region (Fig. 2).

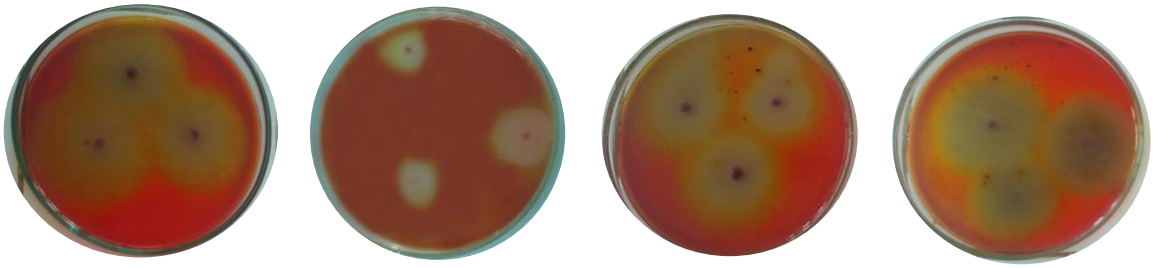


Fig. 2. Clear zones on media with the addition of hydrocarbons: a. used cooking oil; b. diesel fuel; c. used oil; d. kerosene

The diameter of the clear zone formed varies depending on the bacterial strain's ability to absorb hydrocarbons. With a lipolytic index of 6.31, kerosene was the most successful. With a lipolytic index of 5.48, used cooking oil came in second, followed by used lubricant with a lipolytic index of 5.37, and diesel with a lipolytic index of 3.63 (Table 2 and Fig. 3).

Table 2. Results of the lipolytic index of *Serratia marcescens* MBC1 strain on various substrate hydrocarbons

Types of hydrocarbons	x̄ diameter of colony ± SDev	x̄ diameter of clear zone ± SDev	Lipolytic index
Kerosene	0.37 ± 0.06	2.75 ± 0.61	6.31[c]
Used cooking oil	0.39 ± 0.03	2.50 ± 0.22	5.48[b]
Diesel	0.52 ± 0.10	2.37 ± 0.34	3.63[a]
Used lubricants	0.48 ± 0.04	3.03 ± 0.39	5.37[b]

Using multivariate analysis of variance, data on colony diameter, clear zone, and lopylitic index were investigated, and it was discovered that there were differences in the lipolytic index in the hydrolysis of diesel oil compared to kerosene (Sig. < 0.05). In addition, each kind of hydrocarbon substrate tested show no differences in diameter colony and clear zone formation (Sig. > 0.05).

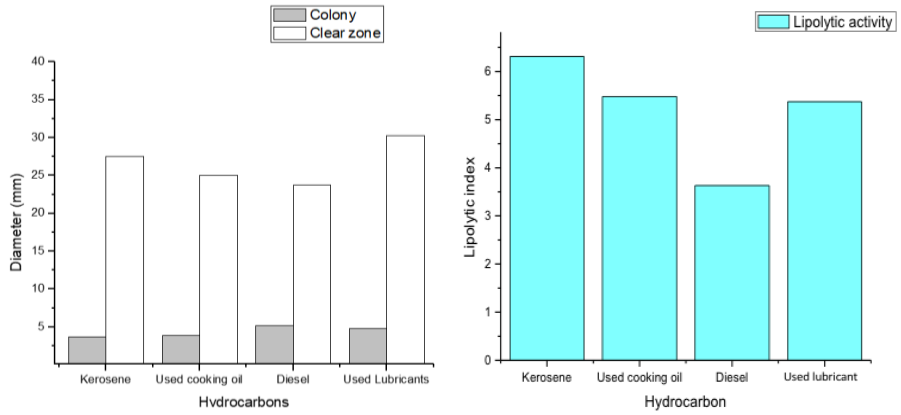
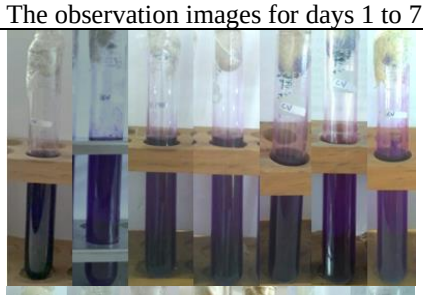
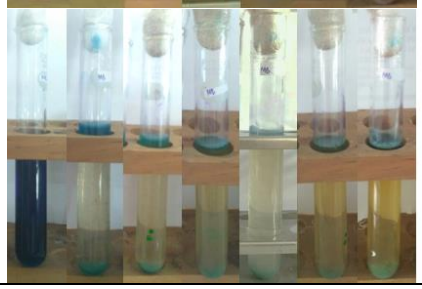


