iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** IV **Month of publication:** April 2019

DOI: https://doi.org/10.22214/ijraset.2019.4092

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 7 Issue IV, Apr 2019- Available at www.ijraset.com

Microbial Degradation of Methylene Blue Dye Bye Bacterial Strain

Komal Sisodiya[1], Chirag Shah[2], Megha Fulwadiya[3], Krisha Talati[4], Linz-Buoy George[5]
[1,2,3,4]*Department of Environmental Science, Government Science College, Ahmedabad-380026*
[5]*Department of Zoology, Gujarat University, Ahmedabad.*

Abstract: ***In the present study the untreated wastewater produce from the dye industry. In the textile industry azo dyes are used widely. Textile industry is removing the wastewater which is containing color, and it is very serious issue. Manufacturing of different types of dyes. The physic-chemical process is available and also biological process can be applied. In this task bacteria are used to degrade the methylene blue (mb). During the procedure the bacterial organisms are grow and found the decolorization. By the using of surface methodology optimization of dye decolorization at different dye concentration and also optimization of inoculums load of dye decolorization. The wastewater analyzed for – carbon sources, nitrogen sources, ph, static & shaking condition, temperature toxicity, and etc. After the treatment of wastewater the sludge are generated in high amount by the study. In industry many stages of manufacturing process are produced wastewater. This wastewater is not discharged properly by the norms of GPCB. Wastewater contains toxic substances. This toxic substance is harm to aquatic organisms as well as animals. Under the natural condition they are not easy degrade. By the treatment system the toxicity are removed by the waste.***

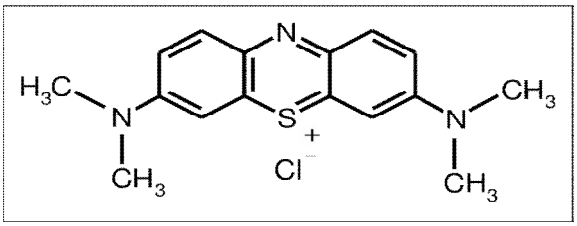
Keywords: Degradation, Microbes, Dye.

I. INTRODUCTION

A **dye** is a colored substance that has an affinity to the substrate to which it is being applied. The dye is generally applied in an aqueous solution, and may require a mordant to improve the fastness of the dye on the fibre.[1] Both dyes and pigments are colored, because they absorb only some wavelengths of visible light. Dyes are usually soluble in water whereas pigments are insoluble. Some dyes can be rendered insoluble with the addition of salt to produce a lake pigment. The majority of natural dyes are derived from plant sources: roots, berries, bark, leaves, and wood, fungi, and lichens.[2] Most dyes are synthetic, i.e., are man-made from petrochemicals. Other than pigmentation, they have a range of applications including organic dye lasers,[3] optical media (cd-r) and camera sensors (color filter array). Dyes produced by the textile, printing and paper industries can end up in waste waters and are therefore a potential source of pollution of rivers and waterways[4].various porous materials, often used to adsorb harmful chemicals in general, have been specifically tested to remove dyes from aqueous environments, especially those who could combine wide availability, fast kinetics and strong adsorption capacities.[5] possible examples include nickel oxide nano plates[6], clays[7], activated carbons[8], composites of hydroxyl compound with organic substrates [9][10], grapheme[11] . Due to rapidly increase in developing technology, population and urbanization there is tremendous increase in pollution. In almost every country air, water, soil pollution is increasing and there is decrease of fertility in soil, the ratio of solid wastes is increasing, the decrease of energy and minerals. The problem arise from increasing urbanization, industrialization etc and due to this the atmosphere is polluted, there is bad quality of the drinking water, the decrease in the areas of greenery, parks and generally the damage is done by local people. Nowadays, different types of pollutants and synthetic chemicals are encountered in the environment due to population growth and industrial development[12-14] synthetic dyes like of conventional wastewater treatment systems methylene blue(mb) are chemical pollutants which are widely employed in textile, paper, leather, cosmetic, plastic, printing and food industries.[15-17]textile industries consume large volume of water during dying process. Consequently, large volume of dyed wastewater is produced. this wastewater must be treated according to environmental regulations until dye concentration is reduced to an acceptable level in effluent.[18-19]dyed materials of industrial wastewaters are important due to toxicity on aquatic organisms, disorder in the performance and aesthetic environment. given that discharging dyed industrial wastewater in receiving water resources can lead to eutrophication and disturbance in ecology, removal of dye from wastewater and aquatic environments is essential for protection of human health and the environment.

