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Land degradation

Characterization of a Metanil Yellow-decolorizing *Pseudomonas* strain Isolated from the Juru Industrial Park

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ABSTRACT

This study examines how the initial concentration of dye, temperature, pH, and NaCl content affect the ability of a bacterial strain, specifically identified as Pseudomonas sp. strain UPM291, to remove color from Metanil Yellow. The decolorization percentage exhibited a distinct trend throughout a range of dye concentrations (0-700 mg/L), with the greatest efficacy (90-100%) recorded at values below 200 mg/L. The efficiency declined at higher concentrations, reaching approximately 20% at a dosage of 700 mg/L. Temperature investigations unveiled a symmetrical curve resembling a bell shape, indicating a range of temperatures that is most favorable for the process of decolorization. The maximum efficiency, approaching 100%, was found at a temperature of 35°C. However, the efficiency decreased considerably as the temperature above 35°C, reaching approximately 20% at 50°C. The impact of pH on decolorization exhibited comparable patterns, with optimal efficacy observed at pH 6.5 and diminished efficacy at both more acidic and more alkaline settings. The decolorization efficiency reached its peak (90-100%) at a pH of 6.5 and decreased to approximately 60% at a pH of 8.0. The influence of the NaCl content on decolorization was shown to follow a certain pattern, with the most effective decolorization (90-100%) occurring at NaCl concentrations of up to 10 g/L. The efficiency declined as the NaCl concentrations increased, reaching around 20% at 30 g/L. The data indicate that the bacterial strain demonstrates the highest effectiveness in removing color within particular ranges of dye concentration, temperature, pH, and NaCl content. There is a noticeable decline in efficiency when the parameters go outside of these optimal ranges. Gaining a comprehensive understanding of these characteristics can assist in optimizing the conditions necessary for the efficient bioremediation of Metanil Yellow utilizing specific bacterial strains.

INTRODUCTION

The textile, leather, paper, plastic, and food sectors are among the many that extensively use dyes. Dyeing is a crucial process in the textile business since it gives materials their color and makes clothing more eye-catching and colorful. Dyeing is also crucial in the leather business to make goods that look good. Food and drink manufacturers use dyes to make their products seem better and attract more customers. Furthermore, dyes are utilized in the printing, cosmetics, and pharmaceutical industries to enhance the visual appeal and overall product quality [1–3]. There are major ecological consequences to the widespread usage of dyes. Severe water contamination occurs when industrial activities dump wastewater containing dyes into bodies of water without treating it properly. Dye colors are both aesthetically unpleasant and harmful to aquatic environments because they prevent sunlight from reaching aquatic plants, which is necessary for photosynthesis. Dye contamination also reduces biodiversity and upsets food webs since many dyes include harmful chemicals that can kill aquatic life. Because they are resistant to degradation and can linger in water bodies for long periods of time, these dyes pose a serious threat to environmental sustainability [4–8]. Dyes' hazardous property is an important problem that impacts human and aquatic health. Heavy metals and aromatic compounds are dangerous components in many synthetic dyes. These substances have the potential to cause cancer, mutations, and birth defects.

Toxic substances have a way of making their way up the food chain, where they can build up in the tissues of fish and then make their way to people who eat seafood that has been poisoned. Toxic dyes are known to induce skin irritation, respiratory difficulties, and cancer if exposed to them for an extended period of time. More specifically, in anaerobic conditions, azo dyes—which make up a large fraction of industrial dyes—can decompose into aromatic amines, which are recognized carcinogens [7,9–12].

Resolving dye pollution is an intricate task that calls for fresh approaches that work. Dye removal from wastewater has been the subject of numerous approaches, each with its own set of pros and cons. Dye removal often uses physical processes like adsorption, filtering, and coagulation-flocculation. The use of activated carbon and other adsorption materials is very effective but can be somewhat costly. Filtration methods, such as membrane filtration, can also remove colors, but they frequently necessitate substantial energy and maintenance input. Advanced oxidation processes (AOPs), reduction, and oxidation are all part of the chemical treatment arsenal. Using these techniques, colors can be broken down into safer substances. One method that has shown promising in breaking down stubborn dyes is the Fenton reaction, which creates hydroxyl radicals. Nevertheless, chemical approaches come with the risk of secondary pollution and can be rather expensive.

Dye degradation by microbes is a key component of biological treatment approaches. This method is thought to be both economical and kind to the environment. Certain bacteria, fungi, and algae have demonstrated the enzymatic decolorization and degradation of dyes. For instance, azoreductase enzymes can decolorize and detoxify azo dyes by breaking down the azo bonds in them. Another encouraging biological approach is phytoremediation, which involves the absorption and accumulation of dyes by plants [13–16]. Dye removal can be made more efficient by combining several approaches, such as physical or chemical processes with biological treatments. To combat dye pollution more effectively, integrated approaches can overcome the shortcomings of standalone techniques. There are serious worries about pollution and toxicity caused by the widespread use of dyes in many different industries. Effective cleanup plans are required to lessen the toll that color contamination takes on ecosystems and people's health. Dye pollution remediation solutions can be developed in a sustainable and efficient manner by combining physical, chemical, and biological processes. This will result in a cleaner and safer environment [17–24].