Fig. 3. The colony growth: a. Diameter and clear zone formation were observed; b. Lipolytic activity of *Serratia marcescens* strain MBC1 on various hydrocarbons were also measured

Decolorization. As shown in Table 3 and Fig. 4, the *S. marcescens* bacterium MBC1 strain decolorizes synthetic dyes such as methylene blue, congo red, methyl red, and methyl orange with a concentration of 25 ppm in just seven days.

Table 3. Color change in the decolorization process from day 1 to day 7. Methyl orange, congo red and methyl red show significant color changes on day 4, then brighter and clearer on day 7. Dye degradation on methylene blue takes only 24 h. Crystal violet, on the other hand, tended to preserve its initial color during fermentation, albeit it appeared to diminish on the seventh day

Synthetic Dyes	The observation images for days 1 to 7
Crystal violet	
Methyl orange	
Congo red	
Methyl red	
Methylene blue	

Methylene blue was the synthetic dye that decolorized the most quickly. In only 24 h, the dark blue methylene blue color became colorless, leaving just a blue residue at the bottom of the test tube, and it became clearer until the 7th day. Methyl red was the second synthetic dye to undergo decolorization. The color of methyl red started to change from red to orange and became clearer after 48 h. The orange solution turned light orange on the third day. The solution's top layer seems more

apparent than the bottom layer. The liquid medium changed to yellowish-white and slightly gloomy on day four. The solvent appears to be even finer until the seventh day. *S. marcescens* is a Gram-negative bacteria which is known to have a good ability to produce biosurfactant lipopeptides and glycolipids. Both types of biosurfactants have lipid groups that act similarly to saponins in emulsifying and reducing the surface tension of compounds (Arifiyanto *et al*., 2021; Riyanto *et al*., 2021). By enhancing the water solubility of hydrophobic substances, it makes it easier for these compounds to be degraded by bacterial enzymes (Hessini *et al*., 2009). Lipase is one of the hydrolytic enzymes reported to play a role in the degradation of textile dyes (Yao *et al*., 2022).

On day 3, Congo red began to decolorize. There was a slight change in the red color that was fading. Until the 6th day, the red color turned clear with the presence of sediment and plague at the top of the test tube, which was blackish red. There was a separate red lump in the middle of the test tube. And until the 7th day, the sediment faded, and the color of the solution became clearer. There were no significant changes in methyl orange up to 48. Only a little faded but still orange. In 72 h, Methyl orange turned clear yellowish. At the bottom of the test tube, there was orange sediment. The yellow color faded and became yellowish-white on the fourth day. The solution was yellowish-white and brighter, and the deposit had reduced by the seventh day. On day 4, the color of crystal violet changed slightly to faded purple, and there was no noticeable change until day 7. It is suspected that the degradation of these dyes is due to the extracellular secretion of the enzymes produced which make the dyes clear, having previously been made more water soluble by the biosurfactants produced (Kabir & Koh, 2021). On day 3, Congo red began to decolorize. There was a slight change in the red color that was fading. Until the 6th day, the red color turned clear with the presence of sediment and plague at the top of the test tube, which was blackish red. There was a separate red lump in the middle of the test tube. And until the 7th day, the sediment faded, and the color of the solution became clearer. There were no significant changes in methyl orange up to 48. Only a little faded but still orange. In 72 h, Methyl orange turned clear yellowish. At the bottom of the test tube, there was orange sediment. The yellow color faded and became yellowish-white on the fourth day. The solution was yellowish-white and brighter, and the deposit had reduced by the seventh day. On day 4, the color of crystal violet changed slightly to faded purple, and there was no noticeable change until day 7. It is suspected that the degradation of these dyes is due to the extracellular secretion of the enzymes produced which make the dyes clear, having previously been made more water soluble by the biosurfactants produced (Kabir & Koh, 2021).