©IJRASET: All Rights are Reserved

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 7 Issue IV, Apr 2019- Available at www.ijraset.com



Methylene Blue is A Basic Aniline Dye With The Molecular Formula $c_{16}h_{18}n_3scl$. At Room Temperature, It Appears As A Solid, Odourless, Dark Green Powder That Yields A Blue Solution When Dissolved In Water. It Has Many Uses In A Number Of Different Fields. For Instance, Chemists Use It To Detect Oxidizing Agents And Biologists Use It To Stain Tissue Samples And Detect Nucleic Acids. In Medicine, It Is Used As A Treatment For Various Illnesses And Disorders, Including Methemoglobinemia, Schizophrenia, Kidney Stones, And Herpes Infections. In Aquaculture, It Is Used To Prevent Freshwater Fish Eggs From Being Infected By Bacteria And Fungi. Trade Names For Methylene Blue Include Desmoid Piller, Pantone. Its Synonyms Include Phenothiazine-5-Ium, 3, 7-Bis (Dimethylamino), Chloride (9CI). Methylene Blue Should Not Be Confused With Methyl Blue, Another Histology Stain, New Methylene Blue, Nor With The Methyl Violets Often Used As Ph Indicators.

II. MATERIALS AND METHODS

A. Dye Sample

Methylene blue (azoic dye) using as model dye.

B. Media

The medium used for isolation and enrichment were nutrient agar medium & complex medium of glucose (1.0gm), peptone (0.5gm), yeast extracts (0.6gm) and glucose peptone yeast agar.

C. Primary Screening of Dye Decolorizing Microorganism

A set of 15 test tubes containing glucose peptone yeast extract (GPY) medium were taken. in all tubes 1ml of 1mm concentration of methylene blue dye was added. the ph was maintained at 7.0 and autoclaved (121^0c, 15lbs, 15min) as per following table different concentration of dyestuff soil sample were added.

D. Secondary Screening of Dye Decolorizing Microorganism

The GPY plate containing 1 mm methylene blue was prepared. The culture was taken from the GPY tube showing maximum decolorization and was spreaded on prepared GPY plate and incubated. After incubation, the plate was observed for zone of decolorization.

E. Optimization of Dye Decolorization at different Dye Concentration

A set of test tubes containing 10ml G.P.Y. medium with different concentration of dyes (10mm to 50mm) was steam sterilized (121^0c, 15 lbs, 15min). The tubes were inoculated with 18 hrs old culture and incubated at 37c for 24 hours. A set of tubes was harvested after 24 and 48 hours and content of the tubes were spun (7000rpm, 15 min) and supernatant was analyzed for any residual dye was estimated spectrophotometrically for residual dye at a 596 nm.

F. Optimization of different Carbon Source Concentration for Dye Decolorization

A set of tubes containing different sugar in like (maltose, lactose, sucrose, dextrose, starch, cellulose) were prepared. We prepared control tubes from each sugar. In control inoculums was not added and dye concentration was same in all tubes. A set of test tubes containing 10ml of medium containing dye were steam sterilized (121^0c, 15lbs, 15min). The tubes were inoculated with 18 hours old culture and incubated at 37^0c. The tubes were spun (7000rpm, 15 min) and supernatant was analyzed for spectrophotometrically for residual dye at a596 nm. And next day's observed result in which tubes sugar was faster decolorized in among all.