Metanil Yellow, often referred to as Acid Yellow 36, is a man-made azo dye predominantly employed for the purpose of coloring in different sectors. However, its utilization is subject to rigorous regulations due to considerable health and environmental risks. Metanil Yellow is employed in the textile industry to color materials like cotton, wool, and silk, imparting a vibrant yellow shade to them. It is also utilized in the paper business to produce colored papers and stationery and in the leather industry to create vivid yellow tones in leather products. Metanil Yellow is utilized in the plastics and polymer sector to dye synthetic polymers and plastics. Traditionally, this dye was additionally employed in the food and beverage sector to pigment confections, drinks, and processed foods, augmenting their visual allure. Nevertheless, numerous nations have implemented bans or limitations on the utilization of this substance in food products due to its inherent health hazards, such as its carcinogenic and poisonous characteristics [25–28].

Metanil Yellow (**Fig. 1**) has also been utilized in cosmetics, including lipsticks, nail polishes, and hair dyes. However, regulatory authorities have implemented limitations due to safety apprehensions. In scientific settings, it functions as a biological dye, aiding in the visualization of cellular structures when observed via a microscope and sometimes as a pH indicator. The main hazards associated with Metanil Yellow revolve around its toxicity and ability to cause cancer, which can lead to allergic reactions and other health problems when exposed to it. Improper disposal of waste can result in water contamination, which has a detrimental impact on aquatic life due to its long-lasting presence in the ecosystem.

Regulatory bodies worldwide have implemented prohibitions or strong limitations on its use in food and cosmetics. They require the industry to strictly follow safety requirements in order to reduce health hazards and minimize environmental effects. Although Metanil Yellow has many practical uses in different industries, its dangerous properties require meticulous handling and adherence to regulatory guidelines to avoid exposure and contamination. The dye is widely utilized for coating turmeric due to its distinctive orange-yellow hue. The dye is exceptionally well-suited for creating water-resistant ink with vibrant colors [25,29,30].

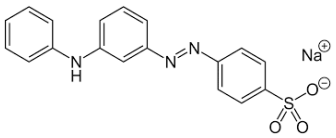

Fig. 1. Structure of Metanil Yellow

Metanil Yellow poses significant health risks, impacting multiple physiological systems. Studies have shown that it can cause severe damage to the nervous system. In animal models, exposure to Metanil Yellow has led to brain damage, affecting neurotransmitter levels in regions such as the striatum, brain stem, and hypothalamus. This damage is often irreversible, even after the cessation of exposure, leading to long-term neurological deficits and impaired learning abilities [29]. The digestive system is also adversely affected by Metanil Yellow. Research indicates that the dye can cause gastrotoxicity, hepatotoxicity, and damage to the intestinal lining. In fish models, exposure resulted in disrupted gastric folds, destroyed epithelial cells, and necrosis of the lamina propria. These effects significantly impair the digestive system's ability to absorb nutrients, leading to malnutrition and other related health issues [25].

Metanil Yellow's impact extends to the cardiovascular system, where it induces cardiotoxicity. Studies on goat heart tissues have shown increased lipid peroxidation and altered levels of endogenous antioxidant enzymes such as catalase, indicating oxidative stress and damage to heart tissues. This oxidative stress is a common mechanism underlying the toxic effects of Metanil Yellow across different organ systems [31]. The excretory and reproductive systems are not spared from the toxic effects of Metanil Yellow. In the kidneys, the dye causes necrosis of tubular epithelium, cloudy swelling of epithelial cells, and disruption of the Bowman's capsule. These pathological changes compromise kidney function, leading to renal failure in severe cases. In the reproductive system, Metanil Yellow disrupts the estrous cycle in female rats, impairs folliculogenesis, and causes oxidative stress in the hypothalamic-pituitary-gonadal axis. Male reproductive toxicity includes testicular degeneration, affecting spermatogenesis and causing long-term reproductive

issues [27]. The toxicity of Metanil Yellow is further exacerbated by its ability to induce oxidative stress, disrupting the body's antioxidant defenses and generating free radicals. This oxidative stress is a critical factor in the dye's harmful effects on various organs and tissues. Chronic exposure to Metanil Yellow, often through contaminated food, poses a significant public health risk, necessitating strict regulatory measures and public awareness campaigns to prevent its use as a food colorant.

