



**UNIVERSITI PUTRA MALAYSIA**

***DECOLORIZATION OF METANIL YELLOW DYE BY FREE AND  
IMMOBILIZED BACTERIAL CELLS***

**FATIN NATASHA AMIRA BINTI MULIADI**

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**By**

**FATIN NATASHA AMIRA BINTI MULIADI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the Degree of Master of  
Science**

**June 2021**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirement for the degree of Master of Science

## DECOLORIZATION OF METANIL YELLOW DYE BY FREE AND IMMOBILIZED BACTERIAL CELLS

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**FATIN NATASHA AMIRA BINTI MULIADI**

June 2021

**Chair : Mohd Izuan Effendi Bin Halmi, PhD**  
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Textile industry is one of the leading industries that contribute to economy. The oldest man-made chemicals and are widely used in the textile industries are a type of azo dyes. Globally,  $2.8 \times 10^5$  tonnes of textile dyes are poured into water ecosystem every year. This has several adverse effects on life including decreased aquatic photosynthesis, ability to exhaust dissolved oxygen and toxic effect on flora, fauna and also humans. The presence of dyes in the textile effluent also causes an unpleasant appearance by imparting the color and also their breakdown products (colorless amines) which are toxic, carcinogenic and mutagenic. One of the examples of azo dyes is Metanil Yellow (MY) dye. MY is a type of azo dyes that is toxic to humans and also environment. Thus, this study is conducted with aims to overcome these problems. For the first objective, which is to isolate, screen and identify MY dye decolorizer from mixed culture and optimization of MY dye decolorization using RSM. The mixed bacterial culture, FN3 was isolated from agriculture soil in palm estate in Universiti Putra Malaysia, (2.9876,101.7234). Forty samples were screened for dye decolorization. The screening process was performed using different dye concentration ranging from 100 mg/L to 400 mg/L. The mixed culture was prepared by dissolving 5.0 mL of the soil suspension (10% v/v) in 50.0 mL of minimal salt medium (MSM) supplemented with desired concentration of MY dye in 250 mL conical flask. The conical flask was incubated at room temperature on a rotary shaker at 120 rpm for 24 hours. The cultures were maintained by subculturing into new MSM media every 3 days and were kept in 8°C. It was later determined that isolate FN3 able to decolorize MY up to 90% of MY dye in 24 hours. Mixed bacterial culture FN3 was then identified using metagenomics analysis. This analysis determined that the mixed bacterial culture FN3 comprised of *Bacillus* sp with percentage of up to 42.6%. The second highest of bacteria found in the mixed culture was from genus *Acinetobacter* with percentage of 14%. Fungi diversity analysis was also performed using Internal Transcribed Sequence (ITS). It was determined that 97% of mixed culture FN3 was "unclassified" fungi and 3% consisted of *Candida*

sp. After that, the optimization of MY decolorization was performed using the methodological approach of Response Surface Methodology (RSM). From the optimization, it was determined that the optimum conditions were 72 mg/L of Metanil Yellow dye concentration, 1.934% of glucose concentration, 0.433 g/L of ammonium sulphate and pH of 7.097. The analysis of variance (ANOVA) demonstrated that the model was significant based on the low probability value ( $F < 0.0001$ ). The goodness of fit of the model was checked using the determination coefficient  $R^2$ . The value of  $R^2$  was 0.9125 that indicated good relation between experimental and predicted values of response. The non-significant value of lack of fit ( $> 0.05$ ) shown that the quadratic model was statistically significant for the response and thus can be used for further analysis. Next, for the second objective which is to optimize the MY dye decolorization of immobilized mixed culture FN3 using RSM and to study the effects of heavy metals ions towards MY dye decolorization. The mixed bacterial culture of FN3 was immobilized using gellan gum and optimized using the same approach, RSM for optimum dye decolorization. It was determined that the optimum conditions were as follows; 130 mg/L of dye concentration, 1.478% of gellan gum concentration, 50 beads and 0.6 cm of beads size. The ANOVA test demonstrated that the model was significant for dye decolorization ( $F < 0.0001$ ). The value of  $R^2$  was 0.9767 which is close to 1 indicating that the correlation between the predicted and experimental values are good. The lack of fit for the model was 5.8 and statistically insignificant implying that the model was statistically significant for the response and can be used for further analysis. The reusability of the microbials beads in dye decolorizing was tested. It is documented that the immobilized beads was able to be reused up to 15 times without substantial loss of catalytic activity. The effects of metals ions were also tested to the free cells and immobilized beads of mixed bacterial culture FN3. It was shown that dye decolorization of MY by the mixed bacterial culture was not affected by the presence of 1 mg/L of the metals ions of argentum, lead, cobalt, copper, zinc, cadmium, chromium, arsenic, nickel and mercury. The ability of the immobilized beads has made this as a great potential of bioremediation tools.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains

## **PENYAHWARNAAN PEWARNA “METANIL YELLOW” OLEH SEL BAKTERIA BEBAS DAN SEL TERSEKAT GERAK**

Oleh

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Industri tekstil merupakan salah satu industry penting yang menyumbang kepada ekonomi. Bahan kimia yang paling tua dan digunakan secara meluas adalah pewarna azo. Di peringkat global, sebanyak  $2.8 \times 10^5$  tan pewarna tekstil dilepaskan ke dalam ekosistem air setiap tahun. Perkara ini memberi kesan kepada kehidupan termasuk pengurangan proses fotosintesis oleh hidupan air, keupayaan menghabiskan kadar oksigen larut dan kesan toksik kepada flora, fauna dan manusia. Kehadiran pewarna di dalam bahan buangan tekstil juga mengakibatkan penampilan yang tidak menyenangkan disebabkan kehadiran warna dan produk penguraian (amina tidak berwarna) yang toksik, karsinogenik dan mutagenik. Salah satu contoh pewarna azo ialah pewarna “Metanil Yellow”. Pewarna “Metanil Yellow” adalah sejenis pewarna azo yang toksik kepada manusia dan alam sekitar. Oleh hal yang demikian, projek ini dijalankan dengan harapan untuk mengatasi masalah sedemikian. Untuk objektif yang pertama iaitu untuk memencilkan, menyaring dan mengenalpasti kultur bakteria campur yang dapat menyahwarnakan pewarna MY dan optimasi penyahwarnaan MY menggunakan RSM. Kultur bakteria campur, FN3 telah berjaya dipencilkan dari tanah pertanian kelapa sawit di Universiti Putra Malaysia, (2.9876,101.7234). Empat puluh sampel tanah telah disaringkan untuk penyahwarna pewarna “Metanil Yellow”. Proses penyaringan dilakukan beberapa kali menggunakan konsentrasi pewarna berbeza iaitu dari 100 mg/L hingga 400 mg/L. Kultur bakteria campur disediakan dengan melarutkan 5.0 mL larutan tanah (10% v/v) di dalam 50.0 mL media “Minimal Salt” (MSM) yang telah disediakan dengan konsentrasi pewarna MY yang dikehendaki di dalam 250 mL kelalang kon. Kelalang kon diinkubasi pada suhu bilik di atas penggoncang bergerak pada kelajuan 120 rpm selama 24 jam. Kultur tersebut dikekalkan dengan subkultur ke dalam media MSM setiap tiga hari dan disimpan pada suhu 8°C. Setelah beberapa kali penyaringan dilakukan, ia dapat ditentukan bahawa kultur FN3 dapat menyahwarnakan pewarna “Metanil Yellow” sehingga sebanyak 90% dalam masa 24 jam. Kemudian, kultur bakteria campur FN3 telah dianalisis menggunakan analisis metagenomik. Analisis ini telah mengenal pasti bahawa

