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Bioremediation of Paper and Pulp Industrial Effluent Using Bacterial Isolates

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Abstract: ***Paper and pulp mill is a source of major pollution generating industry leaving huge amount of intensely colored effluent to the receiving end. Bioremediation is taken to be an attractive option for reducing the pollution load from contaminated water because of its high efficiency and economical impact than the chemical remediation. For solving the above problem, predominant bacterial species were isolated from the premises of agro-based pulp and paper mill which were identified as species of*** **Pseudomonas sp. and Bacillus** ***sp. The effluent was treated with Pseudomonas sp. and Bacillus sp. for about a period of 25days. Followed by the analyses of the physiochemical parameters like pH, BOD, COD, TS, TDS, TSS at the time interval of 5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} days periodically. Pseudomonas sp. showed decreased level of pH, BOD, COD, TS, TDS, TSS with the value of 7.10, 253 mg/L, 975 mg/L, 2670 mg/L, 2460 mg/L, 210 mg/L respectively. Bacillus sp. showed decreased level of pH, BOD, COD, TS, TDS, TSS with the value of 7.15, 250 mg/L, 980 mg/L, 2873 mg/L, 2580 mg/L, 115 mg/L respectively. Besides this the enzyme assay was done to test ability of degrading lignin by both bacterial effluent isolates. Pseudomonas sp. was found to be efficient in degrading the industrial effluent of pulp and paper than Bacillus sp. It was concluded that the isolated bacteria represented a promising application in*** *bioremediation* ***process of paper and pulp industrial effluent.***

Keywords: Bioremediation, effluent, pollution, physiochemical parameters, Pseudomonas, Bacillus.

I. INTRODUCTION

World demand for paper has grown rapidly and was around 5-6% per year. The paper mills have a larger investment and provide employment to 2 lakh people. It is estimated that the capacity of the mills increases from 8.3 million tonnes in 2010 to 14 million tonnes in 2020. In India the total production 70% is from hardwood and bamboo fiber, agro-waste and other 30% is from recycled material. For paper, paperboard and newsprint production, 550 mills in India use wastepaper [1]. Furthermore, the pulp and paper mill effluent is highly coloured. The chemical composition of such effluents depends on the nature of the feedstocks, as well as the treatment procedure. The paper mill wastewater characteristically contains colour, very high level of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD),due to presence of lignin and its derivatives from the raw cellulosic materials , chlorinated compounds, suspended solids (mainly fibers), fatty acids, tannins, resin acids, sulphur and sulphur compounds, etc.

Microbial degradation technique has no negative impact on the environment. It is carried out by different organisms like bacteria, fungus, algae and enzymes as a single step treatment or in combination with other physical and chemical methods. The microorganism treats the effluent mainly by action of enzymes and biosorption [2]. The various enzymes involved in the treatment of pulp and paper mill effluent are lignin peroxidase, manganese peroxidase and laccase. Microorganism showing good production of these enzymes has the potency to treat effluent [3]. Different bacterial species include Bacillus subtilis, Citrobactorfreundi, Alcaligenes, Burkholderia, Pseudomonas aeruginosa, etc and fungal species include Phanerochaetechrysosporium, Rhisopusstolonifer, Pleurotuseryngii, Pleurotusostreatus, etc. Shanthi et al., [4] identified the predominant bacteria and fungi in paper mill effluent and the degradation efficiency of individual isolates and combination of isolates to treat the effluent and effective floc formation and degradation was attained in Pseudomonas alkaligensand Enterobacter spp. combination. Hassan et al., [3] isolated Bacillus sp. from Egyptian soil and achieved maximum lignin degradation on the sixth day at pH 6 was 81.4% which however the lowest lignin degradation rate was observed at pH 13 was 34.2% at the end of the incubation time. Hence, biological treatment has been applied for the decolourization of effluent of pulp and paper mills. An important strategy for effluent treatment is the isolation and characterization of generally significant microorganisms together with designing and optimization of process parameter to deal with specific environment pollutants. Several strains have been proven to modify effluents which are produced during the chemical bleaching of pulps. Although, there is considerable potential for treating these effluents by biological methods. Therefore, present investigation is undertaken initially to collect bacterial strains from pulp and paper mill effluent for bioremediation of the effluent in the invitro condition.