Physicochemical techniques for dye removal, like activated carbon adsorption and advanced oxidation processes, are effective but costly due to expensive materials and high energy needs. These methods can also produce secondary pollutants, increasing complexity and expense. Filtration methods, such as membrane filtration, require significant energy and maintenance, making them less suitable for large-scale operations. Microbial bioremediation offers a cost-effective alternative, using microorganisms to break down azo dyes into non-toxic substances. This approach is generally cheaper, relying on naturally occurring organisms that can be cultivated with low-cost substrates, and reduces the risk of secondary pollution.

Microorganisms, including bacteria, fungi, and algae, degrade dyes through enzymatic processes, such as reductive cleavage by azoreductases, which decolorize and detoxify dyes. Other enzymes, like peroxidases and laccases, further break down these substances. Phytoremediation, involving plants that absorb and accumulate dyes, also plays a role, with rhizospheric microorganisms enhancing degradation. While microbial bioremediation shows great promise, it requires optimization to ensure consistent results. Research should focus on isolating and engineering efficient strains, fine-tuning environmental conditions, and developing integrated systems that combine microbial and physicochemical methods. This strategy provides a sustainable, cost-effective solution for azo dye pollution, leveraging natural processes to achieve minimal environmental impact. In this study, we report a *Pseudomonas* strain that can efficiently decolorize Metanil Yellow.

MATERIALS AND METHODS

Isolation of Metanil Yellow-degrading bacterium

A soil sample of 10 g was taken 5 cm from a dye and pigment effluent industry in Juru, Penang in 2010. The soil was mixed with 100 mL of sterile tap water. The supernatant was collected after allowing the soil to settle down for 30 min. The microorganisms having the ability to degrade dye were screened by inoculating 1 mL of supernatant on the decolorization medium containing (g/L): The ingredients of the growth media (% w/v) were modified from the above to cater for dye-decolorization requirement, which include sodium lactate and sodium nitrate [32,33], and were as follows: glucose (1%), sodium lactate (1%), $(NH_4)_2.SO_4$ (0.3%), $NaNO_3$ (0.2%), $MgSO_4.7H_2O$ (0.05%), yeast extract (0.05%), NaCl (0.5%), Na_2HPO_4 (0.705% or 50 mM). The medium was adjusted to pH 7.0. The buffer helps to prevent decolorization due to a shift in pH causing a shift in the dye colors. This medium was supplemented with yeast extract (2.5 g/L) and Metanil Yellow (200 mg/L). All other chemicals used in this study were analytical [34].

Characterization by morphology, physiology, and biochemistry

The bacterium underwent a battery of biochemical, morphological, and phenotypic analyses. Precise and reliable characterisation was achieved by strictly adhering to the standard techniques specified in Bergey's Manual of Determinative Bacteriology [35]. A thorough analysis of the bacterium's metabolic processes, physiological patterns, and structural features was part of this procedure. The data were then processed utilizing the online platform of the Automated Bacterial Identification System (ABIS) [36] to ensure accurate identification and interpretation of the results. This all-encompassing method enabled a complete comprehension of the bacterium's traits, which in turn allowed for precise categorization and additional research.

Bacterial resting cells preparation

The bacterium was used on a microtiter plate as before, taking into account factors such as carbon sources, heavy metal effects, pH, and temperature [37]. In brief, bacterial cells were cultured in nutrient broth aerobically in a 1 L volume using an orbital shaker (Yihder, Taiwan) set to 120 rpm. The culture was then divided across multiple 250 mL shake flasks. All incubation processes were performed at ambient temperature. The cells were centrifuged for 10-minute at 15,000 x g and at 4 °C. After that, deionized water was used twice to rinse the bacterial pellets. During the characterisation operations, the medium was adjusted appropriately to accommodate changes in dye concentration, phosphate and temperature. The wells of a sterile microplate were filled with approximately 180 (µL) of the correctly adjusted medium through sterile transfer.

Afterward, 20 mL of sterile glucose or another carbon source from a stock solution was added until the final concentration reached 1.0 % (w/v). Exactly 200 µL of culture medium filled each well of the microplate. Following sealing (Corning® microplate), the plates were left to incubate at room temperature for 48 h with periodically readings if needed. The bacterium's capacity to decolorize dyes (final concentration of 200 mg/L) was tested using the microplate above format. Dyes (Sigma-Aldrich (St. Loius, U.S.A.) with maximum wavelength in parentheses were as follows: Ponceau S (C.I. 27195) (352 nm), Naphthol Blue Black (C.I. 20470) (618 nm), Orange II sodium salt (C.I. 15510) (483 nm), Tartrazine (C.I. 19140) (427 nm), Evans Blue (C.I. 23860) (594 nm), Metanil Yellow (C.I. 13065) (414 nm), Remazol Black B (C.I. 20505) (597 nm), Methyl Orange (C.I. 13025) (505 nm), Ponceau 2R (C.I. 16150) (388 nm), Crocein Orange G (C.I. 15970) (482 nm), Congo Red (C.I. 22120) (498 nm), Orange G (C.I. 16230) (476 nm), Methyl Red (C.I. 13020) (493 nm).