kultur bakteria campur FN3 terdiri daripada *Bacillus* sp sebanyak 42.6% manakala mikroorganisma kedua tertinggi yang telah dikenal pasti adalah daripada genus *Acinetobacter* dengan peratusan sebanyak 14%. Analisis kepelbagaian kulat telah dilakukan menggunakan "Internal Transcribed Sequence (ITS)". Ia telah dikenalpasti sebanyak 97% dalam kultur bakteria campur FN3 adalah sejenis kulat yang tidak dapat dikenalpasti manakala 3% lagi adalah terdiri daripada *Candida* sp. Kemudian, pengoptimuman penyahwarna pewarna "Metanil Yellow" telah dilakukan menggunakan pendekatan metodologi "Response Surface Methodology (RSM)". Berdasarkan keputusan pengoptimuman itu, ia telah dapat dikenalpasti bahawa kondisi optimum adalah 72 mg/L kepekatan pewarna "Metanil Yellow", 1.934% kepekatan glukosa, 0.433 g/L ammonium sulfat dan pH 7.097. Analisis varians (ANOVA) menunjukkan model adalah signifikan berdasarkan nilai kebarangkalian rendah ( $F < 0.0001$ ). Ketepatan padanan model telah disemak menggunakan nilai pekali penentuan  $R^2$ . Nilai  $R^2$  untuk model adalah 0.9125 yang memberi indikasi bahawa hubungan antara nilai uji kaji dan nilai ramalan adalah bagus. Nilai padanan kurang tepat yang tidak signifikan ( $> 0.05$ ) menunjukkan bahawa kuadratik model adalah signifikan secara statistik dan boleh digunakan untuk analisis seterusnya. Kemudian, kultur bakteria campur FN3 telah disekat gerak menggunakan "gellan gum". Manik bakteria juga telah mengalami pengoptimuman menggunakan cara yang sama iaitu RSM. Kondisi optimum yang dapat disimpulkan adalah 130 mg/L kepekatan pewarna "Metanil Yellow", 1.478% kepekatan "gellan gum", 50 biji manik bakteria dan 0.6cm size manik. Berdasarkan analisis varians (ANOVA), model adalah signifikan untuk penyahwarna pewarna "Metanil Yellow" ( $F < 0.0001$ ). Nilai  $R^2$  adalah 0.9767 yang hampir dengan nilai 1 memberi indikasi bahawa korelasi antara nilai uji kaji dan nilai ramalan adalah bagus. Nilai padanan kurang tepat untuk model ini adalah 5.8 dan tidak signifikan secara statistik yang menunjukkan model adalah signifikan secara statistik untuk respons dan boleh digunakan untuk analisis seterusnya. Ujian guna semula manik mikrob untuk penyahwarna "Metanil Yellow" telah dilakukan. Berdasarkan ujian tersebut, manik mikrob tersebut dapat diguna semula sebanyak 15 kali tanpa pengurangan keaktifan bermangkin. Kesan ion logam terhadap sel bebas dan sel sekat gerak kultur bakteria campur FN3 juga telah dilakukan. Ia menunjukkan bahawa aktiviti penyahwarna pewarna "Metanil Yellow" oleh kultur bakteria campur FN3 tidak terkesan oleh kehadiran ion logam dengan kepekatan 1 mg/L seperti perak, plumbum, kobalt, zink, kadmium, kromium, arsenik, nikel dan merkuri. Kebolehan manik mikrob sekat gerak ini telah menjadikan ia sebagai alat bioremediasi yang mempunyai potensi yang besar.



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## LIST OF ABBREVIATIONS

%	percent
°C	degree celcius
µL	microlitre
ANOVA	analysis of variance
As	arsenic
BBD	box-benken design
BOD	biological oxygen demand
bp	base pair
CaCl <sub>2</sub>	calcium chloride
CCD	central composite design
Cd	cadmium
cm	centimetre
cm <sup>-1</sup>	per centimetre
CO <sub>2</sub>	carbon dioxide
Co	cobalt
COD	chemical oxygen demand
Cu	copper
DNA	deoxyribonucleic acid
Fe	ferrum
Fe (SO <sub>4</sub> ) <sub>3</sub> .H <sub>2</sub> O	ferrous sulphate
FTIR	fourier-transform infrared spectroscopy
g	gram
g/L	gram per litre

GPS	global positioning system
Hg	mercury
HPLC	high-performance liquid chromatography
ITS	internal transcribed sequence
$K_2HPO_4$	dipotassium phosphate
KBr	potassium bromide
$KH_2PO_4$	potassium dihydrogen phosphate
kPa	kilopascal
M	molar
mg/L	milligram per litre
$MgSO_4 \cdot H_2O$	magnesium sulphate
mL	millilitre
mm	millimetre
Mn	manganese
$MnSO_4 \cdot H_2O$	manganese sulphate
MSM	minimal salt media
$Na_2SO_4$	sodium sulphate
NaCl	sodium chloride
NaOH	sodium hydroxide
$NaMoO_4$	sodium molybdate
$(NH_4)_2SO_4$	ammonium sulphate
Ni	nickel
nm	nanometre
OFAT	one-factor at a time
OTU	operational taxonomy unit

Pb	lead
ppm	parts per million
PUF	polyurethane foam
PVA	polyvinyl-alcohol
R <sup>2</sup>	R squared
ROS	reactive oxygen species
rpm	revolutions per minutes
rRNA	ribosomal ribonucleic acid
RSM	response surface methodology
R <sub>t</sub>	retention time
SEM	scanning electron microscope
v/v	volume per volume
w/v	weight per volume
Zn	zinc

## CHAPTER 1

### INTRODUCTION

The garment and apparel business is one of the oldest industries in Malaysia. It is considered as the tenth largest export earner. In the Industrial Master Plan 3 (2006-2020), the importance of this industry was highlighted by the identification of six key thrusts. Along with these achievements, many drawbacks have arisen. One of them is that the textile industry has been consuming generous amounts of water in its preparation and dyeing processes. In Malaysia, more than 662 licensed and 1000 small scale textile and apparel factories are exempted from the Manufacturing License and this is regarded as a critical source of waste water (Pang & Abdullah, 2013) as 22% of the total volume of industrial wastewater generated in Malaysia comes from wastewater from the textile industry (Idris et al., 2007). The main textile industry in Malaysia is Batik industry. This industry is normally run by the locals in East Coast of Malaysia; Kelantan and Terengganu (Birgani et al., 2016).

Textile processing industries largely utilize the use of azo dyes that includes aromatic hydrocarbons, derivatives of benzene, toluene, naphthalene, phenol and aniline (Puvaneswari et al., 2006). Due to large varieties (more than 3000 different varieties), simple biosynthesis, chemical stability and the variety of colors available compared to natural dyes, azo dyes are chosen (Chang et al., 2004).

Around 80 per cent of azo dyes are used in the textile industry's dyeing process (Singh & Lakhani Singh, 2017). The chemical structure of azo dyes characterized by one or more azo bonds (-N=N-) has permitted the visible region to absorb light (Chang, Chou, et al., 2001b). Even though the textile industry plays an important role in any country's economy, it is still an environmental impediment.

During the dyeing process, 2% of basic dyes and up to as high as 50% for reactive dyes do not bind to the fabric and loss in wastewater. This contributes to significant surface and ground water pollution in the vicinity of the dyeing industries (Pandey et al., 2007). Disposal of these dyes into the environment gives rise to severe damage since the photosynthetic activity of hydrophytes is affected by low light penetration (Aksu et al., 2007) and they may also be toxic to some aquatic organisms due to their breakdown products (Hao et al., 2000; Wang et al., 2009). In addition, the release of colored textile waste into drains and lakes leads to a decrease in the concentration of dissolved oxygen and produces harmful conditions for aquatic flora and fauna (El Bouraie & Salah, 2016).

Metanil Yellow (MY) (Acid Yellow 36) is a highly water-soluble dye and is extensively used for many purposes such as the coloring of soap, spirit lacquer,

shoe polish, bloom sheep dip, for the preparation of wood stains, dyeing of leather, manufacture of pigment lakes and paper staining (Anjaneya et al., 2011a). From the toxicity data, it reveals that oral feeding or intraperitoneal and intratesticular administration of MY in animals produces testicular lesions due to which seminiferous tubules suffer damage and results in the decreased rate of spermatogenesis (Gupta et al., 2008). Studies have shown that 13.6% of the orally administered dose of MY (15mg 200 g<sup>-1</sup> rat) is retained even after 96 h in the gastrointestinal tract, which may be responsible for decreased mucin secretion from the intestinal mucous cells (Ramchandani et al., 1997). On oral consumption, it causes toxic methaemoglobinaemia (Sachdeva et al., 1992) and cyanosis in humans, while for skin contact, it results into allergic dermatitis. MY also has tumour-producing effects and can create intestinal and enzymic disorders in human body (Ramchandani et al., 1997). It is not mutagenic. However, it can alter the genes expression (Gupta et al., 2003).

Removal of the MY dye has become a major concern. Scientists have identified ways to treat the textile wastewater. It can be grouped into three categories: physical, chemical and biological techniques. For physical methods, the most known method is the adsorption method. Due to their greater decolorization efficiency for waste water containing a range of dyes, the adsorption method has significant interest (Holkar, J Jadhav, et al., 2016). Besides that, coagulation, filtration and ion- exchange method are categorized in the physical method as well. The conventional oxidation process, ozonation, Fenton oxidation and electrochemical are some of the methods used in the chemical method category.

These physical and chemical methods have some drawbacks which are in terms of cost, time and production of secondary pollutants (Sudha et al., 2014). Thus, scientists nowadays are searching for other alternative methods. Biological methods have made it ways into recognition. Bioremediation is an advancement in pollution control that utilizes natural biological organisms to catalyze the degradation or transition to less toxic structures of various poisonous synthetic substances (P Shah et al., 2014). The biological materials involved in the bioremediation process are bacteria, fungi, yeasts and algae (Bhatia et al., 2017).

Many pure cultures of microorganisms have been reported to be able to decolorize the azo dyes. However, it has recently been discovered that treatment systems with mixed microbial populations are more efficient and successful because of the mixed microbial populations' concerted metabolic activities (Khehra et al., 2005). This is due to the catabolic activities of the mixed bacterial populations complement with each other and makes the biodegradation, detoxification and mineralization of textile dyes to be higher (Parvin et al., 2015).