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II. MATERIAL AND METHODS

A. *Collection of sample*

The effluents were collected from paper industry in Karur district, Tiruchirapalli in air- tight plastic bottles and stored at 4 ± 1^0c until use.(Fig.1)

B. *Isolation of bacterial culture from effluent sample*

Serial dilution of the effluent was performed upto 10^{-9} dilutions. Isolation of colonies were done by spread plate followed by steak plate for pure culture maintenance (Fig.2).

C. *Identification of isolates*

Isolated strains were identified on the basis of their morphological (simple & gram staining, motility) and biochemical characteristics (indole, methyl red, vogesproskauer, citrate, triple sugar iron, catalase, oxidase) according to Bergey's manual of determinative bacteriology (Table 2 & 3).

D. *Production and extraction of extra cellular enzyme:*

The 2 days old Culture of bacteria was inoculated into the conical flask(250 ml) containing luriabertani agar medium. The flask was incubated at 37°C for 5 days. After 5 days of incubation, culture aliquots were centrifuged at 10,000 rpm for 15 minutes to remove solids. The supernatant was assayed for the enzymatic activity.

Enzyme assay were done by the plate assay method, which allow rapid determination of the presence of enzyme in the extracellular fluid. Luria bertani agar plates was prepared along with the lignin model compound-lignin sulphonate at a concentration of 5g/l. About 1 ul from the supernatant was collected and then placed on the Sterile discs and allowed to dry. It was then placed in the centre of the medium and incubated at 37°C for 24 hrs.

E. *Effluent treatment by isolated organisms*

The bacterial cultures were centrifuged at 10,000 rpm for 10 minutes at 4^0C. The pellet containing cultures were placed in 250 ml conical flask containing 100 ml of effluent and incubated in shaker at 200 rpm at 35^0C. It was then examined for the biodegradation of both bacterial for the next 25 days by UV-Vis spectrophotometer. The effluents were taken at regular intervals (5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} day) to check the level of degradation (Fig. 1 & Fig. 2)

F. *Analysis of physico-chemical parameters*

The physiochemical parameters like pH, BOD, COD, TS, TDS, TSS of the effluent sample was analysed before and after treatment according to standard procedures APHA [5,6] at regular intervals (5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} day).

Measurement of pH

The electrodes of the pH meter were calibrated with standard solution of known pH. 50 ml of effluent were taken in beaker and the electrodes were washed with distilled water and wiped with filter paper. The electrodes were immersed into the beaker containing the effluent and the meter readings were recorded.

G. *Estimation of total solids(TS)*

The silica crucible was accurately weighed and recorded as weight (W_L). About 5 ml of unfiltered sample was poured into the silica crucible. The sample was then evaporated by placing it in a hot air oven at 105°C for 1 hour. The sample was cooled in desiccators.

Calculation:

$$\text{Total solids (mg/L)} = \frac{W_I - W_F}{S}$$

W_I initial weight of the crucible

W_F final weight of the crucible

H. *Estimation of total dissolved solids (TDS)*

The silica crucible was accurately weighed and 100ml of the filtered sample was transferred in the silica crucible (W_I). The sample was then evaporated by placing it in a hot air oven for 1 hour at 180°C. The sample was cooled it in desicator and recorded weight

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as (W_F)

Calculation:

$$\text{TDS mg/l} = \frac{W_I - W_F \times 1000}{S}$$

I. Estimation of total suspended solids(TSS)

Total suspended solids can be obtained by subtracting the total dissolved solids from total solids.