Monitoring of dye decolorization

Three standard wavelengths were used to evaluate decolorization: 405, 490, and 595 nm. These wavelengths cover the maximum absorption values for various dyes [38]. The BioRad 680 microplate reader could accommodate these wavelengths. Furthermore, these predetermined wavelengths are used with the assumption that water-soluble dyes usually have shallow maximum absorption spectra and that a discrepancy of 20 nm from that peak does not result in a significant drop in absorbance [1]. After 48 hours of incubation, the percentage of decolorization was determined by subtracting the starting absorbance values from the final results.

Assay of dye-decolorizing enzyme azoreductase

The activity of azoreductase was determined using the same procedure as described by [39]. After being cultivated in a medium with 200 mg/L of Metanil Yellow, the bacterial cultures were centrifuged at 10,000 × g for 10 min at 4 ◦C.

The cell pellet was rinsed repeatedly with 50 mM potassium phosphate buffer (pH 7.0) to prepare for resuspension. The cells were sonicated in a cold environment by using a Model 505 Sonic Dismembrator for 30 seconds at 70% output and followed by 5 min cooling on ice with a total sonication time of 10 min. The crude enzyme was obtained from the supernatant after centrifuging the sonicated cells at 15,000 × g for 10 min at 4 ◦C.

A reaction mixture comprising 150 µM Metanil Yellow in 50 mM phosphate buffer (pH 7.0) was prepared by adding 100 µL of crude enzyme. After pre-incubating the mixture for 3 minutes, 2 mM NADH was added. At room temperature, the azoreductase activity was measured spectrophotometrically by tracking the drop in absorbance at 340 nm. The amount of enzyme required to catalyze the reaction of 1 µmol of substrate per minute was deemed as one unit of enzyme activity. We performed each experiment three times, including the enzyme assay.

RESULTS AND DISCUSSION

Partial identification of the bacterium

The bacterium was a Gram-negative (**Fig. 2**), rod-shaped, motile microorganism. Culture, morphology, and a battery of biochemical analyses all pointed to the same bacterium, which allowed for its positive identification (**Table 1**) to the Bergey's Manual of Determinative Bacteriology [35] and using the ABIS online software [36]. The software provided two possible identifications for the bacterium, both with similar homology (84%) and accuracy (82%): *Pseudomonas putida* and *Pseudomonas plecoglossicida.*

Numerous species within this genus are recognized for their ability to degrade dyes [40–50]. Consequently, it is not feasible to definitively assign the bacterium to a specific species at this point. Further research is required to accurately identify the species, particularly by employing molecular identification techniques such as 16S rRNA gene comparison. Other Metanil Yellow-degrading bacterium is shown in **Table 2**. At this junction, the bacterium was identified as *Pseudomonas* sp. strain UPM291.

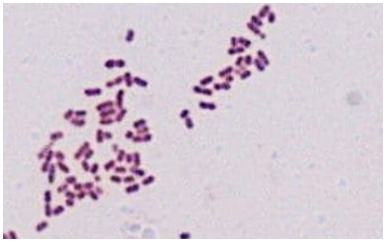

Fig. 2. Gram staining of the bacterium.

Table 1. Biochemical tests.

Motility	+	Utilization of:	
Hemolysis	+	L-Arabinose	+
Growth at 4 ºC	–	Citrate	+
Growth at 41 ºC	+	Fructose	+
Growth on MacConkey agar	–	Glucose	+
Arginine dihydrolase (ADH)	+	meso-Inositol	d
Alkaline phosphatase (PAL)	+	2-Ketogluconate	+
Indole production	-	Mannose	+
Nitrates reduction	–	Mannitol	–
Lecithinase	–	Sorbitol	d
Lysine decarboxylase (LDC)	–	Sucrose	+
Ornithine decarboxylase (ODC)	–	Trehalose	–
ONPG (beta-galactosidase)	–	Xylose	–
Esculin hydrolysis	–		
Gelatin hydrolysis	d		
Starch hydrolysis	–		
Urea hydrolysis	+		
Oxidase reaction	+		

Note: + positive result, − negative result, d indeterminate result

Table 2. Metanil Yellow-decolorizing bacteria.

Bacterium	Best pH	Best Temperature	Maximum concentration Degraded (mg/L)	Time (hours)	Extra Carbon Source/Yeast Extract	Ref
Bacillus sp. strain Neni-10	7.0	37°C	50	48	Yeast Extract	[51]
Bacillus -3330	8.0	30°C	100	24	Glucose	[52]
Halophilic alkalithermophilic consortium	9.0	50°C	200	12	Starch	[34]
Mixed culture FN3	7.5	35°C	75	36	Yeast extract	[53]
Oenococcus oeni ML34	4.5	30°C	60	48	None	[54]
Bacillus sp. AK1	7.2	40	200	27	Yeast extract	[39]
Lysinibacillus sp. AK2	7.2	40	200	12	Yeast extract	[39]