G. Optimization of different Nitrogen Source Concentration for Dye Decolorization

A set of tubes containing different nitrogen source like (peptone, NaCl, urea, yeast, NH_4NO_3, NH_4Cl) were prepared. We prepared control tubes from each nitrogen source. In control inoculums was not added and dye concentration was same in all tubes. A set of

©IJRASET: All Rights are Reserved

test tubes containing 10ml of medium with dye were stem sterilized (121^0c, 15lbs, 15min). The tubes were inoculated with 18 hours old culture and incubated at 37^0c. The tubes were spun (7000rpm, 15 min) and supernatant was analyzed for spectrophotometrically for residual dye at 596 nm.

H. Influence of pH on Dye Decolorization

A set of test tubes containing 10 ml of GPY medium with dye having ph 3-11 were steam sterilized (121^0c, 15 lbs, 15 min) the tubes were inoculated with 18 hours old culture and incubated at 37^0c. Samples were collected after 24 and 48 hours and spun (7000rpm, 15 min) and the supernatant was analyzed spectrophotometricaly for residual dye at 596 nm.

I. Influence of Working Condition [Static & Shaking Condition]

A set of 250 ml flask two containing 100ml GPY medium with dye were steam sterilized (121^0c, 15 lbs, 15 min). The two flasks were inoculated with 18 hours old culture one flask was incubated on shaker (200 rpm) in shaking condition while other was incubated at room temperature in static condition. Contents of the flask were spun (7000rpm, 15 min) and the supernatant was analyzed Spectrophotometricaly for residual dye at 596 nm.

J. Influence of Temperature on Dye Decolourization

A set of test tubes containing 10 ml of GPY medium with dye at different temperature 6°c, 20°c, 37°c, 57°c were steam sterilized (121^0c, 15 lbs, 15 min) the tubes were inoculated with 18 hours old culture and incubated at 37^0c. Samples were collected after 24 and 48 hours and spun (7000rpm, 15 min) and the supernatant was analyzed spectrophotometricaly for residual dye at 596 nm.

K. Influence of Toxicity on Dye Decolorization

A set of test tubes containing 10 ml of GPY medium with dye in presence of different toxic substances that are cadmium, zinc, lead, manganese were use 1gm% and sterilized (121^0c, 15 lbs, 15 min) the tubes were inoculated with 18 hours old culture and incubated at 37^0c. Samples were collected after 24 and 48 hours and spun (7000rpm, 15 min) and the supernatant was analyzed spectrophotometricaly for residual dye at 596 nm.

III. RESULTS

A. Primary Screening of Dye Decolorizing Organism

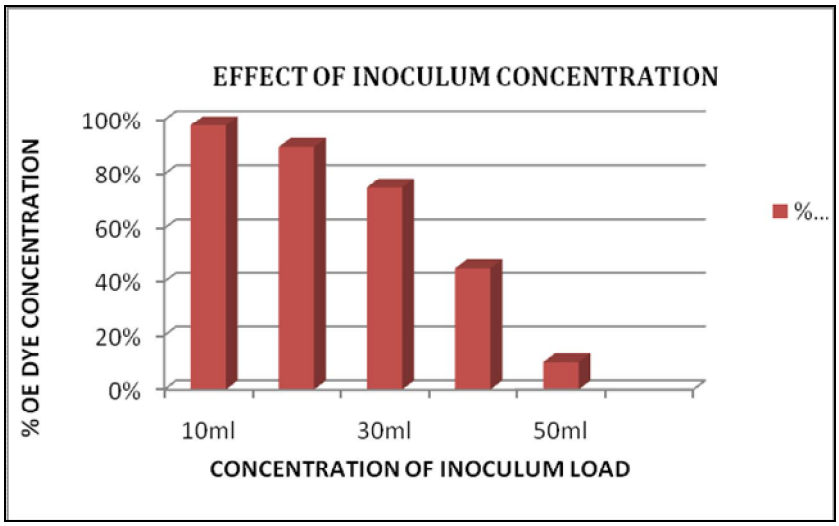
Growth and decolorization of dye occurred in the enrichment culture growing on GPY (Glucose, Peptone, Yeast extract) medium containing dye methylene blue as model dye. A set in which 10 ml soil sample was added got decolorized but the set containing 0.5 ml soil sample were unable to decolorize the dye. The results were only observed in 10 ml soil sample containing set which contain our organism of choice for decolorizing the dye. Now it is transfer on GPY agar plate for further work.