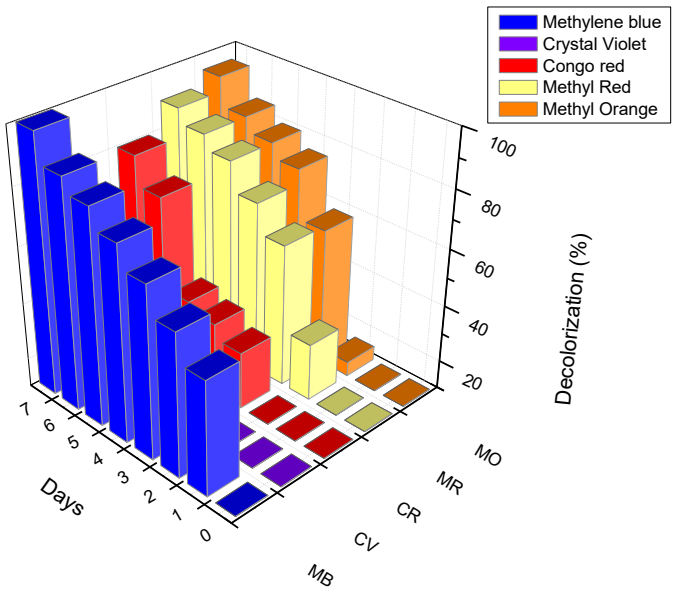


Fig. 4. Decolorization of synthetic dye by *Serratia marcescens* bacterium MBC 1 strain shown on 3DBar. Blue indicates methylene blue (MB), purple represents crystal violet (CV), red denotes congo red (CR), yellow reflects methyl red, and orange is methyl orange (MO)

Lipolytic in hydrocarbons substrate. A clear zone in media treated with hydrocarbons such as used cooking oil, gasoline, used lubricants, and kerosene suggested that *S. marcescens* MBC1 bacteria had already developed lipase enzymes and were breaking down hydrocarbons. The method of decomposing organic matter in the biosphere, which heterotrophic microbial groups carried out, was the bioremediation mechanism. Organic compounds, such as hydrocarbons, can be used as substrates by heterotrophic microbes (Brzeszcz & Kaszycki, 2018). This procedure necessitated an enzymatic chemical reaction, in this case, the lipase enzyme, which hydrolyzed triacylglycerol into free fatty acids and glycerol (Arifiyanto *et al*., 2021). In this test, methyl red is used as a pH indicator. The pH dropped from 7 (medium pH) to a more acidic pH, resulting in a color shift from orange to yellow around the colony. The release of fatty acids due to lipid degradation caused a rise in acidity (Ervina *et al*., 2020).

Decolorization. This study's findings were accurate: a preliminary analysis. It would be helpful in advance of future research. An isolated strain of *S. marcescens* can decolorize a variety of synthetic dyes. *S. marcescens* could be used to help solve stain decolorization challenges and develop promising technologies for extracting color from waste containing harmful synthetic dyes and oil degradation (Fig 5).

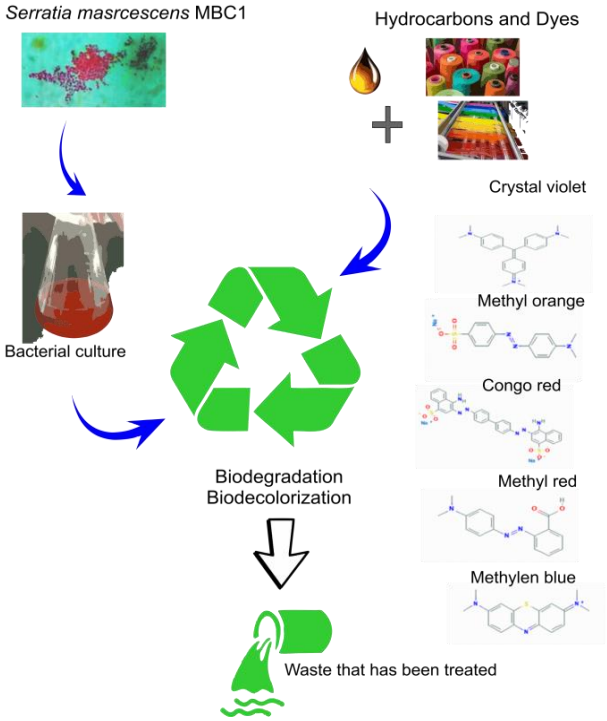


Fig. 5. Treating hydrocarbon waste and synthetic dyes uses the bacterium *Serratia marcescens* MBC1 (Arifiyanto *et al*., 2022)

As compared to other dyes, crystal violet was more challenging to decolorize. The paint was made of triphenylmethane (TPM) and was commonly used in the textile and printing ink industries. TPM dyes were challenging to degrade due to their complex structure. Thus, it often impacts dye accumulation in the environment. Nevertheless, ligninolytic enzymes, such as laccase, lignin peroxidase (LiP), and manganese peroxidase, were involved in the degradation of TPM dye in microorganisms (MnP) (Yang *et al*., 2020). Not only in fungi, laccase, and manganese peroxidase were also generated by *S. marcescens* bacteria. The ligninolytic enzymes described have been widely used in dye decolorization and detoxification.

