IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

# A Study on Optimization Conditions for Biodegradation of Methyl Orange Azo Dye Using *Pseudomonas aeruginosa*

**Vikalp Kumar[1]*, Shehwaz Anwar[2], S. Vijaya Prabhu[3], Ravindra Raut[4], Ehsan Ahmed Yahia[5]**

[1]Mohan Institute of Nursing and Paramedical Sciences, Bareilly, U. P., India

[2] Mohan Institute of Nursing and Paramedical Sciences, Bareilly, U. P., India

[3]Deapartment of Biotechnology, Microbiology and Bioinformatics, National College, Tiruchirappalli, India

[4]National Institute of Technology, Durgapur, West Bengal, India

[5]Faculty of Nursing, Cairo University, Egypt

***Abstract:*** Methyl orange poses a major risk to human and animal life and is poisonous to plants because of its refractory and carcinogenic character. The refractory nature of methyl orange makes physical and chemical methods ineffective for recovering it from industrial effluents. Biological techniques, on the other hand, have the capacity to break down these dyes due to their natural compatibility and less likelihood of having negative environmental consequences. The methyl orange azo dye found in textile effluent was broken down in the current investigation by P. aeruginosa. It was discovered during the first testing that *Pseudomonas aeruginosa* is capable of efficiently breaking down and mineralizing methyl orange. Then, the physicochemical parameters were adjusted to provide the best possible methyl orange degradation. These parameters were 37 °C, pH 7, a low salt content of 0.7 g/l, a high glucose supply of 1200 mg/ml, urea of 0.8 g/l, and a 72-hour trial period. At 5 mM concentrations, Co and Hg had a significant detrimental effect. The suggested process of total mineralization relies on spectrum data, which must be confirmed by ensnaring each stage product separately using the proper inhibitors of each enzyme through future studies.

***Index Terms*** - Methyl Orange; *Pseudomonas aeruginosa*; Bacterial Degradation; Wastewater.

## 1. Introduction

Paper, leather, and textile fibers can all be colored with azo dyes. In several other industries, such as the pharmaceutical and cosmetic ones, they are also utilized (Benkhaya et al., 2020). Azo dyes have higher thermal, chemical, and detergent stability than natural colors. Microbes may readily break them down since they are inexpensive, widely accessible, colorful, and easily degraded. These qualities have significantly expanded their use globally (Pinheiro et al., 2022). The textile industry uses thousands of tons of azo dyes yearly, and thousands of tons of those colors are released into the land and water each year in their unmodified state (Zafar et al., 2022; Kumar et al., 2024; Anwar et al., 2024).

Globally, the textile sector uses over 70% of azo dyes. The breakdown and mineralization of methyl orange are accomplished by a variety of techniques, including physical, chemical, and biological ones (Abdulmohsen et al., 2022; Kumar et al., 2024). Taking into account that the physical and chemical methods provide excess sludge that requires additional processing and are only effective for decolorization of colors;

they cannot be used for dye breakdown. Conversely, the application of biological techniques is economical, environmentally benign, and results in no sludge (Ngo and Tischler, 2022).

Synthetic anionic azo dye methyl orange is sulfonated, heterocyclic, organic, and very soluble in water (Kishor et al., 2021). When applied to living things, including humans, methyl orange refractory dye can induce dermatitis, hypersensitivity reactions, lung cancer, and intestine cancer (Ikram et al., 2022; Khan et al., 2022). Human and animal studies have already established its high toxicity, teratogenicity, carcinogenicity, and mutagenicity. By raising soil salinity, it also has the unintended consequence of lowering agricultural yields, soil fertility, and plant biodiversity (Haider et al., 2022). As a result, methyl orange dye must be properly treated before being dumped into soil and water (Khan et al., 2022).

Profusely growing both aerobically and anaerobically, *Pseudomonas aeruginosa (P. aeruginosa)* is a facultative bacteria that may proliferate quickly in water tainted with fluorescent dyes. *P. aeruginosa* has a notable capacity to degrade methyl red, as our earlier article has reported (Anwar et al., 2024). Therefore, *P. aeruginosa* was first used to investigate the break down the methyl orange dye for confirmation of remediation capability of *P. aeruginosa*. In addition, the impact of numerous physicochemical variables on dye degradation using *P. aeruginosa* was investigated to identify the optimum conditions leading to a high degree of dye breakdown.

## 2. Material and methods

### 2.1. Dye and Other Reagents

The methyl orange textile azo dye was supplied by a textile company located in Delhi. It has been observed that the nutritious broth, sodium hydroxide, glucose, n-hexane, ethyl acetate, hydrochloric acid, redox mediators, and other chemical reagents are of outstanding analytical grade and quality. The final batch of chemical reagents was manufactured by Sigma Aldrich in Germany.

### 2.2. Preparation of Dye Solution

Five hundred milliliters of distilled water and one gram of methyl orange dye were used to make the stock solution. Using stock solution allowed the dye solutions to reach their desired concentrations of 100–800 mg/l. Since azo dyes become unstable when sterilized with moisture and heat, the dyestuff solution was filtered using a 0.22 µm membrane filter (Anwar et al., 2024).

### 2.3. Conditions of medium and culture

A liter of distilled water was used as the base medium, and the contents were as follows: 4 g $K_2HPO_4$; 4 g $KH_2PO_4$; 2 g $(NH_4)_2SO_4$; 0.5 g $MgSO_4 \cdot 7H_2O$; 0.01 g $CaCl_2$; and 0.01 g $FeSO_4 \cdot 7H_2O$. In 250 ml Erlenmeyer flasks, 100 ml of MSM, 0.1% glucose, 0.4% yeast extract, and 50 mg/ml dye were added for most of the assays.

### 2.4 Bacterial Types

Using methyl orange dye, the efficiency of P. aeruginosa for biological degradation was investigated. Our acquaintance who works at Aligarh Muslim University in India provided us with the cultures listed above.

### 2.5. The impact of dye concentration on biodegradation

The selected strain was grown in eight experiment tubes with 10 ml of nutrient broth for the full day in order to figure out the effect of dye concentration on breakdown. When the colony had grown, 5 ml of methyl orange solution (100–800 mg/l) was added to the test tubes. Eight solutions (controls) were prepared in the same manner for each concentration, using 10 milliliters of nutritious broth and just five milliliters of dye solution. The culture mixture with the products that had broken down due to dye was centrifuged for a period of ten minutes at a rate of 10,000 rpm after being preserved at room temperature for three days.