B. Secondary Screening of Potent Dye Decolorizing Organism

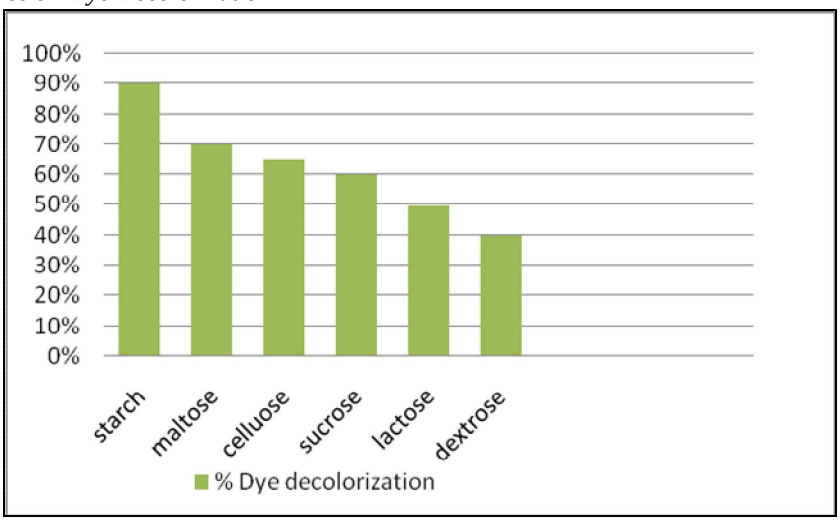
Growth and decolorization of dye occurred in the enrichment culture growing On GPY (Glucose, Peptone, Yeast extract) medium containing dye Methylene Blue as model dye. The dye decolorizing organism giving the decolorization Zone on Petri dish. In control plate culture was not added. The culture of isolated bacteria gives highest (95%) dye decolorization within 24 hours. The isolate had good ability for dye decolorization from 95% at dye concentration of 10mm/ 10ml within 48 hours.