Azoreductase activity

Enzymatic biotransformation is the main process by which microorganisms break down synthetic azo dyes. The reductive cleavage of the azo bond (–N=N–) is the crucial and primary step in this process, facilitated by azoreductase enzymes [39]. The result (**Fig. 3**). shows a consistent profile of Metanil Yellow decolorization and concomitant increase in azoreductase activity, which aligns with findings from other research on bacteria that can decolorize Metanil Yellow. Anjaneya et al. [39] found that *Bacillus* sp. strain AK1 and *Lysinibacillus* sp. strain AK2 were able to quickly remove the color from Metanil Yellow within the first 24 hours, obtaining a significant reduction in color, which is consistent with the findings of the present investigation. Their investigation also observed the maximum level of azoreductase activity at this juncture, which subsequently remained constant as the quantity of dye declined. Guo et al. [34] examined a bacterial consortia that thrives under high salt, alkaline, and hot conditions. They observed that the decolorization of dye occurred rapidly at the beginning, but the rate slowed down as the dye concentration reduced.

The activity of enzymes reached its highest point halfway through the process. In their study, Lal and Chand [52] found that *Bacillus* -3330 was capable of decolorizing Metanil Yellow by more than 90% during a 24-hour period. They also noted that the activity of the enzyme azoreductase reached its highest level at this time, which is consistent with the enzyme activity pattern reported in this study. In addition, Mansur et al. [51] and Muliadi et al. [53] both documented the presence of *Bacillus* strains that exhibit fast decolorization abilities, with azoreductase activity reaching its highest point early in the process and remaining stable as decolorization approaches completion.

These investigations indicate that there is a consistent pattern of quick early removal of dye, followed by a slower phase as the dye concentration decreases. This emphasizes the important involvement of azoreductase in the decolorization process. The observed pattern of azoreductase activity can also be found in other bacteria, such as *Kerstersia* sp. strain VKY1 [55] and *Galactomyces geotrichum* MTCC 1360 [56], during the decolorization of sulfonated azo dyes. This approach highlights these strains' capacity in bioremediation, namely for purifying wastewater that contains artificial azo dyes.

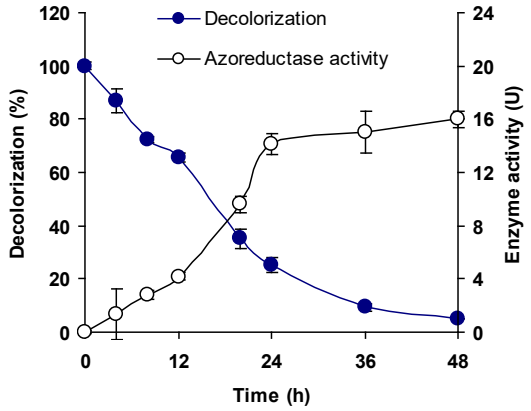

Fig. 3. Azoreductase activity of the Metanil Yellow-decolorizing bacterium. The experiment was replicated thrice, and error bars represent standard deviation.

Most of the reports on this dye decolorization occurred within less than 72 h. For instance, Guo et al. [34] found that high-salinity, high-temperature bacteria degraded over 90% of Metanil Yellow G dye in 48 hours. The consortium can handle high-salt wastewater, as shown by this finding. [52] reported that a newly discovered *Bacillus* strain decolorized Metanil Yellow within 24 hours and completely within 72 hours. Mansur et al. [51] observed that *Bacillus* sp. strain Neni-10 decolorized after 24 hours under optimal circumstances. In addition to decolorization, azoreductase activity increased.

Muliadi et al. [53] increased Malaysian agricultural soil microbial populations via biostimulation. The Metanil Yellow decolorization rate was over 80% in 48 hours. El Ahwany [54] also provided useful information on *Oenococcus oeni* ML34's metabolic pathways for dye decolorization. Fast Red decolorized significantly within 36 hours, which may help explain Metanil Yellow decolorization. Different bacterial strains remove color from Metanil Yellow during 24–72 hours.

Effect of initial dye concentration

The decolorization efficacy of Metanil Yellow by the bacterial strain was evaluated throughout a spectrum of dye concentrations ranging from 0 to 700 mg/L. The decolorization percentage followed a clear pattern, reaching its highest point at a certain concentration and then decreasing at higher concentrations. The decolorization efficiency showed a significant rise at low doses (0 to 100 mg/L), reaching nearly 90% at 100 mg/L. These findings indicate that bacterial isolation significantly reduces the hue of Metanil Yellow at lower doses. The efficiency consistently remained at a high level, ranging from 90% to 100%, at dye concentrations up to 200 mg/L. This plateau represents the ideal range for bacterial activity in the process of decolorizing Metanil Yellow. Nevertheless, when the Concentration of Metanil Yellow was above 200 mg/L, a decrease in decolorization efficiency was noted.