The substance was filtered via filter paper with a pore size of 0.2 µm. The obtained residual mixture's finalized absorbance wavelength has been determined as 430 nm via an ultraviolet-visible spectrophotometer. The following formula was used to get the maximum decolorization percentage (%) at 430 nm for each sample of dye after six days of incubation (Abdulmohsen et al., 2022).

$$\% \text{ Decolorization} = (\text{Initial absorbance} - \text{Final absorbance})/\text{Initial absorbance} \times 100$$

**2.6. Effect of pH on dye biodegradation**

A fixed concentration of methyl orange (50 mg/l) in 50 ml of MSM treated with 0.1% glucose and 0.4% yeast extract was investigated under the stable conditions at a temperature of 30 °C for 24 hours in order to determine just how a range of pH levels (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) altered the decolorization process. *P. aeruginosa* was transferred into 10 experiment tubes which include sterile nutritive broth, all of which was allowed to incubate at 37 °C within the incubation chamber. The experiment was designed because bacteria can survive under a specific pH, only. Each of the experiment tubes had been filled with five milliliters of the dye solution from the initial solution when the cell of the bacterial culture had achieved its full extent, which took twenty-four hours (Abdulmohsen et al., 2022; Anwar et al., 2024).

Control solutions have also been prepared as a reference. The pH levels of the control solutions as well as inoculation tubes have been modified by 1 M HCl solution and 1 M NaOH solution. Using pH indicator strips (Merck KGaA Darmstadt, Germany), the pH levels have been modified by introducing a little volume of base or acid by using a micropipette within the corresponding tubes. Filter paper having a particle size of 0.2 µm had been used for filtering the residue left over after centrifugation. A UV-Visible spectrophotometer and a previous technique have been applied in order to determine the percentage of degradation in the residue from the experiment which had been removed after three days (Anwar et al., 2024).

**2.7. Impact of temperature on dye biodegradation**

A total of 10 test tubes containing 10 ml of solution had been contaminated by the selected culture for the purpose to figure out how temperature affected the methyl orange degradation mechanism. After the bacteria developed, an additional five milliliters of the 40 ppm methyl orange stock solution has been added into every experiment tube. A control solution was also developed, containing 10 ml of nourishing broth and 5 ml of methyl orange. The incubator was to be used to incubate test tubes at 19, 22, 25, 28, 31, 34, 37, 40, 43, and 46 degrees Celsius. The degrading samples have been centrifuged in examination tubes for 10 minutes at 10,000 rpm after having a three-day break. All of the samples were subjected to filtering through a subsequent step by using filter paper that had a 0.2 µm size. This process was performed in order to determine the growth rate of the decolorization process (Abdulmohsen et al., 2022; Anwar et al., 2024).

**2.8. Effect of glucose on dye biodegradation**

Both huge quantities of chemical energy along with carbon, or carbon atoms, have been provided via glucose to bacteria. After a day, 5 ml of the dye solution and a range of concentrations of glucose (100-1200 mg/l) were introduced to every experimental tube having the dye solution. The bacteria were grown in test tubes using nutrient broth medium at 37°C. For all concentrations of glucose and control solutions containing 10 ml of medium and 5 ml of dye solution were additionally added. After centrifugation and filtration by 0.2 µm filter paper, the deteriorating sample was subjected to analysis using UV-visible spectrophotometry. Using the same procedure as in equation (1), the percent decolorization of each tube was determined (Abdulmohsen et al., 2022; Anwar et al., 2024).

**2.9. Impact of incubation time on dye biodegradation**

A bacterial culture was put into a large test tube which contained 30 milliliters of nutritious broth. The tube that contained the broth was then allowed to incubate for a full day. After the culture was established, 15 ml of the dye solution had been introduced. From the first day to the fifteenth, the percentage of dye degradation had been recorded. A control solution with 10 ml of medium and 5 ml of dye only has been created for reference. For the duration of fifteen days, the percentage degradation rate has been determined by the UV-visible spectrophotometer (Anwar et al., 2024).

**2.10. Effect of sodium chloride on dye biodegradation**

Sodium chloride salt alters the dye degradation due to its unique ability to increase the overall salinity of seawater. Most colors and pollutants become decomposed under ideal salinity conditions. A volume of 5 ml had been introduced to examine tubes carrying the *P. aeruginosa* culture which had been cultured. Each of the test tubes which were found to have been contaminated had an extra dose of sodium chloride, varied in the dosage from 100 to 1400 mg/l. For each concentration of sodium chloride, control solutions have also been developed. The percentage of decolorization has been determined by the obtained residue after centrifugation. For effectively breakdown the wastewater that has been treated before being

discharged within the natural environment and its acceptance to recycling, yet additional research needs to be conducted (Anwar et al., 2024).

### 2.11. Effects of heavy metal ions on dye decolorization

Specific metal ions which include Hg ($HgCl_2$), Zn ($ZnSO_4$), Mg ($MnCl_2$), Co ($CoCl_2$), and Mg ($MgCl_2$) have been studied under concentrations ranging from 1 and 5 mM in order to investigate the effect that they had upon degradation capability. The cell suspension had been cultured using metal ions that came from stock solutions for 15 minutes before flasks that contained the dye had been added (Anwar et al., 2024).

### 2.12. Statistical analysis

For the statistical analysis and multifactorial design drawings, Minitab software (Version 17) had been applied. For most statistical investigations, Graph Pad Prism 5 software had been employed, with the use of Dunnett's multiple comparisons post-test at P-value < 0.05 and one method.

## 3. Results

### 3.1. Effect of dye concentrations on bacterial decolorization: Bio-decolorization study and UV–visible spectral analysis

Figure 1 illustrates the association among the dye dosage of mg/l and the dye-degrading property of *P. aeruginosa*. The highest methyl orange degradation has been observed at a dye solution concentration of 100 mg/ml. Once it had been achieved, the capacity to remove the colors eventually reduced. When the dye concentration crosses 100 mg/l, lesser dye has to be broken down through bacteria due to high dye concentrations. This high concentration slows down the growth of bacteria. Therefore, it can be recommended to apply moderate dye concentrations as much as possible when researching degradation of dyes by *P. aeruginosa*.

A relationship among methyl orange metabolites along with elevated dye concentration and the enzyme's active site of azoreductases has been suggested by Khan and colleagues. Additionally, interactions between the azoreductases active site, the elevated levels of dyes, and methyl orange metabolites could also be responsible for the breakdown of dyes. Thus, bacteria may have been capable to break down more dye at less concentration than at greater quantities (Khan et al., 2021).