TSS=Total solids-total dissolved solids

J. Estimation of biological oxygen demand (BOD)

Effluent was collected and 50 ml of effluent sample was added to one litre of 8mg/l o_2 containing effluent sample. BOD bottle was rinsed clearly with water. The pH of the effluent was neutralized using acid or alkali. Two BOD bottles was filled with effluent sample without air bubble and one BOD bottle was used for DO estimation. About 2 ml of manganese sulphate and 2 ml of alkaline iodine azide solution were added to the bottle. The bottle was then closed and observed for brown coloured precipitate. To the sample, 2 or 3 drops of sulphuric acid was added. About 50 ml of acidified sample was titrated against sodium thiosulphate solution. After the formation of pale yellow colour, one or two drops of starch indicator was added to the sample. The sample was titrated again up to the disappearance of blue colour (initial oxygen). The remaining bottle was Incubated at 20-27°C for 3-5 days. The concentration of oxygen was estimated after 5 days of incubation (final oxygen). Dissolved oxygen of the given sample is calculated by the following formula:

Calculation:

$$O_2 \text{ mg/l} = \frac{\text{Titrant value} \times 0.025 \times 8 \times 100}{\text{Volume of the sample}}$$

where, 0.025 = Normality of thetitrant

8 = Molecular weight of oxygen

BOD of the effluent was calculated by the formula: $BOD=D_1-D_2$

K. Estimation of chemical oxygen demand (COD)

Take 20 ml of sample in the flask of reflux unit and 10 ml of potassium dichromate solution was added, a pinch of each silver sulphate and 30 ml of sulphuric acid was also added. Reflux the contents for 2 hours. The flask was cooled, detach from unit and dilute to about 150 ml by adding distilled water. 2 to 3 drops of ferroin indicator solution was added and titrated against ferrous ammonium sulphate solution. At the end point blue green colour of contents changes to reddish blue. Run simultaneously distilled water blank in similar manner.

Calculation:

$$\text{COD (mg/l)} = \frac{(B-T) \times N \times E \times 1000}{\text{Volume of sample(ml)}}$$

Where, T=volume of titrant (FAS) used against sample (ml),
B=volume of titrant used against blank (ml)
N=normality of ferrous ammonium sulphate,
E=equivalent weight of oxygen (8)

III. RESULT AND DISCUSSION

A. Collection of sample

The pulp and paper industry effluent was collected from Karurdistrict ,Tiruchirappalli(Fig. 1. The effluent were brown in colour, which is primarily due to lignin and its derivatives released from the substrate and discharged in the effluents, mainly from the pulping, bleaching and chemical recovery stages [10].

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B. Isolation

The organism were isolated from the effluent sample by spread plate method were shown in figure 2. The plate showed mixed types of colonies on agar medium. Pure culture were obtained by repeated sub-culturing and maintained at 4˚C. The isolates were marked as 1 & 2 and the colony morphology were shown in table 2.

C. Morphological and biochemical characterization

The isolates were subjected to biochemical test, for identification according to the Bergey's manual of systemic bacteriology.The isolates 1 and 2 showed dull white, smooth regular colonies on agar plates. The isolate 1 showed negative results for indole, Citrate and oxidase tests and positive results were observed for MR, VP and urease tests. Acid butt and alkali slant was observed on TSI test (Table. 3).

The isolate 2 showed positive results for Indole, MR, VP, citrate. catalase, urease and oxidase. Alkali butt and alkali slant with H_2s production was observed on TSI test. Based on the colony morphology and biochemicalcharacters, the isolate 1 & 2 were identified as Bacillus&Pseudomonas species, respectively (Fig. 4 & 5).

D. Enzyme assay

the decolourization zone was observed(Fig. 3& 4) after incubation which showed that the bacterial isolates can decompose the monomeric lignin structure models present in the paper and pulp mill effluent.