At a concentration of 300 mg/L, the efficiency experienced a little decline to around 80%, and this decline persisted as the concentrations increased. The decolorization efficiency was diminished to around 40% at a concentration of 600 mg/L, and further declined to roughly 20% at the maximum concentration tested (700 mg/L) (**Fig. 4**). The decrease in decolorization efficiency seen at higher dye concentrations can be attributed to the inhibitory effects of Metanil Yellow on the bacterial isolate. Elevated levels of dye can have detrimental effects by impeding the metabolic activity and enzyme performance required for the decolorization process [47,57–59].

Initial dye concentration and frequent employment of bacterial cells, vital to wastewater treatment, affect the decolorization of synthetic azo dyes like Metanil Yellow. Studies have demonstrated that different bacterial strains degrade Metanil Yellow differently, depending on dye concentration and cell reuse. Anjaneya et al. [39] showed that *Bacillus* sp. strain AK1 and *Lysinibacillus* AK2 could decolorize Metanil Yellow, although larger dye concentrations took longer. The strains' azoreductase activity was high, reaching 1615 and 1630 nmol/mg protein/min, respectively, and they retained their decolorization ability after repeated treatment, but efficiency decreased.

A halophilic alkalithermophilic bacterial consortia decolorized Metanil Yellow G by over 90% in 48 hours, even at high dye concentrations, according to [34]. Its ability to be reused with only a modest drop in decolorization efficiency made the consortium acceptable for high-salinity wastewater treatment. At different initial dye concentrations, a newly obtained *Bacillus* strain decolorized Metanil Yellow within 24 hours and completely within 72 hours, according to Lal and Chand [52]. The strain had high azoreductase activity and decolorized efficiently after multiple reuses.

Bacillus sp. strain Neni-10 decolorized after 24 hours under ideal conditions, even at greater dye concentrations, according to [51]. The strain's azoreductase activity rose and could be reused without losing efficiency. Muliadi et al. [53] used biostimulation to improve Malaysian agricultural soil microbial populations, decolorizing Metanil Yellow by over 80% in 48 hours. Despite frequent application, biostimulated microbial communities tolerated different initial dye concentrations and decolorized. [54] revealed the metabolic pathways of dye decolorization with *Oenococcus oeni* ML34, which decolored Fast Red in 36 hours and Metanil Yellow in similar timeframes.

Initial dye concentration and cell reuse affected the decolorization process, with greater concentrations taking longer and decreasing efficiency over time.

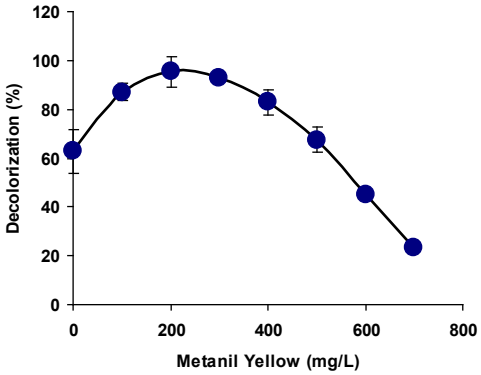

Fig. 4. Effect of initial dye concentration on the Metanil Yellow-decolorizing bacterium. The experiment was replicated thrice, and error bars represent standard deviation.

Effect of temperature on decolorization of Metanil Yellow

The decolorization efficacy of Metanil Yellow by the bacterium was assessed at temperatures ranging from 25°C to 50°C. The percentage of decolorization displayed a symmetrical curve resembling a bell shape, suggesting the presence of an ideal temperature for the decolorization process. The decolorization effectiveness was moderate at temperatures ranging from 25°C to 30°C, with an observed efficiency of around 60% at 25°C. With the rise in temperature to 35°C, the decolorization efficiency experienced a substantial boost, virtually approaching 100%. ANOVA analysis with post hoc Tukey's test indicates that a temperature of between 30 and 35°C is the most favorable for the bacterial isolate to remove the color from Metanil Yellow efficiently. Decolorization efficiency started to decrease above a temperature of 35°C. At a temperature of 40°C, the efficiency experienced a decline to roughly 80%, subsequently dropping to around 60% at 45°C.

At the maximum temperature examined (50°C), the effectiveness of decolorization significantly decreased to approximately 20% (**Fig. 5**). The decrease in the ability to remove color at temperatures beyond 35°C can be ascribed to the susceptibility of the bacterial isolate to heat. Increased temperatures can have a detrimental impact on bacterial metabolism and enzyme function, resulting in a decrease in decolorization ability. On the other hand, temperatures that are lower than the ideal range can decrease the speed of metabolic processes, leading to reduced efficiency in decolorization [60–63].