Besides that, immobilization of microbial cells has received attention these days. In terms of rate, operational stability, cell washout and substrate transfer into the cells, free cells are said to be limited (Cheng et al., 2012b). Thus it is said that immobilized cells frameworks have the potential to debase harmful synthetic



compounds quicker than ordinary wastewater treatment frameworks since high densities of specialized microorganisms are utilized in immobilized cell systems (He et al., 2004). They are often safer than suspension cells for ecological irritations, such as pH or exposure to harmful concentrations of compounds, and are increasingly good for oxygen supply and mass exchange (Cheng et al., 2012b).

There are many different types of methods for immobilization such as adsorption or attachment to inert surfaces, self-aggregation of cells by flocculation, encapsulation in polymer gels or entrapment of different types of matrices (Mathieu Landreau et al., 2016). Some of the gel matrices that are used by researchers are polyvinyl-alcohol (PVA) gel (Chen et al., 2003), combination of calcium alginate and k-carageenan, polyacrylamide (Chang, Chou, & Chen, 2001) and polyurethane foam (PUF) (Srikanlayanukul et al., 2006). Gellan gum is chosen in this study. Gellan gum has higher breakage resistance compared to calcium alginate. Furthermore, when gellan gum is compared to other matrices such as k-carageenan and agar, the activities of cells in latter matrices are lower compared to gellan gum (Survase, Annapure, & S Singhal, 2010; Wang et al., 2007).

In this industrialization era, co-contamination of the environment with heavy metals and other contaminants are common (Olaniran et al., 2013). The presence of heavy metals decreased the activity of microorganisms in the environment and this will inhibit the degradation of toxic waste due to their toxicity.

Thus, the bioremediation process will be affected due to this. Hence, in order to solve this problem, it is important to make use of specific bacteria that able to degrade specific toxic waste and it must also be resistant and tolerant to heavy metals. In a study, *Pseudomonas aeruginosa* ZM130 able to simultaneously removed Cr(VI) and various azo dyes (Maqbool et al., 2016). Another study also has shown that the newly isolated bacterial consortium AIE-2 are efficient in the bioremediation process of Cr(VI) and RV5 (Desai et al., 2009). This proved that specific microorganisms are able to simultaneously removed contaminants without affecting the decolorizing dye ability.

This study aims to find the preliminary ways to treat the textile wastewater using biological method that will cut the cost, reliable and environmental- friendly. This is by means of bacterial isolation that able to decolorize MY dye and later by immobilizing the bacteria using gellan gum.

The objectives of this study are:

1. To isolate, screen and identify the best isolated mixed culture from soil which able to decolorize MY dye and optimizing the MY dye decolorization using Response Surface Methodology (RSM) approach.
2. To optimize the decolorization of MY dye of the selected isolate by immobilising it into porous beads using RSM approach and study the efficiency of the decolorization of immobilised beads and free cells towards heavy metals ions.



## REFERENCES

- Ajaz, M., Rehman, A., Khan, Z., Nisar, M. A., & Hussain, S. (2019). Degradation of azo dyes by *Alcaligenes aquatilis* 3c and its potential use in the wastewater treatment. *AMB Express*, 9(1), 64. <https://doi.org/10.1186/s13568-019-0788-3>
- Aksu, Z., Koçberber KiliÇ, N., Ertuğrul, S., & Dönmez, G. (2007). Inhibitory effects of chromium(VI) and Remazol Black B on chromium(VI) and dyestuff removals by *Trametes versicolor*. *Enzyme and Microbial Technology*, 40 (5). <https://doi.org/10.1016/j.enzmictec.2006.08.024>
- Alabdrraba, W., & Bayati, M. (2014). Biodegradation of Azo Dyes a Review. *Int. J. Environ. Eng. Nat. Resour*, 1(4), 179-189,
- Amim Jr, J., F. S. Petri, D., Maia, F., & Miranda, P. (2009). Ultrathin cellulose ester films: Preparation, characterization and protein immobilization. *Química Nova*, 33, 2064-2069.
- Anjaneya, O., Souche, S. Y., Santoshkumar, M., & Karegoudar, T. B. (2011a). Decolorization of sulfonated azo dye Metanil Yellow by newly isolated bacterial strains: *Bacillus* sp. strain AK1 and *Lysinibacillus* sp. strain AK2. *J Hazard Mater*, 190(1-3), 351-358. <https://doi.org/10.1016/j.jhazmat.2011.03.044>
- Anjaneya, O., Souche, S. Y., Santoshkumar, M., & Karegoudar, T. B. (2011b). Decolorization of sulfonated azo dye Metanil Yellow by newly isolated bacterial strains: *Bacillus* sp. strain AK1 and *Lysinibacillus* sp. strain AK2. *Journal of Hazardous Materials*, 190(1), 351-358. <https://doi.org/https://doi.org/10.1016/j.jhazmat.2011.03.044>
- Bafana Amit, Saravana, D., & Chakrabarti Tapan. (2011). Azo dyes: past, present and the future. *Environmental Reviews*, 19, 350-371. <https://doi.org/10.1139/a11-018>
- Bajaj, I., Survase, S., Saudagar, P., & Singhal, R. (2007). Gellan Gum: Fermentative Production, Downstream Processing and Applications. *Food Technology and Biotechnology*, 45.
- Bajaj, I. B., Survase, S. A., Saudagar, P. S., & Singhal, R. S. (2007). Gellan gum: fermentative production, downstream processing and applications. *Food Technology and Biotechnology*, 45(4), 341-354.
- Banat, I., Nigam, P., Singh, D., & Marchant, R. (1996). Microbial decolorization of textile-dye-containing effluents: A review. *Bioresource Technology*, 58(3), 217-227. [https://doi.org/10.1016/S0960-8524\(96\)00113-7](https://doi.org/10.1016/S0960-8524(96)00113-7)

- Bashir, Y., Singh, S., & Konwar, B. (2014). Review Article Metagenomics: An Application Based Perspective. *Chinese Journal of Biology*, 2014. <https://doi.org/10.1155/2014/146030>
- Bayat, Z., Hassanshahian, M., & Cappello, S. (2015). Immobilization of Microbes for Bioremediation of Crude Oil Polluted Environments: A Mini Review. *The open microbiology journal*, 9, 48-54. <https://doi.org/10.2174/1874285801509010048>
- Bhatia, D., Sharma, n. r., Singh, J., & Kanwar, R. (2017). Biological methods for textile dye removal from wastewater: A Review. *Critical Reviews in Environmental Science and Technology*, 47(19), 1836-1876. <https://doi.org/10.1080/10643389.2017.1393263>
- Bilal, M., & Asgher, M. (2015). Dye decolorization and detoxification potential of Ca-alginate beads immobilized manganese peroxidase. *BMC Biotechnology*, 15(1), 111. <https://doi.org/10.1186/s12896-015-0227-8>
- Birgani, P. M., Ranjbar, N., Abdullah, R. C., Wong, K. T., Lee, G., Ibrahim, S., Jang, M. (2016). An efficient and economical treatment for batik textile wastewater containing high levels of silicate and organic pollutants using a sequential process of acidification, magnesium oxide, and palm shell-based activated carbon application. *Journal of Environmental Management*, 184, 229-239. <https://doi.org/https://doi.org/10.1016/j.jenvman.2016.09.066>
- Boopathy, R. (2000). Factors limiting bioremediation technologies. *Bioresource Technology*, 74(1), 63-67. [https://doi.org/https://doi.org/10.1016/S0960-8524\(99\)00144-3](https://doi.org/https://doi.org/10.1016/S0960-8524(99)00144-3)
- Bouabidi, Z., El-Naas, M., & Zhang, Z. (2018). Immobilization of microbial cells for the biotreatment of wastewater: A review. *Environmental Chemistry Letters*, 17. <https://doi.org/10.1007/s10311-018-0795-7>
- Camelia, I., Peptu, C., Savin, A., Atanase, L., Souidi, K., Mackenzie, G., Popa, M. (2017). Microencapsulation of Baker's Yeast in Gellan Gum Beads Used in Repeated Cycles of Glucose Fermentation. *International Journal of Polymer Science*, 2017. <https://doi.org/10.1155/2017/7610420>
- Camelin, I., Lacroix, C., Paquin, C., Prevost, H., Cachon, R., & Divies, C. (1993). Effect of chelantants on gellan gel rheological properties and setting temperature for immobilization of living bifidobacteria. *Biotechnology Progress*, 9(3), 291-297. <https://doi.org/10.1021/bp00021a008>
- Cassidy, M. B., Lee, H., & Trevors, J. T. (1996). Environmental applications of immobilized microbial cells: A review. *Journal of Industrial Microbiology*, 16(2), 79-101. <https://doi.org/10.1007/BF01570068>
- Chakraborty, S., Jana, S., Gandhi, A., Sen, K. K., Zhiang, W., & Kokare, C. (2014). Gellan gum microspheres containing a novel  $\alpha$ -amylase from marine *Nocardioopsis* sp. strain B2 for immobilization. *International Journal*

of *Biological Macromolecules*, 70, 292-299.  
<https://doi.org/https://doi.org/10.1016/j.ijbiomac.2014.06.046>