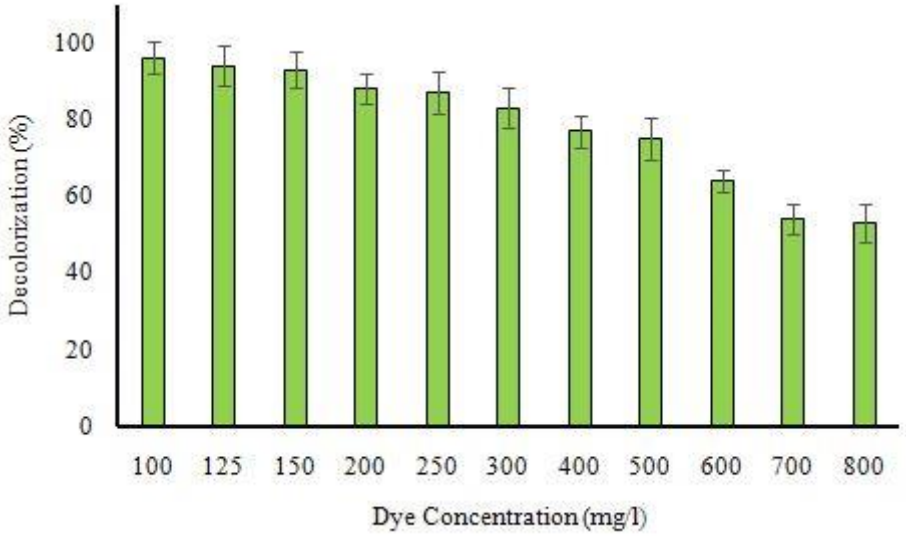


Figure 1- Effect of dye concentration on dye decolorization

### 3.2. Impact of pH on dye biodegradation

Figure 2 demonstrates the breakdown of methyl orange by *P. aeruginosa* when subjected to a range of pH values. The highest dye degradation was seen at a pH of 7. Variations in the pH level of a solution from 7 to less than 7 minimize the efficacy of bacteria to degradation of dyes. Both above and below a normal pH, the growth of bacteria starts to be decreased, that leads to lesser degradation of dye above and below the normal pH. The results we obtained have been confirmed by previous investigations on the degradation of azo dyes at a neutral pH level (Anwar et al., 2024a).

The decolorization capability stayed essentially constant under an alkaline pH. When the pH was below 6, strain does not respond properly to the methyl orange degradation. These strains have been found to be effective in dehydration across a wide pH range of 6-10 (Figure 2). The pH of textile effluents fluctuates from acidic to alkaline as a result of the introduction of acids and salts around the procedure of dyeing, which results in this property highly valuable. The findings show that extreme alkaline and acidic conditions had an impact on bacterial growth and enzymatic activity, and falling at lower pH levels. Alkaline conditions with a pH of 6 to 10 are usually used to decolorize dyes (Saratale et al., 2011).

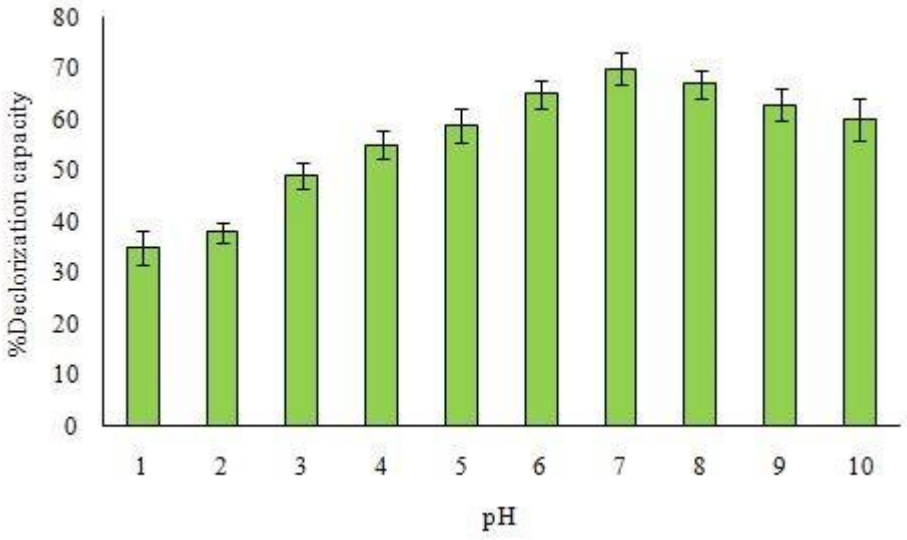


Fig 2- Effect of pH on dye decolorization

### 3.3. Impact of temperature on biodegradation of dye

An appropriate temperature is essential for bacterial development. Figure 3 shows the effect of variations in temperature on the degradation of methyl orange by bacteria. A peak performance azo dye decolorization or degradation has been achieved along 37 °C, or thereabouts, while the proliferation of bacteria seems to have achieved its maximum level. The rate of dye biodegradation decreases above as well as below the recommended temperature as a result of an overall decrease in bacterial proliferation. As temperatures fluctuate either towards or away from the preferred range, the development of bacteria and azoreductases performance simultaneously diminishes.

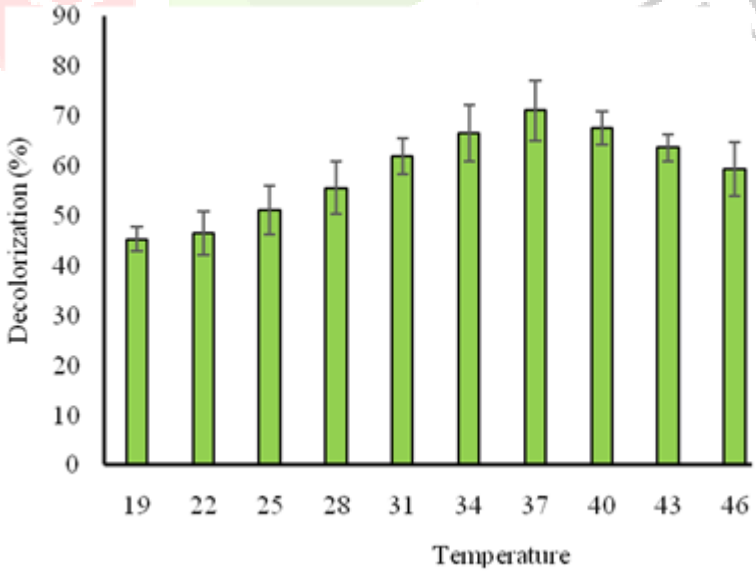


Figure 3- Effect of temperature on % decolorization

### 3.4. Impact of glucose concentration on dye biodegradation

Glucose is a vital and important source of carbon for bacteria. Additions of carbon cause an increase in the biomass of bacteria. This leads to the dye's biodegradation rate to rise. A large number of microorganisms are required to break down certain azo dyes due to their complicated structures. The final result turned out to be the supply of carbon has accelerated the rate of bacterial growth and development. Figure 4 demonstrates the degradation of methyl orange by *P. aeruginosa* under various concentrations of the glucose that functions as an important source of carbon to bacteria. Our results show that whenever the glucose content gets higher, the rate of dye breakdown was continuously accelerated. Due to the complicated chemical structures of methyl orange dyes, a number of bacteria have to be present to facilitate their degradation. As a consequence, the supply of carbon has accelerated the rate of bacterial growth and development.