Lignin peroxidase (LiP) was an extracellular peroxidase enzyme that required the presence of H_2O_2 in crystal violet to function. Laccase worked as a multicopper enzyme that could oxidize a wide range of phenolic and non-phenolic compounds. MnP, on the other hand, was an extracellular

peroxidase enzyme that used Mn^{2+} as a reducing substrate. MnP converted Mn^{2+} to Mn^{3+} and then to phenoxyl radicals by oxidizing the phenolic structure. The Mn^{3+} form was highly reactive, forming complexes with organic acids, including oxalic and malic acid. Finally, the Mn^{3+} ion was stabilized and penetrated the substrate tissue with the aid of a chelator (Falade *et al.*, 2017).

Another dye used in this analysis is azo. The bonding properties of azo (-N = N-) compounds are the main characteristics used as dyes in textiles, tanneries, sheets, and indicators (Popli & Patel, 2015). Azo dyes included methyl orange, Congo red, Methyl red, and methylene blue (Valica & Hostin, 2016). Because of their many benefits, they were commonly used, but 10-15% of dyes were discarded and could not be reused. Benzidine-containing Azo group dyes have been reported to be tumorigenic and carcinogenic (Pillai, 2017). Under anaerobic conditions, azoreductase hydrolyzes azo dyes reductively. The enzyme degraded the dye's azo bonds and was inactivated by oxygen (Sarkar *et al*., 2017). In *Serratia* sp. strain S2, many azoreductase genes were discovered, all of which had the potential to reduce chromium, but the established gene needed to be confirmed (Dong *et al*., 2018). Bacterial ligninolytic peroxidase (LiP) enzymes frequently played a part in the bio-decolorization of azo dyes (Akansha *et al.*, 2019). The overhaul of stains by ligninolytic enzymes started with the ligninolytic enzymes being oxidized by oxygen, and instead, the ligninolytic enzymes deteriorated the textile dyes in this oxidized state. Aromatic compounds and synthetic polymers were remodeled by ligninolytic enzymes. By redox reactions, the ligninolytic enzymes renovated aromatic compounds, synthetic polymers, and dyes, in which the ligninolytic enzymes fully oxidized carbon compounds into CO_2 and H_2O. During the decolorization of bacteria from pulp and paper mill effluents, *S. liquefaciens* bacteria showed levels of LiP induced. On LiP activity of 20.4 U/mL, enzyme activity increased for 72 h before steadily decreasing (Haq *et al*., 2016).

The biodegradation of dyes altered the chemical structure of the chromophore or auxochrome groups, as well as the two groups. A reduction occurred in which HN-HN substituted the N-N bonds in azo dyes from NH_2. The substance did not absorb visible light, suggesting that the azo dye reduction and decolorization process had occurred (Benkhaya *et al*., 2020).

CONCLUSION

The *Serratia marcescens* MBC 1 strain generated lipase enzymes, which caused clear zones to form in all media exposed to different forms of hydrocarbon, including used cooking oil, diesel, kerosene, and used lubricant. With a lipolytic index of 6.313 cm, kerosene was the largest region. *S. marcescens* MBC 1 strain could also bio-decolorize various synthetic dyes, including methylene blue, congo red, methyl red, methyl orange, and crystal violet (MnP). Methylene blue was the synthetic dye that decolorized the fastest, taking just 24 h. Since it was able to generate reducing enzymes such as azoreductase, lignin peroxidase (LiP), laccase, and manganese peroxidase, based on the literature, further research regarding these activities is required.

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REFERENCES

Abdullah AH, Al-Ammiri H, Al-Ammiri HH. 2017. Isolation and identification of *Serratia marcescens* from bovine mastitis infections in Iraq and their susceptibility to antibiotics. *Journal of Entomology and Zoology Studies*. vol 5(2): 489–492.