- Chakravorty, S., Helb, D., Burday, M., Connell, N., & Alland, D. (2007). A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *Journal of Microbiological Methods*, 69(2), 330-339. <https://doi.org/https://doi.org/10.1016/j.mimet.2007.02.005>
- Chan, G. F., Abdul Rashid, N. A., Koay, L., Chang, S., & Tan, W. (2011). Identification and Optimization of Novel NAR-1 Bacterial Consortium for the Biodegradation of Orange II. *Insight Biotechnology*, 1, 7-16. <https://doi.org/10.5567/IBIOT-IK.2011.7.16>
- Chang, J.-S., Chen, B.-Y., & Lin, Y. S. (2004). Stimulation of bacterial decolorization of an azo dye by extracellular metabolites from *Escherichia coli* strain NO3. *Bioresource Technology*, 91(3), 243-248. [https://doi.org/https://doi.org/10.1016/S0960-8524\(03\)00196-2](https://doi.org/https://doi.org/10.1016/S0960-8524(03)00196-2)
- Chang, J.-S., Chou, C., & Chen, S.-Y. (2001). Decolorization of azo dyes with immobilized *Pseudomonas luteola*. *Process Biochemistry*, 36(8), 757-763. [https://doi.org/https://doi.org/10.1016/S0032-9592\(00\)00274-0](https://doi.org/https://doi.org/10.1016/S0032-9592(00)00274-0)
- Chang, J.-S., Chou, C., Lin, Y.-C., Lin, P.-J., Ho, J.-Y., & Hu, T.-L. (2001a). Kinetic characteristics of bacterial azo-dye decolorization by *Pseudomonas luteola*. *Water research*, 35, 2841-2850. [https://doi.org/10.1016/S0043-1354\(00\)00581-9](https://doi.org/10.1016/S0043-1354(00)00581-9)
- Chang, J.-S., Chou, C., Lin, Y.-C., Lin, P.-J., Ho, J.-Y., & Hu, T.-L. (2001b). Kinetic characteristics of bacterial azo-dye decolorization by *Pseudomonas luteola*. *Water Research*, 35(12), 2841-2850. [https://doi.org/10.1016/S0043-1354\(00\)00581-9](https://doi.org/10.1016/S0043-1354(00)00581-9)
- Chen, B.-Y., & Chang, J.-S. (2007). Assessment upon species evolution of mixed consortia for azo dye decolorization. *Journal of the Chinese Institute of Chemical Engineers*, 38, 259-266. <https://doi.org/10.1016/j.jcice.2007.04.002>
- Chen, J.-P., & Lin, Y.-S. (2007a). Decolorization of azo dye by immobilized *Pseudomonas luteola* entrapped in alginate-silicate sol-gel beads. *Process Biochemistry*, 42, 934-942. <https://doi.org/10.1016/j.procbio.2007.03.001>
- Chen, J.-P., & Lin, Y.-S. (2007b). Decolorization of azo dye by immobilized *Pseudomonas luteola* entrapped in alginate-silicate sol-gel beads. *Process Biochemistry*, 42(6), 934-942. <https://doi.org/https://doi.org/10.1016/j.procbio.2007.03.001>
- Chen, K.-C., Wu, J.-Y., Huang, C.-C., Liang, Y.-M., & Hwang, S.-C. (2003). Decolorization of Azo Dye Using PVA-Immobilized Microorganisms. *Journal of Biotechnology*, 101(3), 241-252. [https://doi.org/10.1016/S0168-1656\(02\)00362-0](https://doi.org/10.1016/S0168-1656(02)00362-0)



- Chen, Y., Cheng, J. J., & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: A review. *Bioresource technology*, 99(10), 4044-4064. <https://doi.org/https://doi.org/10.1016/j.biortech.2007.01.057>
- Cheng, Y., Lin, H., Chen, Z., Mallavarapu, M., & Naidu, R. (2012a). Biodegradation of crystal violet using *Burkholderia vietnamiensis* C09V immobilized on PVA-sodium alginate-kaolin gel beads. *Ecotoxicology and environmental safety*, 83, 108-114. <https://doi.org/10.1016/j.ecoenv.2012.06.017>
- Cheng, Y., Lin, H., Chen, Z., Mallavarapu, M., & Naidu, R. (2012b). *Biodegradation of crystal violet using Burkholderia vietnamiensis C09V immobilized on PVA-sodium alginate-kaolin gel beads* (Vol. 83). <https://doi.org/10.1016/j.ecoenv.2012.06.017>
- Chibuikwe, G. U., & Obiora, S. C. (2014). Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods. *Applied and Environmental Soil Science*, 2014, 12, Article 752708. <https://doi.org/10.1155/2014/752708>
- De Mandal, S., Sanga, Z., & Nachimuthu, S. K. (2014). Metagenomic analysis of bacterial community composition among the cave sediments of Indo-Burman biodiversity hotspot region. *PeerJ PrePrints*, 2. doi: 10.7287/peerj.preprints.631v1.
- Desai, C., Jain, K., Patel, B., & Madamwar, D. (2009). Efficacy of bacterial consortium-AIE2 for contemporaneous Cr (VI) and azo dye bioremediation in batch and continuous bioreactor systems, monitoring steady-state bacterial dynamics using qPCR assays. *Biodegradation*, 20(6), 813-826.
- Dey, G., Mitra, A., Banerjee, R., & Maiti, B. R. (2001). Enhanced production of amylase by optimization of nutritional constituents using response surface methodology. *Biochemical Engineering Journal*, 7(3), 227-231. [https://doi.org/https://doi.org/10.1016/S1369-703X\(00\)00139-X](https://doi.org/https://doi.org/10.1016/S1369-703X(00)00139-X)
- Dixit, S., Purshottam, S., Khanna, S., & Das, M. (2009). Surveillance of the quality of turmeric powders from city markets of India on the basis of curcumin content and the presence of extraneous colours. *Food Additives & Contaminants: Part A*, 26, 1227-1231. <https://doi.org/10.1080/02652030903016586>
- Doner, L. W., & Douds, D. D. (1995). Purification of commercial gellan to monovalent cation salts results in acute modification of solution and gel-forming properties. *Carbohydrate Research*, 273(2), 225-233. [https://doi.org/https://doi.org/10.1016/0008-6215\(95\)00115-A](https://doi.org/https://doi.org/10.1016/0008-6215(95)00115-A)
- El Ahwany, A. M. D. (2008). Decolorization of Fast red by metabolizing cells of *Oenococcus oeni* ML34. *World Journal of Microbiology and Biotechnology*, 24(8), 1521-1527. <https://doi.org/10.1007/s11274-007-9640-z>

- El Bouraie, M., & Salah, W. (2016). Biodegradation of Reactive Black 5 by *Aeromonas hydrophila* strain isolated from dye-contaminated textile wastewater. *Sustainable Environment Research*, 26(5), 209-216. <https://doi.org/10.1016/j.serj.2016.04.014>
- Elakkiya, M., Prabhakaran, D., & Thirumarimurugan, M. (2016). Methods of cell immobilization and its applications. *Methods*, 5(4), 211-216.
- Elksibi, I., Haddar, W., Ben Ticha, M., gharbi, R., & Mhenni, M. F. (2014). Development and optimisation of a non conventional extraction process of natural dye from olive solid waste using response surface methodology (RSM). *Food Chemistry*, 161, 345-352. <https://doi.org/https://doi.org/10.1016/j.foodchem.2014.03.108>
- Ewida, A., El-Sesy, M., & Abou Zeid, A. (2019). Complete degradation of azo dye acid red 337 by *Bacillus megaterium* KY848339.1 isolated from textile wastewater. *Water Science*, 33, 154-161. <https://doi.org/10.1080/11104929.2019.1688996>
- Fang, H., Wenrong, H., & Yuezhong, L. (2004). Investigation of isolation and immobilization of a microbial consortium for decoloring of azo dye 4BS. *Water Research*, 38(16), 3596-3604.
- Ferreira, S. L. C., Bruns, R. E., Ferreira, H. S., Matos, G. D., David, J. M., Brandão, G. C., dos Santos, W. N. L. (2007). Box-Behnken design: An alternative for the optimization of analytical methods. *Analytica Chimica Acta*, 597(2), 179-186. <https://doi.org/https://doi.org/10.1016/j.aca.2007.07.011>
- Gadow, S. I., & Li, Y.-Y. (2020). Development of an integrated anaerobic/aerobic bioreactor for biodegradation of recalcitrant azo dye and bioenergy recovery: HRT effects and functional resilience. *Bioresource Technology Reports*, 9, 100388. <https://doi.org/https://doi.org/10.1016/j.biteb.2020.100388>
- Gauthier, P. T., Norwood, W. P., Prepas, E. E., & Pyle, G. G. (2014). Metal-PAH mixtures in the aquatic environment: A review of co-toxic mechanisms leading to more-than-additive outcomes. *Aquatic Toxicology*, 154, 253-269. <https://doi.org/https://doi.org/10.1016/j.aquatox.2014.05.026>
- Ge, X., Yang, L., & Xu, J. (2017). Cell Immobilization: Fundamentals, Technologies, and Applications. *Industrial Biotechnology: Products and Processes*, 205-235.
- Ghosh, A., Mehta, A., & Khan, A. M. (2019). Metagenomic Analysis and its Applications. In S. Ranganathan, M. Gribskov, K. Nakai, & C. Schönbach (Eds.), *Encyclopedia of Bioinformatics and Computational Biology* (pp. 184-193). Academic Press. <https://doi.org/https://doi.org/10.1016/B978-0-12-809633-8.20178-7>