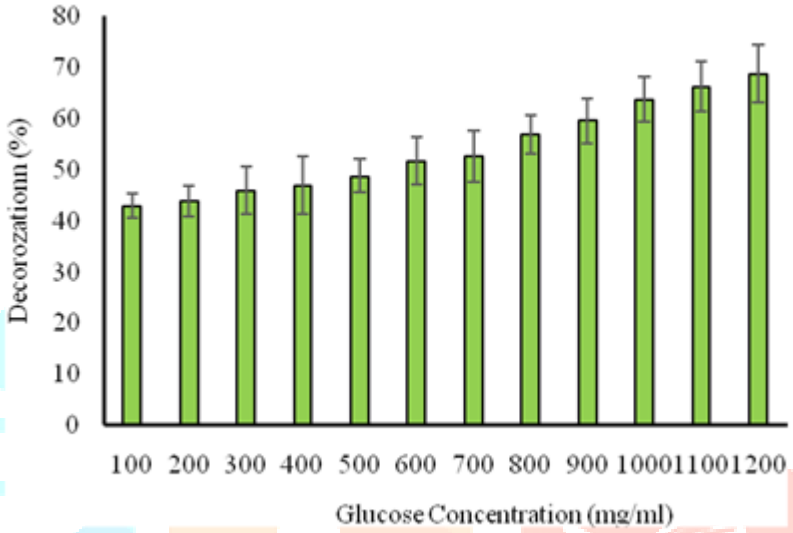


Figure 4- Effect of glucose concentration on % decolorization

### 3.5. Impact of Urea concentration on dye biodegradation

A bacterial cell needs an abundance of urea in order to breakdown the dye so that they can make use of the substance as an important source of nitrogen. Figure 5 demonstrates the manner in which *P. aeruginosa*'s rate of methyl orange degradation potential is influenced by urea content. At 800 mg/l, a significant rate of the breakdown (70.23%) had been observed with respect to the given dye. The degradation efficiency diminished due to triggered toxicity when the urea content becomes raised. Whenever urea concentrations became substantially increased, the rate of degradation efficiency reduced as a result of a rise in urea concentration along with toxicity.

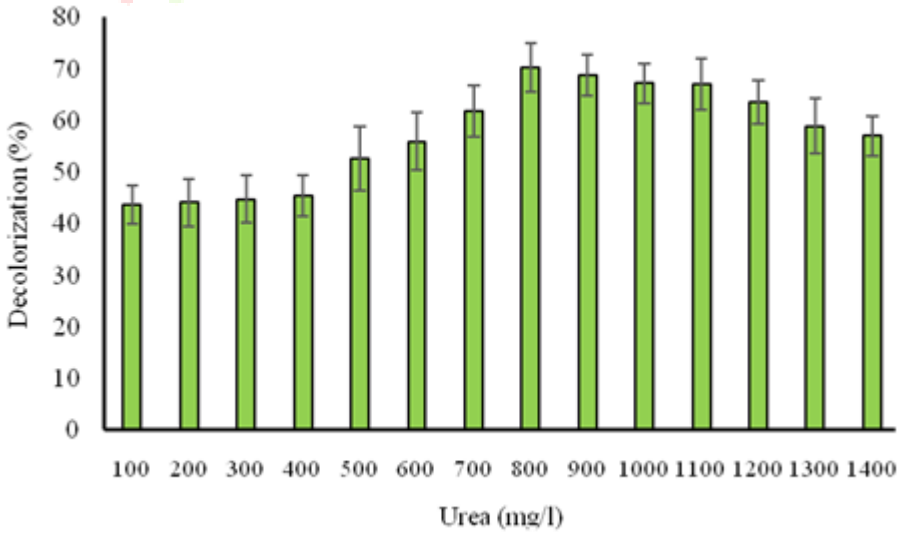


Figure 5- Effect of urea concentration on % decolorization

### 3.6. Impact of incubation time on dye biodegradation

Time affects dye degradation considerably because the rate of bacterial growth is time-dependent. Excellent proliferation of bacteria and a sufficient period lead to in huge amounts of biomass, which will eventually encourage a high rate of degradation. According to such standards *P. aeruginosa* is capable of developing under circumstances having minimal nutrition, yet degradation may occur after an interval of 28 days. This species can survive and grow over a hundred days or even longer within water.

After each 24-hour period of incubation, 10 ml of samples had been centrifuged for 20 minutes at 5000 rpm in order to separate the resulting pellets and the residue. The remaining liquid had been employed to figure out the percentage of degradation by means of a UV/visible spectrophotometer. Because there was no noticeable rise observed after 6 days, the rate of degradation was determined each 3 days for a maximum duration of 21 days. Its highest possible level of degradation had been observed after three consecutive days of incubation (Figure 6). After these three days, there was little declining condition. We are becoming nearer to reaching the stationery and mortality stage, which happens to be the most possible reason behind this.

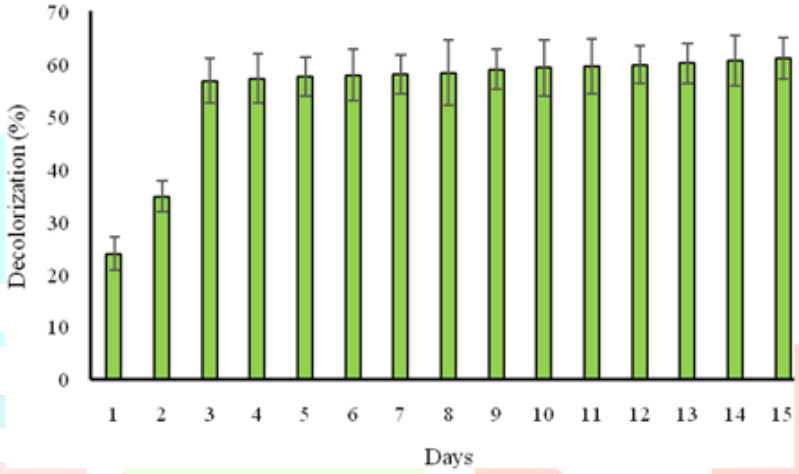


Figure 6. Impact of incubation time (days) on % decolorization.

### 3.7. Effect of salinity (sodium chloride) on dye biodegradation

Sodium chloride (NaCl), one of the fundamental components of water from the sea, may influence how bacteria breakdown dyes. To examine the effect of salt content, 14 test tubes containing 100-1400 mg/ml of NaCl were prepared that underwent incubation for three days at 37 °C. Formula 1 has been applied to figure out the rate of dye degradation during three days of incubation. *P. aeruginosa* has been demonstrated to breakdown down methyl orange at the highest rate of 70.16% under a salt concentration of 700 mg/l. Plasmolysis, the process that bacteria encounter under highly salty environments, inhibits their growth as well as a result, decreases their ability for degradation substances (Ikram et al., 2022) (Figure7).