Temperature affects the decolorization of synthetic azo dyes like Metanil Yellow, which optimizes wastewater treatment biodegradation. Many research have studied how different bacterial strains react to temperature changes during Metanil Yellow decolorization. [39] observed that *Bacillus* sp. strain AK1 and *Lysinibacillus* strain AK2 decolorized best from 30°C to 40°C. A halophilic alkalithermophilic bacterial consortia decolorized Metanil Yellow G efficiently at higher temperatures, particularly at 50°C, according to [34]. The study found that consortium enzymatic activity and metabolic rates rose with temperature, improving decolorization. Lal and Chand [52] found that a newly obtained *Bacillus* strain decolorized Metanil Yellow at 30–35°C. *Bacillus* sp. strain Neni-10 decolorized best at 37°C, according to Mansur et al. [51]. The study found that temperatures above 40°C hindered the strain's decolorization, emphasizing the need of appropriate temperature. Muliadi et al. (2021) used biostimulation to improve Malaysian agricultural soil microbial populations, decolorizing Metanil Yellow at 30°C. *Oenococcus oeni* ML34 decolorized Fast Red at 30°C, according to [54].

Effect of pH on decolorization of Metanil Yellow

The ability of the bacterial isolate to remove color from Metanil Yellow was assessed across a pH range of 5.5 to 8.0. The decolorization percentage displayed a symmetrical curve resembling a bell shape, suggesting the existence of a certain pH range where the decolorization process reaches its maximum efficiency.

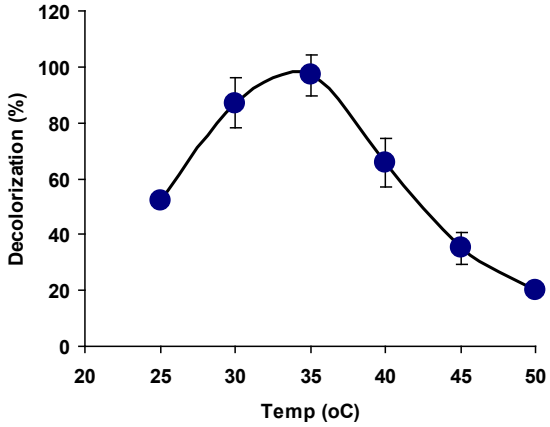

Fig. 5. Effect of temperature on the Metanil Yellow-decolorizing bacterium. The experiment was replicated thrice, and error bars represent standard deviation.

The decolorization efficiency was moderate at lower pH values (5.5 to 6.0), with an initial level of roughly 60% at pH 5.5 and a subsequent increase to nearly 80% at pH 6.0. At a pH of 6.5, the decolorization efficiency reached its highest point, almost reaching 100%. This suggests that the bacterial isolate are most successful at decolorizing Metanil Yellow at this pH level. Decolorization efficiency started to decrease beyond a pH of 6.5. At a pH of 7.0, efficiency experienced a little fall to roughly 90%, and further declined to around 80% at a pH of 7.5. At the pH level of 8.0, the decolorization efficiency decreased to around 60% (**Fig. 6**). decolorization effectiveness at pH levels higher than 6.5 can be ascribed to the impact of pH on bacterial metabolism and enzyme function. The enzymes involved in the decolorization process may experience changes in their ionization state under alkaline conditions, which can decrease their efficacy. On the other hand, acidic environments can also hinder enzyme activity, resulting in reduced efficacy in removing color [60–63].

Decolorization of synthetic azo dyes like Metanil Yellow is affected by pH, a key element in wastewater treatment biodegradation optimization. Various research has studied how different bacterial strains react to pH changes during Metanil Yellow decolorization. [39] observed that *Bacillus* sp. strain AK1 and *Lysinibacillus* sp. strain AK2 decolorized best at pH ranges of 7–9. [34] found that the halophilic alkalithermophilic bacterial consortia could decolorize Metanil Yellow G in alkaline circumstances, notably at pH 10. Lal and Chand [52] found that a newly isolated *Bacillus* strain decolorized Metanil Yellow at pH 7–8. [51] reported that *Bacillus* sp. strain Neni-10 decolorized best at pH 8. The strain's decolorization was negatively influenced by pH levels below 6 or over 9, emphasizing the

necessity of appropriate pH. Muliadi et al. Muliadi et al. (2021) used biostimulation to improve Malaysian agricultural soil microbial populations and decolorize Metanil Yellow best at pH 7.5. *Oenococcus oeni* ML34 decolorized Fast Red at pH levels around 7 according to [54].

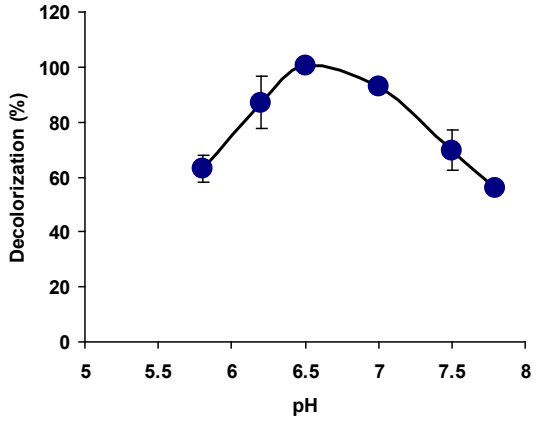

Fig. 6. Effect of pH on the Metanil Yellow-decolorizing bacterium. The experiment was replicated thrice, and error bars represent standard deviation.