- Ghosh, D., Singha, P., Firdaus, S., & Ghosh, S. (2017). Metanil yellow: The toxic food colorant. *Asian Pacific Journal of Health Sciences*, 4. <https://doi.org/10.21276/apjhs.2017.4.4.16>
- Gopinath, K. P., Kathiravan, M. N., Srinivasan, R., & Sankaranarayanan, S. (2011). Evaluation and elimination of inhibitory effects of salts and heavy metal ions on biodegradation of Congo red by *Pseudomonas* sp. mutant. *Bioresource technology*, 102(4), 3687-3693. <https://doi.org/https://doi.org/10.1016/j.biortech.2010.11.072>
- Gowsalya, A., Ponnusami, V., & Sugumaran, K. (2014). Isolation of bacteria from soil sample for exo-polysaccharide production. *International Journal of ChemTech Research*, 6(5), 2925-2928.
- Guerra, E., Llompарт, M., & Garcia-Jares, C. (2017). Miniaturized matrix solid-phase dispersion followed by liquid chromatography-tandem mass spectrometry for the quantification of synthetic dyes in cosmetics and foodstuffs used or consumed by children. *Journal of Chromatography A*, 1529, 29-38. <https://doi.org/https://doi.org/10.1016/j.chroma.2017.10.063>
- Gunawan, E., Basri, M., Rahman, M., Bakar Salleh, A., & Rahman, R. (2005). Study on response surface methodology (RSM) of lipase-catalyzed synthesis of palm-based wax ester. *Enzyme and Microbial Technology*, 37(7), 739-744. <https://doi.org/10.1016/j.enzmictec.2005.04.010>
- Gupta, S., Sundarrajan, M., & Kurapati, K. (2003). Tumor promotion by metanil yellow and malachite green during rat hepatocarcinogenesis is associated with dysregulated expression of cell cycle regulatory proteins. *Teratog. Carcinog. Mutagen*, 23, 301-312. <https://doi.org/10.1002/tcm.10056>
- Gupta, V., Mittal, A., Kurup, L., & Mittal, J. (2008). Process development for removal and recovery of Metanil Yellow by adsorption on waste materials-bottom ash and de-oiled soya. *J. Hazard. Mater*, 151, 834-845.
- Halmi, M. I. E. b., Abdullah, S. R. S., Wasoh, H., Johari, W. L. W., Ali, M. S. b. M., Shahrudin, N. A., & Shukor, M. Y. (2016). Optimization and maximization of hexavalent molybdenum reduction to Mo-blue by *Serratia* sp. strain MIE2 using response surface methodology. *Rendiconti Lincei*, 27(4), 697-709. <https://doi.org/10.1007/s12210-016-0552-4>
- Hao, O. J., Kim, H., & Chiang, P.-C. (2000). Decolorization of Wastewater. *Critical Reviews in Environmental Science and Technology*, 30(4), 449-505. <https://doi.org/10.1080/10643380091184237>
- He, F., Hu, W., & Li, Y. (2004). Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium. *Chemosphere*, 57(4), 293-301. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2004.06.036>
- Holkar, C., J Jadhav, A., Pinjari, D., M Mahamuni, N., & Pandit, A. (2016). A critical review on textile wastewater treatments: Possible approaches.

*Journal of Environmental Management*, 182, 351-366.  
<https://doi.org/10.1016/j.jenvman.2016.07.090>

- Holkar, C., Jadhav, A., Pinjari, D., Mahamuni, N., & Pandit, A. (2016). A critical review on textile wastewater treatments: Possible approaches. *Journal of Environmental Management*, 182, 351-366.  
<https://doi.org/10.1016/j.jenvman.2016.07.090>
- Idris, A., Hashim, R., Abdul Rahman, R., Ahmad, W., Ibrahim, Z., Abdul Razak, P., Bakar, I. (2007). Application of Bioremediation Process for Textile Wastewater Treatment Using Pilot Plant. *Int. J. Eng. Technol*, 4.
- Igiri, B. E., Okoduwa, S. I. R., Idoko, G. O., Akabuogu, E. P., Adeyi, A. O., & Ejiogu, I. K. (2018). Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review. *Journal of Toxicology*, 2018, 16, Article 2568038.  
<https://doi.org/10.1155/2018/2568038>
- Islahuddin, N. K. S., Halmi, M. I. E., Manogaran, M., & Shukor, M. Y. (2017). Isolation and culture medium optimisation using one-factor-at-time and Response Surface Methodology on the biodegradation of the azo-dye amaranth. *Bioremediation Science and Technology Research*, 5(2), 25-31.
- Islam, T., Rahman, M. S., & Hussain, M. S. (2017). Heavy Metal Tolerance Pattern of Textile Dye Degrading Native Bacteria: A Bioremediation Viewpoint. *Annals of Medical and Health Sciences Research*.
- Jafari, N., Kasra-Kermanshahi, R., & Soudi, M. R. (2013). Screening, identification and optimization of a yeast strain, *Candida palmiophila* JKS4, capable of azo dye decolorization. *Iranian journal of microbiology*, 5(4), 434.
- Jain, K., Shah, V., Chapla, D., & Madamwar, D. (2012). Decolorization and degradation of azo dye – Reactive Violet 5R by an acclimatized indigenous bacterial mixed cultures-SB4 isolated from anthropogenic dye contaminated soil. *J Hazard Mater*, 213-214, 378-386.  
<https://doi.org/https://doi.org/10.1016/j.jhazmat.2012.02.010>
- Jain, R., Sharma, N., & Radhapyari, K. (2004). Removal of hazardous azo dye metanil yellow from industrial wastewater using electrochemical technique. *European Water*, 27(28), 43-52.
- Johari, W. L. W., Isa, R. I. M., Ghazali, N., Arif, N. M., & Shukor, M. Y. A. (2014). Decolorization of Azo Dyes by Local Microorganisms. *From Sources to Solution, Singapore*. [https://doi.org/10.1007/978-981-4560-70-2\\_65](https://doi.org/10.1007/978-981-4560-70-2_65)
- Jonstrup, M., Kumar, R. N., Murto, M., & Mattiasson, B. (2011). Sequential anaerobic-aerobic treatment of azo dyes: Decolourisation and amine degradability. *Desalination*, 280, 339-346.  
<https://doi.org/10.1016/j.desal.2011.07.022>

- Junnarkar, N., Murty, D. S., Bhatt, N. S., & Madamwar, D. (2006). Decolorization of diazo dye Direct Red 81 by a novel bacterial consortium. *World Journal of Microbiology and Biotechnology*, 22(2), 163-168.
- Kanmani, P., Satish Kumar, R., Yuvaraj, N., Paari, K. A., Pattukumar, V., & Arul, V. (2011). Optimization of media components for enhanced production of *streptococcus phocae* pi80 and its bacteriocin using response surface methodology. *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*, 42(2), 716-720. <https://doi.org/10.1590/S1517-838220110002000038>
- Kar, S., Tudu, B., Bag, A., & Bandyopadhyay, R. (2018). Application of Near-Infrared Spectroscopy for the Detection of Metanil Yellow in Turmeric Powder. *Food Analytical Methods*, 11. <https://doi.org/10.1007/s12161-017-1106-9>
- Karel, S. F., Libicki, S. B., & Robertson, C. R. (1985). The immobilization of whole cells: Engineering principles. *Chemical Engineering Science*, 40(8), 1321-1354. [https://doi.org/https://doi.org/10.1016/0009-2509\(85\)80074-9](https://doi.org/https://doi.org/10.1016/0009-2509(85)80074-9)
- Karimifard, S., & Alavi Moghaddam, M. R. (2018). Application of response surface methodology in physicochemical removal of dyes from wastewater: A critical review. *Science of The Total Environment*, 640-641, 772-797. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.05.355>
- Kaur, B., Kumar, B., Garg, N., & Kaur, N. (2015). Statistical optimization of conditions for decolorization of synthetic dyes by *Cordyceps militaris* MTCC 3936 using RSM. *BioMed research international*, 2015, 536745-536745. <https://doi.org/10.1155/2015/536745>
- Khan, R., Patel, V., & Khan, Z. (2020). Chapter 5 - Bioremediation of dyes from textile and dye manufacturing industry effluent. In P. Singh, A. Kumar, & A. Borthakur (Eds.), *Abatement of Environmental Pollutants* (pp. 107-125). Elsevier. <https://doi.org/https://doi.org/10.1016/B978-0-12-818095-2.00005-9>
- Khanna, S., & Srivastava, A. K. (2005). Statistical media optimization studies for growth and PHB production by *Ralstonia eutropha*. *Process Biochemistry*, 40(6), 2173-2182. <https://doi.org/https://doi.org/10.1016/j.procbio.2004.08.011>
- Khehra, M. S., Saini, H. S., Sharma, D. K., Chadha, B. S., & Chimni, S. S. (2005). Decolorization of various azo dyes by bacterial consortium. *Dyes and Pigments*, 67(1), 55-61. <https://doi.org/https://doi.org/10.1016/j.dyepig.2004.10.008>
- Kurade, M. B., Waghmode, T. R., Jadhav, M. U., Jeon, B.-H., & Govindwar, S. P. (2015). Bacterial–yeast consortium as an effective biocatalyst for biodegradation of sulphonated azo dye Reactive Red 198