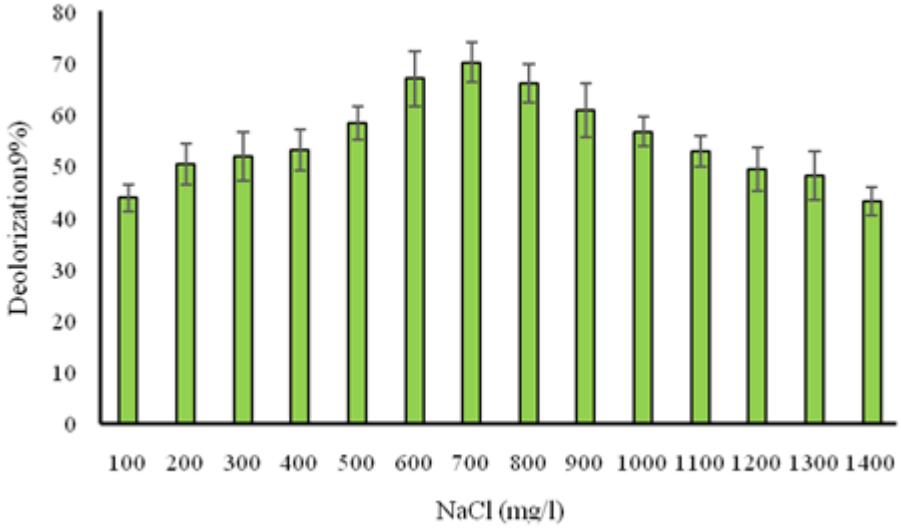


Figure 7- Effect of NaCl concentration on % decolorization

### 3.8. Impact of presence of heavy metal ions on dye biodegradation

Considering the high incidence of contamination by heavy metals in textile pollutants, these impose quite extremely high risk. A few metal ions are possibly helpful for bacteria, while other kinds of metal ions may be harmful. Because heavy metals bind with protein components and form complexes, they may have immediate impacts upon proteins as well as enzymes (Balali-Mood et al., 2021). We consequently investigated the impact of a number of metal ions at 1 and 5 mM concentrations on *P. aeruginosa*'s efficiency to degrade. Only Mn has a little triggering effect on degradation efficiency, as illustrated by Figure 8. Among all of the substances which had been studied, zinc demonstrated the highest level of inhibition at both concentrations. At 5 mM concentrations, Co and Hg likewise demonstrated a substantial harmful effect. The effects of magnesium were not statistically significant on decolorization of methyl orange (Figure 8).

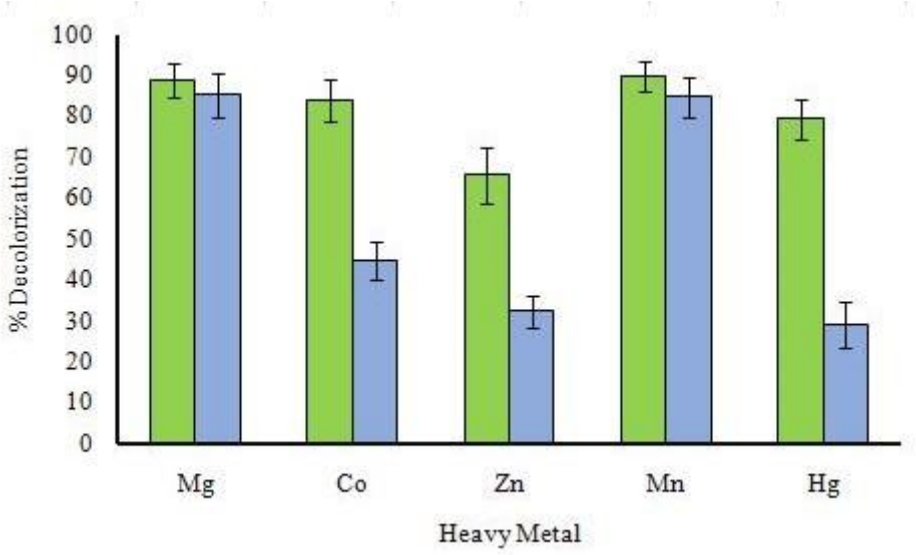


Figure 8. Effect of presence of metal ions on decolorization of methyl orange

## 4. Discussion

Because dyes are tenacious chemicals that defy biodegradation, the amount of pollution created by dye waste is rapidly rising daily. At the moment, dyes and how to remove their color are of great scientific interest (Ismail et al., 2019). In several distinct industries which include textiles, leather, dyeing, as well as paper, methyl orange dye is commonly used. As a pH indicator, methyl orange is widely utilized in research labs (Khan et al., 2022). In the absence of proper remediation, the immediate release of methyl orange in both water and soil sources offers an imminent danger to not only people in addition to organisms but to all life on land and in water (Wu et al., 2021). Methyl orange, even in low concentrations, blocks light from entering deep water, resulting in an adverse effect on photosynthesis (Arshadi et al., 2016). Additionally, it impacts gaseous solubility in water, and this is essential to aquatic life for survival (Haidar et al., 2022).

According to Kovacic and Somanathan (2014), dye toxicity may be attributed to electron transport, reactive oxygen species, and oxidative stress (Kovacic and Somanathan, 2014). The imbalance between the generation and removal of reactive oxygen species is the cause of oxidative stress (Rahmani et al., 2023; Rahmani et al., 2022; Younus and Anwar, 2018). The body constantly produces reactive oxygen species, which are extremely reactive chemical species that contain oxygen and are a product of cell metabolism (Anwar et al. 2023; Anwar et al., 2022a; Anwar et al., 2022b). Superoxide dismutase is an important antioxidant defence molecule of body (Rahmani et al., 2022; Younus and Anwar, 2018; Younus, 2018). The activity of superoxide dismutase was reported to be affected by methyl orange (Yildirim and Yaman, 2019). In addition, the changes in lipid peroxidation, detoxification and antioxidant enzymes, and other biomarkers may be employed as sensitive indicators to evaluate the environmental danger of methyl orange (Yildirim and Yaman, 2019). Many times, antioxidants stop or lessen toxicity (Kovacic and Somanathan, 2008; Tan et al., 2018). Oxidative stress causes a significant damage to important biomolecules (Anwar et al., 2020).