C. Effect of Inoculums Load at different Concentration

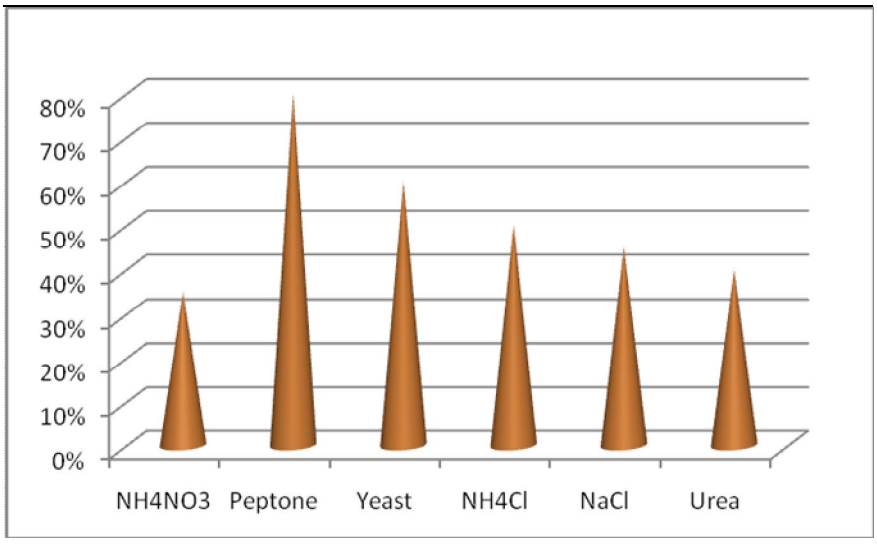
©IJRASET: All Rights are Reserved



D. Effect of different Carbon Source on Dye Decolorization

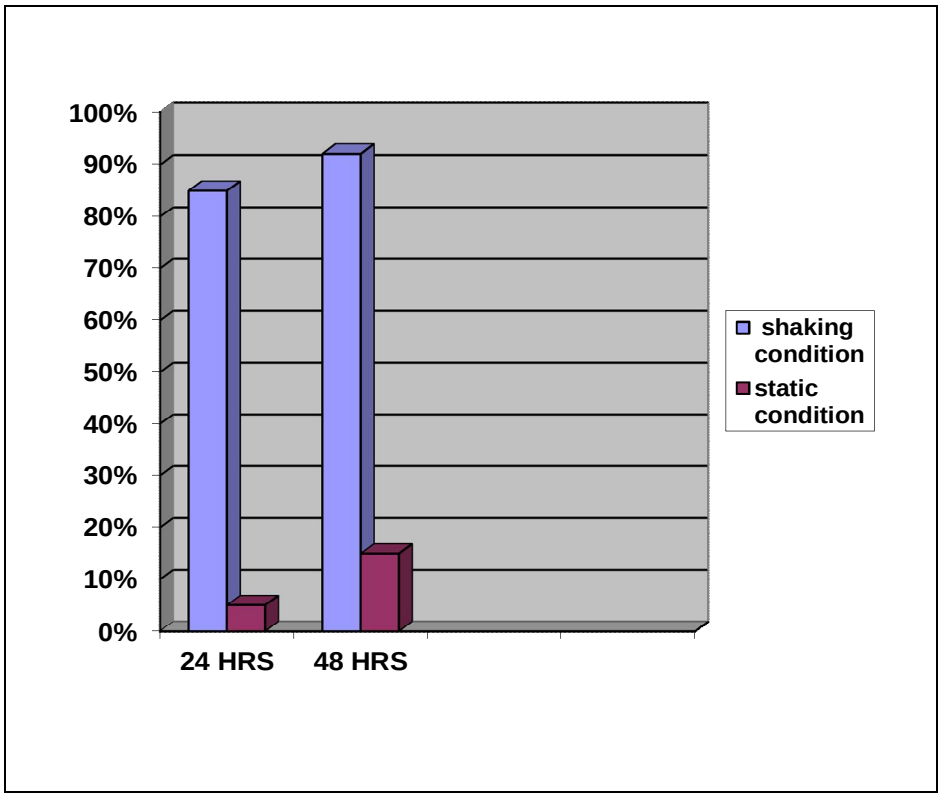


E. Effect of different Nitrogen Source

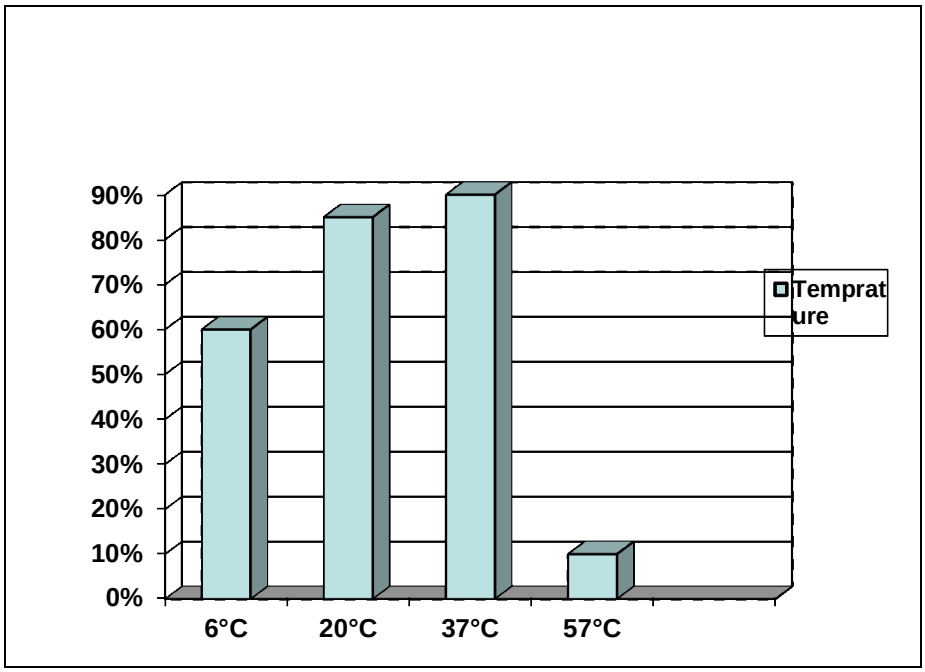


©IJRASET: All Rights are Reserved

F. Effect of Culture Condition on Dye Decolorization [Shaking & Static Condition]

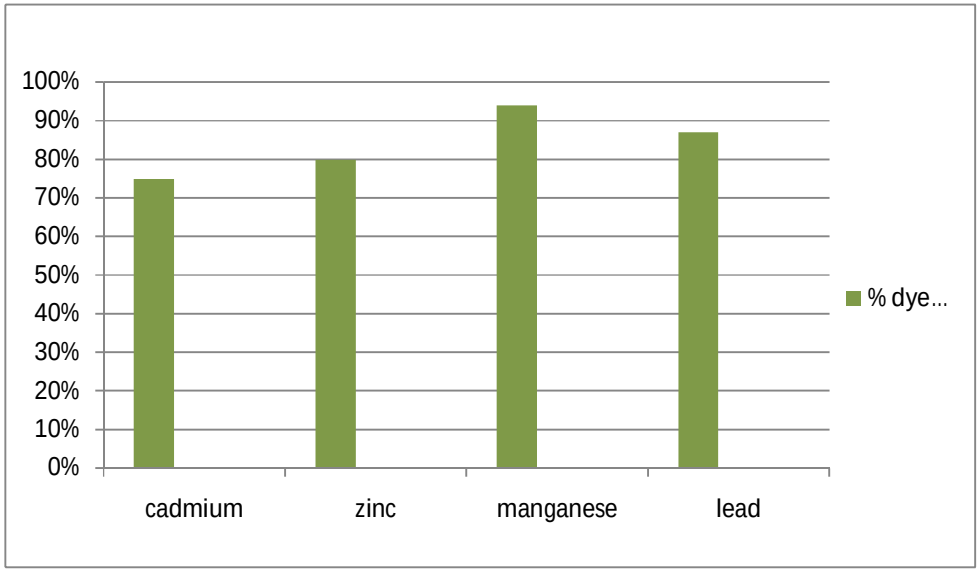


G. Effect of Temperature on Dye Decolorization



©IJRASET: All Rights are Reserved

H. Effect of Toxicity on Dye Decolorization



IV. DISCUSSION

Effect of optimization of various parameters for decolorization of methylene blue dye using bacterial culture:

A. Optimization of Carbon Source

The effect of lactose, maltose, starch, cellulose, dextrose and starch was studied and the results showed maximum decolorization and degradation occurred with starch at the concentration of 1gm%.However other sugar appeared to support the decolorization activity, each at the concentration of 1gm% (w/v), during the initial phase of the life cycle. The second best carbon source was found to be maltose at the concentration of 1gm%. The different carbon source like starch, cellulose, dextrose, sucrose, lactose and maltose were in combination with Yeast Extract, at the concentration of 1gm%.

B. Optimization of Nitrogen Source

Effect of various nitrogen on decolorization of methylene blue was studied using organic source like Peptone, NH_4NO_3, Yeast, NH_4Cl, Urea, NaCl each at concentration of 1gm %(w/v). Peptone, at the concentration of 1gm% was found to be giving the highest decolorization activity followed by Yeast, NH_4Cl, NaCl, Urea, NH_4NO_3 at the concentration 1gm%(w/v) in 24 hrs. So peptone at the concentration of 1gm% (w/v) was selected as N-source of further experiments.

C. Effect of Initial Dye Concentration on Decolorization

The decolorization of Methylene Blue was studied at various increasing concentration of dye i.e from 10mM, 20mM, 30mM, 40mM, 50mM/L. We found that the rate of decolorization was decreased with increasing concentration of dye.

D. Decolorization at various pH

Bacillus strain is able to grow over a wide range of pH. Decolourization increased as the initial pH of the medium was raised. Decolourization of the dyes in the acidic pH of the medium was lowered. The strain also decolorized the dyes over a broad pH range but decolorizing activity occurs optimally in the alkaline pH. Highest decolorization was observed at pH 7.0 in 24 hrs.