[10.1039/C4RA15834B]. *RSC Advances*, 5(29), 23046-23056.  
<https://doi.org/10.1039/C4RA15834B>

- Lade, H., Govindwar, S., & Paul, D. (2015). Low-Cost Biodegradation and Detoxification of Textile Azo Dye C.I. Reactive Blue 172 by *Providencia rettgeri* Strain HSL1. *Journal of Chemistry*, Article ID 894109, 1-10.  
<https://doi.org/10.1155/2015/894109>
- Landreau, M., Duthoit, F., Claeys-Bruno, M., Vandenaabeele-Trambouze, O., Aubry, T., Godfroy, A., & Le Blay, G. (2016). Entrapment of anaerobic thermophilic and hyperthermophilic marine micro-organisms in a gellan/xanthan matrix. *J Appl Microbiol*, 120(6), 1531-1541.  
<https://doi.org/10.1111/jam.13118>
- Landreau, M., Duthoit, F., Claeys-Bruno, M., Vandenaabeele-Trambouze, O., Aubry, T., Godfroy, A., & Le Blay, G. (2016). Entrapment of anaerobic thermophilic and hyperthermophilic marine microorganisms in a gellan/xanthan matrix. *J Appl Microbiol*, 120, 1531-1541.  
<https://doi.org/10.1111/jam.13118>
- Lázaro, N., Sevilla, A., Morales, S., & Marques, A. (2003). Heavy metal biosorption by gellan gum gel beads. *Water research*, 37, 2118-2126.  
[https://doi.org/10.1016/S0043-1354\(02\)00575-4](https://doi.org/10.1016/S0043-1354(02)00575-4)
- Leelakriangsak, M., & Borisut, S. (2012). Characterization of the decolorizing activity of azo dyes by *Bacillus subtilis* azoreductase AzoR1. *Songklanakarinn Journal of Science and Technology*, 34, 509-516.
- Liu, Y. J., Zhang, A. N., & Wang, X. C. (2009). Biodegradation of phenol by using free and immobilized cells of *Acinetobacter* sp. XA05 and *Sphingomonas* sp. FG03. *Biochemical Engineering Journal*, 44(2), 187-192.  
<https://doi.org/https://doi.org/10.1016/j.bej.2008.12.001>
- Lone, T. A., Revathi, C., & Lone, R. A. (2015). Isolation of dye degrading *Bacillus* species from the soil near dyeing industry and its potential application in dye effluent treatment. *Am-Euras J Toxicol Sci*, 7, 129-135.
- Lucas, J. (2010). Response Surface Methodology: Process and Product Optimization Using Designed Experiments, 3rd edition. *Journal of Quality Technology*, 42, 228-230.  
<https://doi.org/10.1080/00224065.2010.11917819>
- Lucas, M. S., Amaral, C., Sampaio, A., Peres, J. A., & Dias, A. A. (2006). Biodegradation of the diazo dye Reactive Black 5 by a wild isolate of *Candida oleophila*. *Enzyme and Microbial Technology*, 39(1), 51-55.
- Mabrouk, M., & Yusef, H. (2008). Decolorization of fast red by *Bacillus subtilis* HM. *Journal of Applied Sciences Research*, 4, 262-269.
- Mansur, R., Gusmanizar, N., Roslan, M. A. H., Ahmad, S. A., & Shukor, M. Y. (2017). Isolation and Characterisation of a Molybdenum-reducing and



Metanil Yellow Dye-decolourising *Bacillus* sp. strain Neni-10 in Soils from West Sumatera, Indonesia. *Tropical life sciences research*, 28(1), 69-90. <https://doi.org/10.21315/tlsr2017.28.1.5>

Maqbool, Z., Hussain, S., Ahmad, T., Nadeem, H., Imran, M., Khalid, A., Martin-Laurent, F. (2016). Use of RSM modeling for optimizing decolorization of simulated textile wastewater by *Pseudomonas aeruginosa* strain ZM130 capable of simultaneous removal of reactive dyes and hexavalent chromium. *Environmental science and pollution research international*, 23. <https://doi.org/10.1007/s11356-016-6275-3>

Mittal, A., Gupta, V., Malviya, A., & Mittal, J. (2008). Process development for the batch and bulk removal and recovery of a hazardous, water-soluble azo dye (Metanil Yellow) by adsorption over waste materials (Bottom Ash and De-Oiled Soya). *Journal of Hazardous Materials*, 151(2-3), 821-832. <https://doi.org/10.1016/j.jhazmat.2007.06.059>

Mohamad said, K. a., Afizal, M., & Amin, M. (2015). Overview on the Response Surface Methodology (RSM) in Extraction Processes. *Journal of Applied Science & Process Engineering (JASPE)*, 2, 8-17.

Mohana, S., Shrivastava, S., Divecha, J., & Madamwar, D. (2008a). Response surface methodology for optimization of medium for decolorization of textile dye Direct Black 22 by a novel bacterial consortium. *Bioresource Technology*, 99(3), 562-569. <https://doi.org/https://doi.org/10.1016/j.biortech.2006.12.033>

Mohana, S., Shrivastava, S., Divecha, J., & Madamwar, D. (2008b). Response Surface Methodology for Optimization of Medium for Decolorization of Textile Dye Direct Black 22 by a Novel Bacterial Consortium. *Bioresource technology*, 99, 562-569. <https://doi.org/10.1016/j.biortech.2006.12.033>

Mokhtar, M., & Wan Ismail, W. N. S. (2012). Marketing Strategies and the Difference Level of Sales and Profits Performance of the Batik SMEs in Malaysia. *International Journal of business and management*, 7, 96-111. <https://doi.org/10.5539/ijbm.v7n23p96>

Moradi Birgani, P., Ranjbar, N., Che Abdullah, R., Tiek, W., Lee, G., Ibrahim, S., Jang, M. (2016). An efficient and economical treatment for batik textile wastewater containing high levels of silicate and organic pollutants using a sequential process of acidification, magnesium oxide, and palm shell-based activated carbon application. *Journal of Environmental Management*, 184(2), 229-239. <https://doi.org/10.1016/j.jenvman.2016.09.066>

Moslemy, P., Guiot, S., & J. Neufeld, R. (2006). Encapsulation of Bacteria for Biodegradation of Gasoline Hydrocarbons. In: *Guisan J.M.(eds) Immobilization of Enzymes and Cells. Methods in Biotechnology*, 22, 415-426. [https://doi.org/10.1007/978-1-59745-053-9\\_36](https://doi.org/10.1007/978-1-59745-053-9_36)

- Moslemy, P., Guiot, S. R., & Neufeld, R. J. (2006). Encapsulation of bacteria for biodegradation of gasoline hydrocarbons. In *Immobilization of Enzymes and Cells*, 415-426.
- Moslemy, P., Neufeld, R. J., & Guiot, S. R. (2002). Biodegradation of gasoline by gellan gum-encapsulated bacterial cells. *Biotechnology and Bioengineering*, 80(2), 175-184.
- Murugesan, K., Dhamija, A., Nam, I.-H., Kim, Y.-M., & Chang, Y.-S. (2007a). Decolourization of reactive black 5 by laccase: Optimization by response surface methodology. *Dyes and Pigments*, 75, 176-184. <https://doi.org/10.1016/j.dyepig.2006.04.020>
- Murugesan, K., Dhamija, A., Nam, I.-H., Kim, Y.-M., & Chang, Y.-S. (2007b). Decolourization of reactive black 5 by laccase: Optimization by response surface methodology. *Dyes and Pigments*, 75(1), 176-184. <https://doi.org/https://doi.org/10.1016/j.dyepig.2006.04.020>
- Murugesan, K., Kim, Y.-M., Jeon, J.-R., & Chang, Y.-S. (2009). Effect of metal ions on reactive dye decolorization by laccase from *Ganoderma lucidum*. *Journal of Hazardous Materials*, 168(1), 523-529. <https://doi.org/https://doi.org/10.1016/j.jhazmat.2009.02.075>
- Nallapan Maniyam, M., Ibrahim, A., & Cass, T. (2017). Enhanced cyanide biodegradation by immobilized crude extract of *Rhodococcus* UKMP-5M. *Environmental Technology*, 40, 1-41. <https://doi.org/10.1080/09593330.2017.1393015>
- Nallapan maniyam, M., Latif Ibrahim, A., & Cass, T. (2017). Enhanced cyanide biodegradation by immobilized crude extract of *Rhodococcus* UKMP-5M. *Environmental technology*, 40, 1-41. <https://doi.org/10.1080/09593330.2017.1393015>
- Nath, D., Sarkar, K., Tarafder, P., Mondal, M., Das, K., & Paul, G. (2015a). Practice of using metanil yellow as food colour to process food in unorganized sector of West Bengal - A case study. *International Food Research Journal*, 22, 1421-1428.
- Nath, D., Sarkar, K., Tarafder, P., Mondal, M., Das, K., & Paul, G. (2015b). Practice of using metanil yellow as food colour to process food in unorganized sector of West Bengal - A case study. *International Food Research Journal*, 22, 1424-1428.
- National Center for Biotechnology Information. *Metanil yellow*, CID=3935589, Pubchem Database. <https://pubchem.ncbi.nlm.nih.gov/compound/Metanil-yellow>
- Oh, Y.-S., Maeng, J. S., & Kim, S.-J. (2000). Use of microorganism-immobilized polyurethane foams to absorb and degrade oil on water surface. *Applied Microbiology and Biotechnology*, 54, 418-423.