In a variety of industries, including the paper printing, rubber, grocery, and cosmetic product sectors as well as infrastructure, synthetic coloring agents, such as dyes, are widely employed (Guerra et al., 2018; Mohan and Sharma, 2022). A class of synthetic dyes known as "azo dyes" is widely employed as a vital component in the textile production sector (Reza et al., 2019; Alsukaibi, 2022). It has already been

demonstrated that azo dyes are hepatotoxic (Hussain et al., 2022).) The liver is a vital organ of the body (Yahia and Anwar, 2020). Thus, azo dye's hepatotoxicity can pose a serious hazard to life. An investigation assessed the toxicity of the azo dyes disperse orange 1 (DO1), disperse red 1 (DR1), and disperse red 13 (DR13) in HepG2 cells cultured in three dimensions (3D) or monolayers. Following 24, 48, and 72 hours of incubation of cells with 3 different doses of the azo dyes, the hepatotoxicity of the dyes was assessed using 3-(4,5-dimethylthiazol-2yl)2,5-diphenyltetrazolium (MTT) and cell counting kit 8 (CCK-8) assays. The outcomes unequivocally showed that HepG2 cells were cytotoxically exposed to the dyes under study (Ferraz et al., 2012).

Long term exposure of methyl orange may contribute to liver, heart and kidney damage. Besides, repeated skin contact may cause dermatitis (Australian Chemical Reagents- Safety data sheet 2023: https://www.chemsupply.com.au/uploads/sds/0219.pdf).

When using biological techniques, bacteria having rapid growth cycles usually among the most appropriate organisms for use because they produce huge amounts of biomass (Mauerhofer et al., 2022), which is essential for the proper degradation process (Casau et al., 2022). Bioremediation (Butani et al., 2013) is an environmentally friendly method that has been used to break down dyes; the microorganism's ability to adapt and act is what makes it effective. There have been numerous reports of microorganisms engaged in the degradation of dyes, including bacteria, fungi, algae, and plants (Ali, 2010; Srinivasan and Viraraghavan, 2010). Out of all of them, bacteria turned out to be a good option because they grow in less expensive sources, have a short incubation period, and produce less sludge. Bacterial degradation is much preferable over fungal degradation since it has a longer incubation period and is less durable at high temperatures, which limits its use in dye decolorization (Thangaraj et al, 2021). Certain types of algae, fungus, and bacteria may be able to decolorize wastewater from textiles more effectively than most other costly methods by utilizing enzymes including azoreductase, laccase, and peroxidase (Das et al., 2023).

In addition, bacteria tend to be better adapted for breaking down azo dyes in comparison to fungus, algae, or plants because they are capable of withstanding adverse circumstances in a wide range of environments even having an extremely fast rate of reproduction (Lellis et al., 2019). Enzymes which are capable of breaking and mineralize azo dyes include azoreductases, peroxidases, and lasses, which are released by bacteria (Chen, 2006). Methyl orange's breakdown and degradation have been considered the subject of numerous academic investigations (Ibrahim et al., 2022). It has previously been documented that biological enzyme systems break down and decolorize dyes with extraordinary efficacy. They are also significantly less expensive and useful in natural settings (Yaseen and Scholz, 2019).

The main goal of textile dye biodegradation is color removal, but it also changes dangerous textile dyes into less dangerous forms that are safe to discharge into the environment. It has apparently been demonstrated that a variety of bacterial strains are able to decolorize the dyes. The quantities of various dyes that are stained by various bacterial strains can be changed because each dye has a unique structure and degree of complexity (Kamal et al., 2022).

Although the extent of methyl orange degradation has already been documented in previous investigations, no study has been conducted upon improving the factors which contribute to a higher rate of methyl orange degradation by *P. aeruginosa*. That is why we performed this research study in order to determine how our developed technique may have been maintained in extremely salinized circumstances. In accordance with the current research, the most suitable pH along with temperature for *P. aeruginosa* to successfully break down the dye methyl orange within the medium used for culture has been determined to be 7.0 and 37°C, respectively. As the temperature increased, the bacterial cells grew more rapidly while the methyl orange dye degradation maximum at 37°. Current research suggests that 100 mg/l dye concentration, 1200 mg/l glucose concentration, 700 mg/l sodium chloride concentration, pH 7, temperature 37 °C, 800 mg/l urea concentration, and a 3-day period of incubation are the ideal parameters for methyl orange degradation. These findings might be due to temperature-related increases in growth and enzyme activity which previous researchers have observed (Ikram et al., 2022).

Continued discoloration of methyl orange has been demonstrated to be severely impeded with additional temperature increase. The bacterium discolored methyl orange most effectively under 100 mg/l of dye concentration. As the concentration of dye got increased, the rate of discoloration dropped. This can have happened as a consequence of the dye's adverse effects that interact with the processes of metabolism (Kamal et al., 2022).

Because they include pollutants like heavy metals, textile wastewaters can be particularly dangerous. According to our research, all of the metal ions exhibit adverse effects on bacteria, although some of them can even encourage the growth of these particular bacteria. This study demonstrates that, at a 5 mM concentration, heavy metals can have a negative impact on the rate of decolorization procedure. On the

other hand, heavy metals can also affect bacterial cells in a number of ways, including altering the functioning of enzymes, severely reducing growth, and inhibiting replication (Aljerf and AlMasri, 2018). The efficiency of discoloration may be diminished by the existence of contaminants such as heavy metals (Velusamy et al., 2021).

Our research helps to advance the knowledge of the ideal conditions for significant discoloration of effluent having azo dye as contaminants and supports the results of previous research focusing on *P. aeruginosa's* efficiency against dyes. The work we conducted shows proof for the validity of previous investigations on *P. aeruginosa*'s performance against methyl orange dye along with throws more light on the most suitable circumstances enabling a significant discoloration of effluent including the azo dye as pollutants.

## 5. Conclusion

*P. aeruginosa* has the capacity to survive and grow under both aerobic and inert environments, entirely breaking down the heterocyclic portion of dye molecule. The efficacy of the discoloration process, which begins via the enzymatic decrease in azo interactions, depends upon glucose and amino acid supply. Methyl orange appears to be discolored by *P. aeruginosa* might be through a full breakdown of the azo bond, leading to in the synthesis of two aromatic amines, which are followed by further azo bond breakdown. This investigation was conducted to assess *P. aeruginosa*'s capability to biodegrade methyl orange. The chosen bacterial strain degraded the methyl orange textile azo dye more satisfactorily. Through one-at-a-time factor optimization, the optimum circumstances have been found to be that of the following: a three-day incubation period; a temperature of 37 °C; a dye concentration of 100 mg/l; a pH of 7; maximum breaking down of methyl orange was affected by the ideal parameters for pH, incubation period, temperature, and dye concentration. Our research suggested *P. aeruginosa*'s methyl orange discoloring ability across an extensive variety of dye concentrations, pH levels, salt concentrations, and temperatures. This capacity may make it feasible options for effluents from the textile industries.

**Funding:** None.

**Conflict of interest:** The authors declare no conflict of interest.

**Author contributions-** Conceptualization: S. A., V. K., E. A. Y.; Experimental part: S. A., V. K., R. R., S. V. P.; Writing and Editing- S. A., V. K., S. V. P., R. R., E. A.Y.; Final draft- S. A., V. K., E. A. Y., S. V. P., R. R.