E. Decolorization at Static & Shaking Condition

In shaking condition 85% of decolorization of Methylene Blue (10mM/ml) occurs. But in static condition only 5% of decolorization occurs. Thus best culture condition for the highest decolorization activity was as follow:

Carbon source = starch, at the concentration of 1gm%, Nitrogen source = peptone, at the concentration of 1gm%, Medium pH= 7.0, Growth temperature = 37^0C, Methylene Blue dye, concentration = 1mM

©IJRASET: All Rights are Reserved

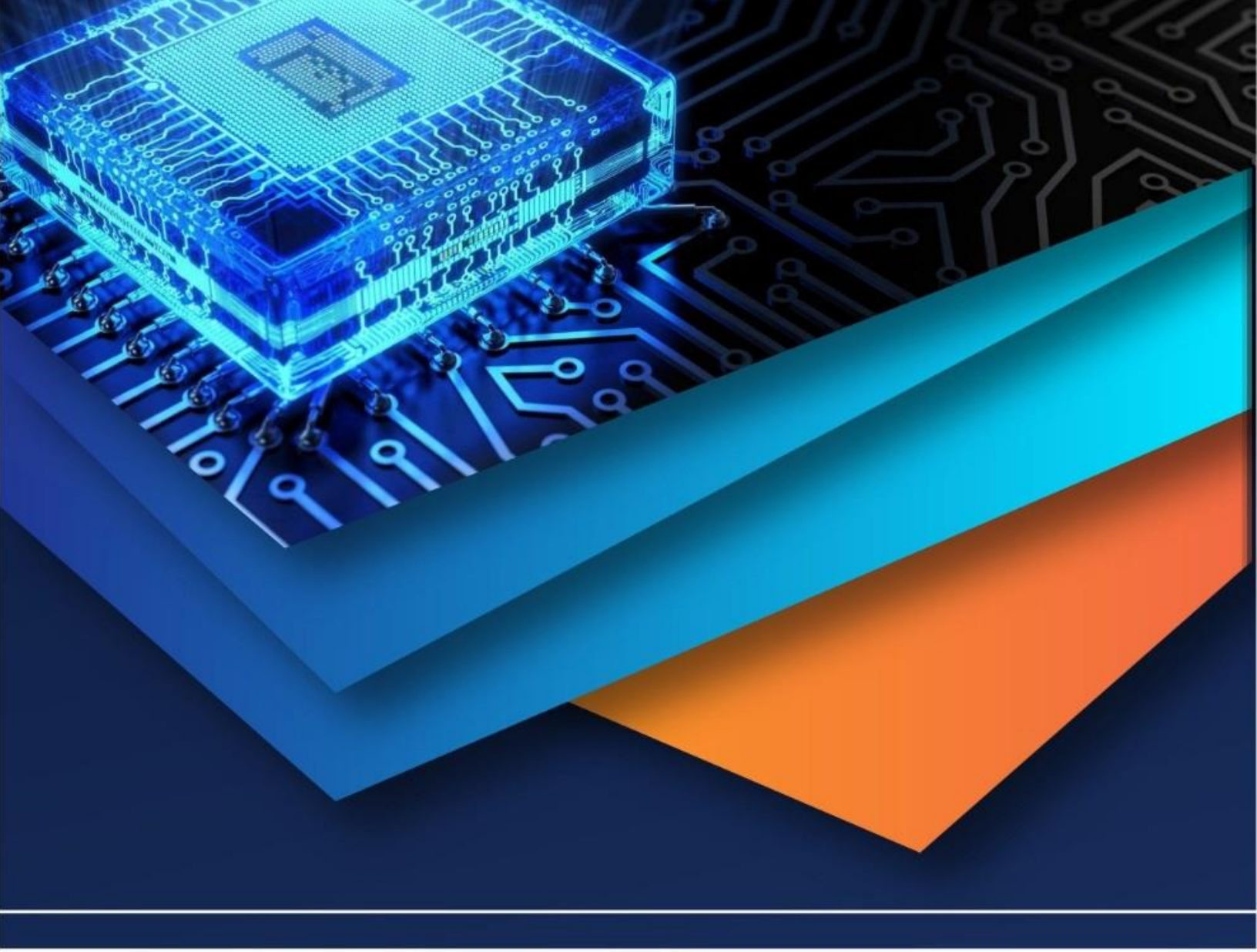
V. CONCLUSION

The selected isolate which was identified as *Bacillus* strain CN-25 gave complete decolorization of Methylene Blue dye within 24 hours. The isolated culture can decolorize Methylene Blue concentration up to 30mM in natural condition. 2ml inoculums dose of Bacillus culture is required for efficient decolorization. Starch (1g %) is found to be the best Carbon source while Peptone (1g %) As well as Yeast (1g %) were found as the best Nitrogen source for maximum biodecolorization procedure. Methylene Blue was decolorized within 24hrs at pH 7.0. Results also indicate better decolorization under Shaking condition rather than Static condition.

REFRENCES

[1] Arminder Kaur, Siddharth Vats, Sumit Rekhi, AnkitBhardwaj, JharnaGoel, Ranjeet S. Tanwar and Komal. K.Gaur, Procedia Environmental Sciences, **2010**, 2, 595–599.
[2] Thoker Farook Ahmed, Manderia Sushil1 and Manderia Krishna, *International Research Journal of Environment Sciences*, **2012**, 1(2), 41-45.
[3] N. Manikandan, S. SurumbarKuzhali and R. Kumuthakalavalli, *J. Microbiol. Biotech. Res.*,**2012**, 2 (1), 57-62.
[4] K. Rajeswari, R. Subashkumar and K. Vijayaraman, *J. Microbiol. Biotech. Res.*, **2013**, 3 (5), 37-41.