Akansha K. Chakraborty D, Sachan SG. 2019. Decolorization and degradation of methyl orange by Bacillus stratosphericus SCA1007. *Biocatalysis and Agricultural Biotechnology*. vol 18: 1–29. doi: https://doi.org/10.1016/j.bcab.2019.101044.

Almansoory AF, Hasan HA, Idris M, Sheikh Abdullah SR, Anuar N, Tibin EMM. 2017. Biosurfactant production by the hydrocarbon-degrading bacteria (HDB) *Serratia marcescens*: Optimization using central composite design (CCD). *Journal of Industrial and Engineering Chemistry.* vol 47: 272–280. doi: https://doi.org/10.1016/j.jiec.2016.11.043.

Arifiyanto A, Afriani H, Putri MH, Damayanti B, Riyanto CLR. 2021. The biological prospective of red-pigmented bacteria cultured from contaminated agar media. *Biodiversitas, Journal of Biological Diversity*. vol 22(3): 1152–1159. doi: https://doi.org/10.13057/biodiv/d220310.

Arifiyanto A, Damayanti B, Putri MH, Riyanto CLR. 2022. Screening of the bio-decolorization ability of synthetic dyes and the degradation of hydrocarbon bacteria *Serratia marcescens* MBC1. *Biogenesis: Jurnal Ilmiah Biologi*. vol 10(2): 244–252. doi: https://doi.org/10.24252/bio.v10i2.32095.

Benkhaya S, M'rabet S, El Harfi A. 2020. Classifications, properties, recent synthesis and applications of azo dyes. Heliyon. vol 6(1): 1–26. doi: https://doi.org/10.1016/j.heliyon.2020.e03271.

Brzeszcz J, Kaszycki P. 2018. Aerobic bacteria degrading both n-alkanes and aromatic hydrocarbons: an undervalued strategy for metabolic diversity and flexibility. *Biodegradation*. vol 29(4): 359–407. https://doi.org/10.1007/s10532-018-9837-x.

Damayanti B, Sumardi S, Arifiyanto A, Handayani K, Kanedi M, Putri MH, Riyanto CLR. 2022. Pengaruh media pertumbuhan dan ph terhadap aktivitas biosurfaktan dari bakteri *Serratia marcescens* strain MBC 1 pada minyak jelantah. *Indonesian Journal of Chemical Analysis (IJCA).* vol 5(1): 1–8. doi: https://doi.org/10.20885/ijca.vol5.iss1.art1.

Dong L, Zhou S, He Y, Jia Y, Bai Q, Deng P, Gao J, Li, Y, Xiao H. 2018. Analysis of the genome and chromium metabolism-related genes of *Serratia* sp. S2. *Applied Biochemistry and Biotechnology*. vol 185(1): 140–152. doi: https://doi.org/10.1007/s12010-017-2639-5.

Ervina E, Ekowati CN, Sumardi S, Rosa E. 2020. Lipolytic-screening of *Bacillus* genera as biocontrol candidate in coffee plantation. *Jurnal Ilmiah Biologi Eksperimen dan Keanekaragaman Hayati*. vol 7(1): 31–34. doi: http://dx.doi.org/10.23960%2Fj_bekh.v7i1.2496.

Falade AO, Nwodo UU, Iweriebor BC, Green E, Mabinya LV, Okoh AI. 2017. Lignin peroxidase functionalities and prospective applications. *MicrobiologyOpen*. vol 6(1): 1–14. doi: https://doi.org/10.1002/mbo3.394.

Fitri RD, Illavi G, Renjana E, Pratiwi IA, Sumarsih S. 2019. Cell wall response of bacteria Serratia marcescens LII61–lipase and protease enzymes producer–in gram staining. *Ecology, Environment and Conservation*. vol 25: 76–80.

Gazzola P, Pavione E, Pezzetti R, Grechi D. 2020. Trends in the fashion industry. The perception of sustainability and circular economy: A gender/generation quantitative approach. *Sustainability*. vol 12(7): 1–19. doi: https://doi.org/10.3390/su12072809.

Gondaliya N, Parikh SC. 2013. Bioremediation of textile azo dyes by *Serratia marcescens* and optimized conditions for efficient decolorization. *The Microbes*. vol 5: 44–56. doi: https://doi.org/10.3390/su12072809.