**Acknowledgement:** The authors are very thankful to Mr. S. D. Maurya, Chairman, Mohan Group of Nursing, Pharmacy and Paramedical Sciences for their support and providing facilities for laboratory working and internet facility.

## Reference

1. Abdulmohsen KDA, Alimi FR, Mechi L, Ahmed A O, Asma K A A A, Eida Mohammad A, Ali Khan MW. Optimization of *Pseudomonas aeruginosa* isolated for bioremediation from Ha'il region of Saudi Arabia. Bioinformation. 2023 Sep 30;19(9):893-900. doi: 10.6026/97320630019893. PMID: 37928487; PMCID: PMC10625362.

2. Ali, H., 2010. Biodegradation of synthetic dyes—a review. Water Air Soil Pollut. 213, 251–273.

3. Aljerf L, AlMasri N. A Gateway to Metal Resistance: Bacterial Response to Heavy Metal Toxicity inthe Biological Environment. Ann Adv Chem. 2018; 2: 032-044.https://doi.org/10.29328/journal.aac.1001012.

4. Alsukaibi AKD. Various Approaches for the Detoxification of Toxic Dyes in Wastewater. *Processes*. 2022; 10(10):1968. https://doi.org/10.3390/pr10101968

5. Anwar S, Almatroudi A, Alsahli MA, Khan MA, Khan AA, Rahmani AH. Natural Products: Implication in Cancer Prevention and Treatment through Modulating Various Biological Activities.Anticancer Agents Med Chem. 2020;20(17):2025-2040. doi: 10.2174/1871520620666200705220307.PMID: 32628596.

6. Anwar, S., Raut, R., Kanwal, B., Yahiya, E.A. Vitamin B12 Deficiency And Increased Susceptibility And Severity Of Covid-19: A Review. IJCRT. 2022a, 10, 8, b623-b651

7. Anwar S, Kumar V, Yahia EA, Prabhu SV. Optimization of Various Factors Affecting Pseudomonas aeruginosa Mediated Removal of Azo Dye. IJCRT, 2024, 12(1), 538-549.

8. Anwar S, Kumar V, Kanwal B, Yahia EA. (2023). A Review on Utilization of Ashoka Plant in Oxidative Stress Induced Women Reproductive Health Complications. IJCRT, 11(9), f249-f258.

9. Anwar S, Raut R, Kanwal B, Yahia EA. Kumar V, (2022b). In Vitro Investigation of Anti-inflammatory and Antioxidant Activities of Curcuma Longa Rhizome Methanol Extract. IJCRT, 10(10), c559-c581.

10. Arshadi, M., Abdolmaleki, M.K., Mousavinia, F., Khalafi-Nezhad, A., Firouzabadi, H., Gil, A. Degradation of methyl orange by heterogeneous Fenton-like oxidation on a nano-organometallic compound in the presence of multi-walled carbon nanotubes. Chemical Engineering Research and Design. 2016, 112, 113-121.

11. Australian Chemical Reagents Safety data sheet, 2023, https://www.chemsupply.com.au/uploads/sds/0219.pdf.

12. Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR and Sadeghi M (2021) Toxic Mechanisms ofFive Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. Front. Pharmacol. 12:643972.doi: 10.3389/fphar.2021.643972.

13. Benkhaya S, M'rabet S, El Harfi A. Classifications, properties, recent synthesis and applications of azo dyes. Heliyon. 2020 Jan 31;6(1):e03271. doi: 10.1016/j.heliyon.2020.e03271. PMID: 32042981; PMCID: PMC7002841.

14. Butani, N., Jobanputra, J., Bhatiya, P., Pater, R., 2013. Recent biological technologies for textile effluent treatment. Int. Res. J. Biol. Sci. 2 (6), 77–82.

15. Casau M, Dias MF, Matias JCO, Nunes LJR. Residual Biomass: A Comprehensive Review on the Importance, Uses and Potential in a Circular Bioeconomy Approach. *Resources*. 2022; 11(4):35. https://doi.org/10.3390/resources11040035.

16. Chen H. Recent advances in azo dye degrading enzyme research. Curr Protein Pept Sci. 2006 Apr;7(2):101-11. doi: 10.2174/138920306776359786. PMID: 16611136; PMCID: PMC5863238.

17. Das S, Cherwoo L, Singh R. Decoding dye degradation: Microbial remediation of textile industry effluents. Biotechnology Notes. 2023 Oct 26.

18. Ferraz ER, Li Z, Boubriak O, de Oliveira DP. Hepatotoxicity assessment of the azo dyes disperse orange 1 (DO1), disperse red 1 (DR1) and disperse red 13 (DR13) in HEPG2 cells. J Toxicol Environ Health A. 2012;75(16-17):991-9. doi: 10.1080/15287394.2012.696513. PMID: 22852849.

19. Guerra E, Llompart M, Garcia-Jares C. Analysis of Dyes in Cosmetics: Challenges and Recent Developments. *Cosmetics*. 2018; 5(3):47. https://doi.org/10.3390/cosmetics5030047

20. Haider FU, Wang X, Zulfiqar U, Farooq M, Hussain S, Mehmood T, Naveed M, Li Y, Liqun C, Saeed Q, Ahmad I. Biochar application for remediation of organic toxic pollutants in contaminated soils; An update. Ecotoxicology and Environmental Safety. 2022 Dec 15;248:114322.

21. Ibrahim A, El-Fakharany EM, Abu-Serie MM, ElKady MF, Eltarahony M. Methyl Orange Biodegradation by Immobilized Consortium Microspheres: Experimental Design Approach, Toxicity Study and Bioaugmentation Potential. Biology (Basel). 2022 Jan 5;11(1):76. doi: 10.3390/biology11010076. PMID: 35053074; PMCID: PMC8772785.

22. Ikram M, Naeem M, Zahoor M, Rahim A, Hanafiah MM, Oyekanmi AA, Shah AB, Mahnashi MH, AlAli A, Jalal NA, Bantun F, Sadiq A. Biodegradation of Azo Dye Methyl Red by

Pseudomonas aeruginosa: Optimization of Process Conditions. Int J Environ Res Public Health. 2022 Aug 12;19(16):9962. doi: 10.3390/ijerph19169962.