[5] K. Varunprasath and A.N. Daniel, *Iranica. J. Energy Environ.*, **2010**, 1, 315-320.
[6] D. Suteu, C. Zaharia, D. Bibla, A. Muresan, R. Muresan and A. Popescu, *Industria Textila*, **2009**, 5, 254-263.
[7] Praveen Sharma, G.R. Chaudry and Thomes Edison, Applied Environmental Microbiology, **2009**, 42(4): 641-648.
[8] Sriram, N., D. Reetha and P. Saranraj, *Middle-East Journal of Scientific Research*, **2013**, 17 (12), 1695-1700.
[9] N. Ramamurthy, S. Balasaraswathy and P. Sivasakthivelan. *Romanian J. Biophys.*, **2011**, 21 (2), 113–123.
[10] F. J. Cervantes, F.P.Van der Zee, G. Lettinga. *Water Science and Technology*, **2001**, 44, 123-128.
[11] J.L. Bragger, A.W. Lloyd, S.H. Soozandehfar, *International Journal of Pharmacy*, **1997**, 157: 61-71.
[12] O. Khadijah, K. K. Lee. and Mohd Faiz F. Abdullah. *Malaysian Journal of Microbiology*, **2009**, 5(1), 25-32.
[13] Mir Tariq Ahmad, Manderia Sushil and Manderia Krishna, *Internation Research Journal of Environment Science*, **2012**, 1(1), 50-53.
[14] Sofia Nosheen, Haq Nawaz and Khalil-UR-Rehman, *International Journal of Agriculture and Biology*, **2000**, 2(3), 232-233.
[15] P. Saranraj, V. Sumathi, D. Reetha and D. Stella. *Journal of Ecobiotechnology*, **2010**, 2 (7): 12 – 16.
[16] M.M. Hassan, M.Z. Alam and M.N. Anwar., *International Research Journal of Biological Sciences*, **2013**,2(8):27-31.
[17] A. Karthikeyan and N. Anbusaravanan. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, **2013**, 7 (2): 51-57.
[18] Rashid Mahmood, Faiza Sharif, Sikander Ali, Muhammad Umar Hayyat, Tanzeem Akbar Cheema. *Biologia.*,**2012**, 58 (1&2) 53-60.

©IJRASET: All Rights are Reserved



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089 (24*7 Support on Whatsapp)