- Olaniran, A. O., Balgobind, A., & Pillay, B. (2009). Impacts of heavy metals on 1,2-dichloroethane biodegradation in co-contaminated soil. *Journal of Environmental Sciences*, 21(5), 661-666. [https://doi.org/https://doi.org/10.1016/S1001-0742\(08\)62322-0](https://doi.org/https://doi.org/10.1016/S1001-0742(08)62322-0)
- Olaniran, A. O., Balgobind, A., & Pillay, B. (2013). Bioavailability of Heavy Metals in Soil: Impact on Microbial Biodegradation of Organic Compounds and Possible Improvement Strategies. *International Journal of Molecular Sciences*, 14(5). <https://doi.org/10.3390/ijms140510197>
- Ozdemir, G., Pazarbasi, B., Kocyigit, A., Omeroglu, E. E., Yasa, I., & Karaboz, I. (2008). Decolorization of Acid Black 210 by *Vibrio harveyi* TEMS1, a newly isolated bioluminescent bacterium from Izmir Bay, Turkey. *World journal of microbiology & biotechnology*, 24(8), 1375-1381. <https://doi.org/10.1007/s11274-007-9619-9>
- P Shah, M., Ka, P., Ss, N., & M Darji, A. (2014). Microbial Decolorization of Textile Dye by *Bacillus* Spp. ETL-79: An Innovative Biotechnological Aspect to Combat Textile Effluents. *American Journal of Microbiological Research*, 1(3), 57-61. <https://doi.org/10.12691/ajmr-1-3-5>
- Pandey, A., Gaur, V., Udayan, A., Varjani, S., Kim, S.-H., & Wong, J. (2021). Biocatalytic remediation of industrial pollutants for environmental sustainability: Research needs and opportunities. *Chemosphere*, 272, 129936. <https://doi.org/10.1016/j.chemosphere.2021.129936>
- Pandey, A., Singh, P., & Iyengar, L. (2007). Bacterial decolorization and degradation of azo dyes. *International Biodeterioration & Biodegradation*, 59(2), 73-84. <https://doi.org/https://doi.org/10.1016/j.ibiod.2006.08.006>
- Pang, Y., & Abdullah, A. Z. (2013). Current Status of Textile Industry Wastewater Management and Research Progress in Malaysia: A Review. *Clean Soil Air Water*, 41, 751-764. <https://doi.org/10.1002/clen.201000318>
- Partovinia, A., & Rasekh, B. (2018). Review of the immobilized microbial cell systems for bioremediation of petroleum hydrocarbons polluted environments. *Critical Reviews in Environmental Science and Technology*, 48(1), 1-38. <https://doi.org/10.1080/10643389.2018.1439652>
- Parvin, F., Rahman, M., Islam, M., Jahan, N., Pallob, M., Shaekh, E., Salah Uddin, M. (2015). Isolation of Mixed Bacterial Culture from Rajshahi Silk Industrial Zone and Their Efficiency in Azo Dye Decolorization. *Indian Journal of Science and Technology*, 8(10), 950-957. <https://doi.org/10.17485/ijst/2015/v8i10/57152>
- Patil, P. S., Shedbalkar, U. U., Kalyani, D. C., & Jadhav, J. P. (2008). Biodegradation of Reactive Blue 59 by isolated bacterial consortium PMB11. *Journal of Industrial Microbiology & Biotechnology*, 35(10), 1181-1190. <https://doi.org/10.1007/s10295-008-0398-6>



- Peinado, R. A., Moreno, J. J., Maestre, O., & Mauricio, J. C. (2005). Use of a Novel Immobilization Yeast System for Winemaking. *Biotechnology Letters*, 27(18), 1421-1424. <https://doi.org/10.1007/s10529-005-0939-2>
- Permana, R., Ihsan, Y. N., & Rizal, A. (2021). Bioremediation Enhancement of Oil-Polluted Water by Bacterial Immobilization: A Review. *World Scientific News*, 159, 210-223.
- Pokharia, A., & Singh, S. (2016). Biodecolorization and degradation of xenobiotic azo dye -Basic Red 46 by *Staphylococcus epidermidis* MTCC 10623. 5, 10-2310.
- Purba, M., & Shukla, S. (2015). Detection of Non-Permitted Food Colors in Edibles. *Journal of Forensic Research*, s4. <https://doi.org/10.4172/2157-7145.1000S4-003>
- Puvaneswari, N., Jayarama, M., & Gunasekaran, P. (2006). Toxicity assessment and microbial degradation of azo dyes. *Indian J Exp Biol*, 44(8).
- Rahman, S., Alif, F., & Hossain, M. (2018). Optimization of conditions for the biological treatment of textile dyes using isolated soil bacteria. *F1000Research*, 7, 351. <https://doi.org/10.12688/f1000research.13757.1>
- Raj, D., Prabha, R., & Leena, R. (2012). Analysis of bacterial degradation of azo dye congo red using HPLC. *Journal of Industrial Pollution Control*, 28, 57-62.
- Ramchandani, S., Das, M., Joshi, A., & K. Khanna, S. (1997). Effect of Oral and Parenteral Administration of Metanil Yellow on Some Hepatic and Intestinal Biochemical Parameters. *J. Appl. Toxicol*, 17, 85-91. [https://doi.org/10.1002/\(SICI\)1099-1263\(199701\)17:1<85::AID-JAT394>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1099-1263(199701)17:1<85::AID-JAT394>3.0.CO;2-K)
- Ramchandani, S., Das, M., & Khanna, S. K. (1994). Effect of metanil yellow, orange II and their blend on hepatic xenobiotic metabolizing enzymes in rats. *Food and Chemical Toxicology*, 32(6), 559-563. [https://doi.org/https://doi.org/10.1016/0278-6915\(94\)90114-7](https://doi.org/https://doi.org/10.1016/0278-6915(94)90114-7)
- Rathore, S., Desai, P. M., Liew, C. V., Chan, L. W., & Heng, P. W. S. (2013). Microencapsulation of microbial cells. *Journal of Food Engineering*, 116(2), 369-381. <https://doi.org/https://doi.org/10.1016/j.jfoodeng.2012.12.022>
- Sachdeva, S. M., Mani, K. V., Adaval, S. K., Jalpota, Y. P., Rasela, K. C., & Chadha, D. S. (1992). Acquired toxic methaemoglobinaemia. *The Journal of the Association of Physicians of India*, 40(4), 239-240. <http://europepmc.org/abstract/MED/1452530>
- Santa Cruz Biotechnology, I. (2010, June 10). *Metanil Yellow (Material Safety Data Sheet)*. <http://datasheets.scbt.com/sc-215306.pdf>