23. Ismail, M., Gul, S, Khan, M.I., Khan, M. A., Asiri, A. M., Khan, S. B. "*Medicago polymorpha*-mediated antibacterial silver nanoparticles in the reduction of methyl orange" *Green Processing and Synthesis*, vol. 8, no. 1, 2019, pp. 118-127. https://doi.org/10.1515/gps-2018-0030

24. Kamal IM, Abdeltawab NF, Ragab YM, Farag MA, Ramadan MA. Biodegradation, Decolorization,and Detoxification of Di-Azo Dye Direct Red 81 by Halotolerant, Alkali-Thermo-Tolerant Bacterial Mixed Cultures. Microorganisms. 2022 May 9;10(5):994. doi: 10.3390/microorganisms10050994.PMID: 35630437; PMCID: PMC9147255

25. Khan AU, Zahoor M, Rehman MU, Shah AB, Zekker I, Khan FA, Ullah R, Albadrani GM, Bayram R, Mohamed HRH. Biological Mineralization of Methyl Orange by *Pseudomonas aeruginosa*. *Water*. 2022; 14(10):1551. https://doi.org/10.3390/w14101551.

26. Kishor R, Purchase D, Saratale GD, Ferreira LF, Hussain CM, Mulla SI, Bharagava RN. Degradation mechanism and toxicity reduction of methyl orange dye by a newly isolated bacterium Pseudomonas aeruginosa MZ520730. Journal of Water Process Engineering. 2021 Oct 1;43:102300.

27. Kumar V, Anwar S, Prabhu SV. A Discussion On Physiochemical And Biomediated Removal Approaches Of Dyes From Textile Effluents: A Review. IJCRT. 2024, 12(2), 62-75.

28. Kovacic P, Somanathan R. Integrated approach to immunotoxicity: electron transfer, reactive oxygen species, antioxidants, cell signaling, and receptors. J Recept Signal Transduct Res. 2008;28(4):323-46. doi: 10.1080/10799890802305217. PMID: 18702007.

29. Kovacic P, Somanathan R. Toxicity of imine-iminium dyes and pigments: electron transfer, radicals, oxidative stress and other physiological effects. J Appl Toxicol. 2014 Aug;34(8):825-34. doi: 10.1002/jat.3005. Epub 2014 May 22. PMID: 24852913.

30. Lellis B, Fávaro-Polonio CZ, Pamphile JA, Polonio JC. Effects of textile dyes on health and the environment and bioremediation potential of living organisms. Biotechnology Research and Innovation. 2019 Jul 1;3(2):275-90.

31. Mauerhofer LM, Pappenreiter P, Paulik C, Seifert AH, Bernacchi S, Rittmann SKR. Methods for quantification of growth and productivity in anaerobic microbiology and biotechnology. Folia Microbiol (Praha). 2019 May;64(3):321-360. doi: 10.1007/s12223-018-0658-4. Epub 2018 Nov 16. PMID: 30446943; PMCID: PMC6529396.

32. Ngo ACR, Tischler D. Microbial Degradation of Azo Dyes: Approaches and Prospects for a Hazard-Free Conversion by Microorganisms. Int J Environ Res Public Health. 2022 Apr 14;19(8):4740. doi:10.3390/ijerph19084740. PMID: 35457607; PMCID: PMC9026373

33. Pinheiro LRS, Gradíssimo DG, Xavier LP, Santos AV. Degradation of Azo Dyes: Bacterial Potential for Bioremediation. *Sustainability*. 2022; 14(3):1510. https://doi.org/10.3390/su14031510.

34. Rahmani AH, Babiker AY, Anwar S. Hesperidin, a Bioflavonoid in Cancer Therapy: A Review for aMechanism of Action through the Modulation of Cell Signaling Pathways. Molecules. 2023;28(13):5152.

35. Rahmani AH, Anwar S, Raut R, Almatroudi A, Babiker AY, Khan AA, Alsahli MA, Almatroodi SA. Therapeutic Potential of Myrrh, a Natural Resin, in Health Management through Modulation ofOxidative Stress, Inflammation, and Advanced Glycation End Products Formation Using In Vitroand In Silico Analysis. Applied Sciences. 2022; 12(18):9175.

36. Reza MSA, Hasan MM, Kamruzzaman M, Hossain MI, Zubair MA, Bari L, Abedin MZ, Reza MA, Khalid-Bin-Ferdaus KM, Haque KMF, Islam K, Ahmed MU, Hossain MK. Study of a common azo food dye in mice model: Toxicity reports and its relation to carcinogenicity. Food Sci Nutr. 2019 Jan 29;7(2):667-677. doi: 10.1002/fsn3.906.

37. Srinivasan, A., Viraraghavan, T., 2010. Decolorization of dye wastewater by biosorbents: a review. J. Environ. Manage. 91, 1915–1929.

38. Tan BL, Norhaizan ME, Liew WP, Sulaiman Rahman H. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front Pharmacol. 2018 Oct 16;9:1162. doi: 10.3389/fphar.2018.01162. PMID: 30405405; PMCID: PMC6204759.

39. Thangaraj S, Bankole PO, Sadasivam SK. Microbial degradation of azo dyes by textile effluent adapted, Enterobacter hormaechei under microaerophilic condition. Microbiological Research. 2021 Sep 1;250:126805.

40. Velusamy S, Roy A, Sundaram S, Kumar Mallick T. A Review on Heavy Metal Ions and ContainingDyes Removal Through Graphene Oxide-Based Adsorption Strategies for Textile WastewaterTreatment. Chem Rec. 2021 Jul; 21(7):1570-1610. doi: 10.1002/tcr.202000153.

41. Wu L, Liu X, Lv G, Zhu R, Tian L, Liu M, Li Y, Rao W, Liu T, Liao L. Study on the adsorption properties of methyl orange by natural one-dimensional nano-mineral materials with different structures. Sci Rep. 2021 May 20;11(1):10640. doi: 10.1038/s41598-021-90235-1. PMID: 34017049; PMCID: PMC8138017.

42. Yaseen, D.A., Scholz, M. Textile dye wastewater characteristics and constituents of synthetic effluents:a critical review. Int. J. Environ. Sci. Technol. 16, 1193–1226 (2019). https://doi.org/10.1007/s13762-018-2130-z.

43. Yahia, E.A., & Anwar, S. (2020). Indicators and risk factors associated with Malnutrition amongpatients with Liver Cirrhosis: Nursing Perspective. IJCRT, 8(3), 3255-3269.

44. Yildirim NC, Yaman M. (2019) The usability of oxidative stress and detoxification biomarkers in *Gammarus pulex* for ecological risk assessment of textile dye methyl orange, Chemistry and Ecology, 35:4, 319-329, DOI: 10.1080/02757540.2019.1579199.

45. Younus, H.; Anwar, S. Antiglycating activity of Aloe vera gel extract and its active componentaloin. J.P.P. 2018, 9, 115–125.39.

46. Younus H. Therapeutic potentials of superoxide dismutase. Int J Health Sci (Qassim). 2018 May-Jun;12(3):88-93.

47. Zafar S, Bukhari DA, Rehman A. Azo dyes degradation by microorganisms-An efficient and sustainable approach. Saudi Journal of Biological Sciences. 2022 Sep 6:103437.