Effect of NaCl concentration on decolorization of Metanil Yellow

An investigation was conducted to examine the impact of NaCl content on the efficacy of decolorizing Metanil Yellow by the bacterium. The findings suggest that the effectiveness of decolorization is affected by the concentration of salt, with the best results reported at moderate levels of NaCl. The effectiveness of the bacterium in removing color from Metanil Yellow was evaluated at various NaCl concentrations ranging from 0 to 30 g/L. The decolorization efficiency increased at low NaCl concentrations (0 to 5 g/L), reaching nearly 90% at 5 g/L. The efficiency consistently remained at a high level, ranging from 90% to 100%, at NaCl concentrations of up to 10 g/L. ANOVA analysis showed no difference in decolorizing ability between 5 and 10 g/L. The plateau indicates that the bacterial isolate exhibits a high efficacy level in decolorizing Metanil Yellow under moderately salty circumstances.

Nevertheless, when the Concentration of NaCl exceeded 10 g/L, a decrease in decolorization efficiency was noted. The efficiency reduced to around 70% at a concentration of 15 g/L and dropped to around 40% at a concentration of 20 g/L. At the maximum dose tested (30 g/L), the effectiveness of decolorization decreased to approximately 20% (**Fig. 7**). The decrease in decolorization efficacy at elevated NaCl concentrations can be ascribed to the osmotic stress caused by high salt levels, which can have a detrimental impact on bacterial metabolism and enzyme function essential for the decolorization process. High salt levels can cause cellular dehydration and hinder metabolic processes, decreasing bacterial effectiveness [64–66].

Synthetic azo dyes like Metanil Yellow decolorize depending on NaCl concentration, which optimizes biodegradation in saline wastewater. Various research has studied how different bacterial strains react to NaCl levels during Metanil Yellow decolorization. Anjaneya et al. [39] observed that *Bacillus* sp. strain AK1 and *Lysinibacillus* strain AK2 decolorized best at NaCl concentrations up to 5%. A halophilic alkalithermophilic bacterial consortia decolorized Metanil Yellow G efficiently at increased NaCl concentrations, particularly 10%, according to [34]. Lal and Chand [52] found that a newly obtained *Bacillus* strain decolorized Metanil Yellow at 3% NaCl. Mansur et al. [51] reported that *Bacillus* sp. strain Neni-10 decolorized within 24 hours at NaCl concentrations up to 4%. Muliadi et al. (2021) reported decolorization at NaCl concentrations up to 5%. *Oenococcus oeni* ML34 decolorized Fast Red at 3% NaCl concentrations, according to El Ahwany [54].

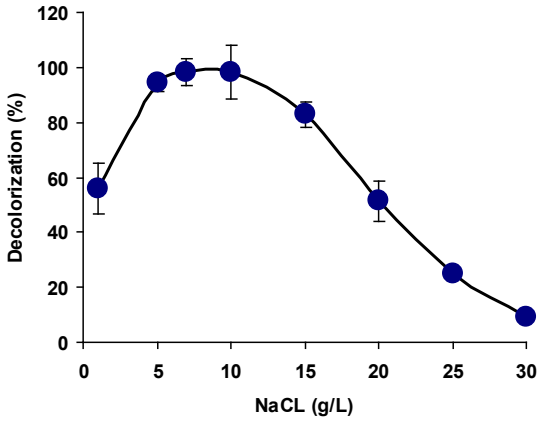

Fig. 7. Effect of salinity on the Metanil Yellow-decolorizing bacterium. The experiment was replicated thrice, and error bars represent standard deviation.

CONCLUSION

The results of this study show that the ability of the bacterial strain to remove color from Metanil Yellow is greatly affected by the initial concentration of the dye, temperature, pH, and the amount of NaCl present. The most effective decolorization occurred when the dye concentrations were below 200 mg/L, the temperatures were approximately 35°C, the pH was 6.5, and the NaCl concentrations were below 10 g/L. Decolorization efficiency significantly decreased beyond these ideal conditions, suggesting that the strain is sensitive to higher dye concentrations, extreme temperatures, non-neutral pH levels, and elevated salt content. These findings emphasize the significance of maintaining precise environmental conditions to optimize the decolorization capacity of the bacterial strain. Applying this knowledge can optimize bioremediation processes to enhance the removal of dyes in wastewater treatment, thus promoting environmental sustainability and pollution control. Future research should prioritize the investigation of the molecular mechanisms that govern the bacterial response to these conditions and the exploration of possible genetic modifications to improve the strain's ability to withstand and perform well in a broader range of conditions.

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