- Saratale, R. G., Saratale, G. D., Chang, J. S., & Govindwar, S. P. (2011). Bacterial decolorization and degradation of azo dyes: A review. *Journal of the Taiwan Institute of Chemical Engineers*, 42(1), 138-157. <https://doi.org/https://doi.org/10.1016/j.jtice.2010.06.006>
- Saratale, R. G., Saratale, G. D., Kalyani, D. C., Chang, J. S., & Govindwar, S. P. (2009). Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium-GR. *Bioresource Technology*, 100(9), 2493-2500. <https://doi.org/https://doi.org/10.1016/j.biortech.2008.12.013>
- 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*, 2(4), 121-131. <https://doi.org/10.1007/s41101-017-0031-5>
- Shah, M. P., Patel, K. A., Nair, S. S., & Darji, A. (2013). Microbial decolorization of methyl orange dye by *Pseudomonas* spp. ETL-M. *International Journal of Environmental Bioremediation and Biodegradation*, 1(2), 54-59.
- Sharma, D. C., & Satyanarayana, T. (2006). A marked enhancement in the production of a highly alkaline and thermostable pectinase by *Bacillus pumilus* dcsr1 in submerged fermentation by using statistical methods. *Bioresource Technology*, 97(5), 727-733. <https://doi.org/https://doi.org/10.1016/j.biortech.2005.04.012>
- Sharma, P., Singh, L., & Dilbaghi, N. (2009). Optimization of process variables for decolorization of Disperse Yellow 211 by *Bacillus subtilis* using Box-Behnken design. *J Hazard Mater*, 164(2), 1024-1029. <https://doi.org/https://doi.org/10.1016/j.jhazmat.2008.08.104>
- Shi, S., Qu, Y., Ma, F., & Zhou, J. (2014). Bioremediation of cooking wastewater containing carbazole, dibenzofuran and dibenzothiphenone by immobilized naphthalene-cultivated *Arthrobacter* sp. W1 in magnetic gellan gum. *Bioresource Technology*, 166, 79-86. <https://doi.org/https://doi.org/10.1016/j.biortech.2014.05.036>
- Singh, M., Saini, H., Sharma, D., Chadha, B., & Chimni, S. (2005). Decolorization of Various Azo Dyes by Bacterial Consortium. *Dyes and Pigments*, 67, 55-61. <https://doi.org/10.1016/j.dyepig.2004.10.008>
- Singh, P., & Lakhani Singh, R. (2017). Bio-removal of Azo Dyes: A Review. *International Journal of Applied Sciences and Biotechnology*, 5(2), 108-126. <https://doi.org/10.3126/ijasbt.v5i2.16881>
- Singh Rai, H., Mani, Bhattacharyya, S., Singh, J., Bansal, T. K., Vats, P., Banerjee, P. (2005). Removal of Dyes from the Effluent of Textile and Dyestuff Manufacturing Industry: A Review of Emerging Techniques With Reference to Biological Treatment. *Critical Reviews in Environmental Science and Technology*, 35, 219-238. <https://doi.org/10.1080/10643380590917932>

- Singh, R. P., Singh, P. K., & Singh, R. L. (2014). Bacterial Decolorization of Textile Azo Dye Acid Orange by *Staphylococcus hominis* RMLRT03. *Toxicology international*, 21(2), 160-166. <https://doi.org/10.4103/0971-6580.139797>
- Sivashankar, R., Velmurugan, S., A.B.Sathya, & Pallipad, S. (2013). Biosorption of Hazardous Azo Dye Metanil Yellow using Immobilized Aquatic weed.
- Sleiman, M., Vildoza, D., Ferronato, C., & Chovelon, J.-M. (2007). Photocatalytic degradation of azo dye Metanil Yellow: Optimization and kinetic modeling using a chemometric approach. *Applied Catalysis B: Environmental*, 77(1), 1-11. <https://doi.org/https://doi.org/10.1016/j.apcatb.2007.06.015>
- Srikanlayanukul, M., Khanongnuch, C., & Lumyong, S. (2006). Decolorization of Textile Wastewater by Immobilized *Coriolus versicolor* RC3 in Repeated-Batch System with the Effect of Sugar Addition. *CMU J*, 5.
- Stolz, A. (2001). Basic and Applied Aspects in the Microbial Degradation of Azo Dyes. *Applied Microbiology and Biotechnology*, 56, 69-80. <https://doi.org/10.1007/s002530100686>
- Subki, N. S. (2011). A Preliminary Study on Batik Effluent in Kelantan State: A Water Quality Perspective.
- Sudha, M., Saranya, A., Gopal, S., & Natesan, S. (2014). Microbial degradation of Azo Dyes: A review. *Int. J. Curr. Microbiol. App. Sci*, 3, 670-690.
- Suganya, K., & Revathi, K. (2016). Decolorization of Reactive Dyes by Immobilized Bacterial Cells from Textile Effluents. *International Journal of Current Microbiology and Applied Sciences*, 5, 528-532. <https://doi.org/10.20546/ijcmas.2016.501.053>
- Sun, J., Liu, J., Liu, Y., Li, Z., & Nan, J. (2011). Optimization of Entrapping Conditions of Nitrifying Bacteria and Selection of Entrapping Agent. *Procedia Environmental Sciences*, 8, 166-172. <https://doi.org/10.1016/j.proenv.2011.10.027>
- Sun, W., & Griffiths, M. W. (2000). Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan-xanthan beads. *International Journal of Food Microbiology*, 61(1), 17-25. [https://doi.org/https://doi.org/10.1016/S0168-1605\(00\)00327-5](https://doi.org/https://doi.org/10.1016/S0168-1605(00)00327-5)
- Survase, S., Annapure, U., & S Singhal, R. (2010). Gellan Gum as Immobilization Matrix for Production of Cyclosporin A. *J Microbiol Biotechnol*, 20(7). <https://doi.org/10.4014/jmb.1001.01006>
- Survase, S., Annapure, U., & Singhal, R. (2010). Gellan Gum as Immobilization Matrix for Production of Cyclosporin A. *Journal of microbiology and biotechnology*, 20, 1086-1091. <https://doi.org/10.4014/jmb.1001.01006>

The columbia encyclopedia, t. E. *Adulteration of food*. Retrieved 22 September from <https://www.encyclopedia.com/sports-and-everyday-life/food-and-drink/food-and-cooking/food-adulteration>

Thomas, T., Jack, G., & Meyer, F. (2012). Metagenomics - A guide from sampling to data analysis. *Microb Informatics*, 2(3). <https://doi.org/10.1186/2042-5783-2-3>

Tony, B. D., Goyal, D., & Khanna, S. (2009). Decolorization of textile azo dyes by aerobic bacterial consortium. *International Biodeterioration & Biodegradation*, 63(4), 462-469. <https://doi.org/https://doi.org/10.1016/j.ibiod.2009.01.003>

Toolabi, A., Malakootian, M., Taghi Ghaneian, M., Esrafil, A., Ehrampoush, M., Askarishahi, M., Khatami, M. (2018). Optimizing the photocatalytic process of removing diazinon pesticide from aqueous solutions and effluent toxicity assessment via a response surface methodology approach. *Rend. Fis. Acc. Lincei*, 30, 155-165. <https://doi.org/10.1007/s12210-018-0751-2>

Unnikrishnan, S., Ramamoorthi, P., & S, D. S. (2015). Studies on decolorization of malachite green using immobilized *Pseudomonas putida*. *J. Chem. Pharm. Res*, 7(12), 589-596.

Vitor, V., & Corso, C. R. (2008). Decolorization of textile dye by *Candida albicans* isolated from industrial effluents. *Journal of Industrial Microbiology & Biotechnology*, 35(11), 1353-1357.

Wan Mohd Khalik, W. F., Ho, L.-N., Ong, S.-A., Wong, Y., Nik Yusoff, N. N. A., & Ridwan, F. (2015). Decolorization and Mineralization of Batik Wastewater through Solar Photocatalytic Process. *Sains Malaysiana*, 44(4), 607-612. <https://doi.org/10.17576/jsm-2015-4404-16>

Wang, H., Su, J., Zheng, X., Tian, Y., Xiong, X., & Zheng, T. (2009). Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by *Citrobacter* sp. CK3. *International Biodeterioration & Biodegradation*, 63(4), 395-399. <https://doi.org/10.1016/j.ibiod.2008.11.006>

Wang, X., Gai, Z., Yu, B., Feng, J., Xu, C., Yuan, Y., Xu, P. (2007). Degradation of Carbazole by Microbial Cells Immobilized in Magnetic Gellan Gum Gel Beads. *Applied and Environmental Microbiology*, 73(20), 6421. <https://doi.org/10.1128/AEM.01051-07>

WU, K.-S., WU, A.-B., HUANG, M.-C., & CHEN, C.-Y. (1999). Identification of illegal coal tar dyes constituents in mucous cosmetics by HPLC method. *Journal of Food and Drug Analysis*, 7(2).

Yang, P. Y., Cai, T., & Wang, M.-L. (1988). Immobilized mixed microbial cells for wastewater treatment. *Biological Wastes*, 23(4), 295-312. [https://doi.org/https://doi.org/10.1016/0269-7483\(88\)90017-1](https://doi.org/https://doi.org/10.1016/0269-7483(88)90017-1)

- Zaharia, C., & Suteu, D. (2012). Textile Organic Dyes; Characteristics, Polluting Effects and Separation/Elimination Procedures from Industrial Effluents; A Critical Overview. In: T. Puzyn, & A. Mostrag-Szlichtyng (Eds). *Organic Pollutants Ten Years After the Stockholm Convention- Environmental and Analytical Update*. <https://doi.org/10.5772/32373>
- Zaharia, C., Suteu, D., Muresan, A., Muresan, R., & Popescu, A. (2009). Textile wastewater treatment by homogenous oxidation with hydrogen peroxide. *Environmental engineering and management journal*, 8, 1359-1369. <https://doi.org/10.30638/eemj.2009.199>
- Zhang, Z. Y., Pan, L. P., & Li, H. H. (2010). Isolation, identification and characterization of soil microbes which degrade phenolic allelochemicals. *Journal of Applied Microbiology*, 108(5), 1839-1849.
- Zheng, Y., & Wang, A. (2010). Removal of heavy metals using polyvinyl alcohol semi-IPN poly(acrylic acid)/tourmaline composite optimized with response surface methodology. *Chemical Engineering Journal*, 162(1), 186-193. <https://doi.org/https://doi.org/10.1016/j.cej.2010.05.027>