Haq I, Kumar S, Kumari V, Singh SK, Raj A. 2016. Evaluation of bioremediation potentiality of ligninolytic *Serratia liquefaciens* for detoxification of pulp and paper mill effluent. *Journal of Hazardous Materials*. vol 305: 190–199. doi: https://doi.org/10.1016/j.jhazmat.2015.11.046.

Karim ME, Dhar K, Hossain MT. 2018. Decolorization of textile reactive dyes by bacterial monoculture and consortium screened from textile dyeing effluent. *Journal of Genetic Engineering and Biotechnology*. vol 16(2): 375–380. doi: https://doi.org/https://doi.org/10.1016/j.jgeb.2018.02.005.

Koshlaf E, Ball AS. 2017. Soil bioremediation approaches for petroleum hydrocarbon polluted environments. *AIMS Microbiology.* vol 3(1): 25–49. doi: https://doi.org/10.3934/microbiol.2017.1.25.

Lestari MD, Nukmal N, Setyaningrum E, Farisi S, Arifiyanto A. 2022. Larvicide effects of *Serratia marcescens* strain MBC1 extract on instar III larvae of *Aedes aegypti*. *Jurnal Ilmiah Biologi Eksperimen Dan Keanekaragaman Hayati (J-BEKH).* vol 9(1): 42–48. doi: https://doi.org/10.23960/jbekh.v9i1.219.

Lim G, Tan TK, Rahim NA. 1987. Variations in amylase and protease activities among *Rhizopus* isolates. *MIRCEN Journal of Applied Microbiology and Biotechnology*. vol 3: 319–322. doi: https://doi.org/10.1007/BF00933585.

Perera F. 2017. Pollution from fossil-fuel combustion is the leading environmental threat to global pediatric health and equity: Solutions exist. *International Journal of Environmental Research and Public Health*. vol 15(1): 1–17. doi: https://doi.org/10.3390/ijerph15010016.

Pillai HPJS. 2017. Optimization of process conditions for effective degradation of azo blue dye by *Streptomyces* DJP15. *Journal of Pure and Applied Microbiology*. vol 11(4): 1757–1765. doi: https://doi.org/10.22207/JPAM.11.4.14.

Popli S, Patel UD. 2015. Destruction of azo dyes by anaerobic–aerobic sequential biological treatment: A review. *International Journal of Environmental Science and Technology*. vol 12(1): 405–420. doi: https://doi.org/10.1007/s13762-014-0499-x.

Putri MH, Handayani K, Setiawan WA, Damayanti B, Ratih CL, Arifiyanto A. 2021. Screening of extracellular enzymes on *Serratia marcescens* strain MBC1. *Jurnal Riset Biologi dan Aplikasinya*. vol 3(1): 23–29. doi: https://doi.org/10.26740/jrba.v3n1.p23-29.

Sarkar S, Banerjee A, Halder U, Biswas R, Bandopadhyay R. 2017. Degradation of synthetic azo dyes of textile industry: A sustainable approach using microbial enzymes. *Water Conservation Science and Engineering*. vol 2(4): 121–131. doi: https://doi.org/10.1007/s41101-017-0031-5.

Valica M, Hostin S. 2016. Electrochemical treatment of water contaminated with methylorange. *Nova Biotechnologica et Chimica.* vol 15(1): 55–64. doi: https://doi.org/10.1515/nbec-2016-0006.

Variani YA, Setyaningrum E, Handayani K, Nukmal N, Arifiyanto A. 2021. Analisis senyawa bioaktif ekstrak metabolit sekunder *Serratia marcescens* strain MBC1. *IJCA (Indonesian Journal of Chemical Analysis).* vol 4(2): 64–71. doi: https://doi.org/10.20885/ijca.vol4.iss2.art3.

Yadav VG, Yadav GD, Patankar SC. 2020. The production of fuels and chemicals in the new world: critical analysis of the choice between crude oil and biomass vis-à-vis sustainability and the environment. *Clean Technologies and Environmental Policy*. vol 22(9): 1757–1774. doi: https://doi.org/10.1007/s10098-020-01945-5.

Yang J, Zhang Y, Wang S, Li S, Wang Y, Wang S, Li H. 2020. Biodegradation of crystal violet mediated by CotA from Bacillus amyloliquefaciens. *Journal of Bioscience and Bioengineering*. vol 130(4): 347–351. doi: https://doi.org/10.1016/j.jbiosc.2020.05.005.