E. Biodegradation of effluent

The effluents were analysed for the physicochemical characters like pH, BOD, COD, TS, TDS, TSS. Initial physicochemical characteristics were carried out on the day of collection. Biodegradation of the effluents was carried out by using *Bacillus* sp and *Pseudomonas* sp for a period of 25 days (Table.4 & 5).

F. Analyses of physicochemical paprameters of the effluentpH

The hydrogen-ion concentration is an important quality parameter of wastewater. Fig.1 shows that the pH of the influent (raw wastewater) was measured to be 7.8. The treated effluent using *Bacillus* sp and *Pseudomonas* sp were found to be 7.15 and 7.10, respectively. Low value of pH is due to the metabolism of bacteria and also metabolic production of acids by indigenous micro flora [8-9].

G. BOD and COD

BOD measure the amount of oxygen requires by bacteria for breakingdown the simpler substances from the decomposable organic matter present in any water and COD test is useful in pinpointing toxic condition and presence of biological resistant substances

In the present study, the untreated BOD and COD values were found to be 318mg /L and 1256 mg/L, respectively. The result of the effluent treated by *Bacillu*s sp and *Pseudomonas* sp were shown in table 4 & 5. Biological treatment process results in oxidation of organic matter, which provides energy for microbial metabolic process [11]. BOD and COD showed slow degradation rates until the 5^{th} day (Fig. 7 & 8). After 5^{th} day fast degradation rates were observed. The first five days of incubation may be considered as adaptation time for the organism to degrade it.

H. Total dissolved solids

Total dissolved solid is the measure of total inorganic salts and other substances that are dissolved in water**.** The effluents with high TDS value may cause salinity problem if discharged to irrigation water. The TDS value of the untreated effluent were found to be 3000 mg/l. *Bacillus* sp and *Pseudomonas* sp treated effluent showed the value of 2460 mg/l and 2580 mg/l, respectively.

I. Total suspended solids

The total suspended solid present in untreated effluent were found to be 320 mg/L .*Bacillus* sp and *Pseudomonas* sp treated effluent showed the value of210 mg/l and 115 mg/l. Degradation rate was more in *Pseudomonas* sp when compared with the *Bacillus* sp. This might be due to plasmid conferring resistence gene [12]. cell wall modification capacity and formation of bio precipitation are other mechanisms employed by the bacterial cells to reduce the toxic effect [13]. Study revealed that the effect of various stress conditions resulting in the induction of outer membrane protein (OMP) in Pseudomonas sp.

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Table: 1Isolation of bacterial culture from effluent sample

Soil Sample	Dilutions	No.of Colonies
Paper and pulp effluent	10^{-5}	50
	10^{-6}	45
	10^{-7}	55
	10^{-8}	47
	10^{-9}	37

Table: 2Morphological characterizations of microorganisms:

Tests	Isolate 1	Isolate 2
Color	Dull white	Dull white
Shape	Regular	Regular
Texture	Smooth	Smooth
Elevation	Undulate	Undulate
Margin	Convex	Convex

Table: 3Identification of Biochemical Tests

S. NO	Biochemical tests	Isolate 1	Isolate 2
1	Indole	Negative	Positive
2	Methyl red	Positive	Positive
2	Vogesproskaur	Positive	Positive
4	Citrate utilization	Negative	Positive
5	Trible sugar iron	A/K	k/k H_2S
6	Catalase	Positive	Positive
7	Oxidase	Negative	Positive
8	Urease	Positive	Positive

Table: 4Treatment of pulp and paper effluent by *Bacillus* sp

S.No	Parameters	Initial	Day 5	Day10	Day15	Day20	Day25
1	pH	7.8	7.76	7.40	7.24	7.15	7.15
2	BOD	318	296	275	264	259	253
3	COD	1256	1168	1127	1013	983	975
4	TS	3320	3126	3112	3010	2870	2670
5	TDS	3000	2845	2835	2775	2645	2460
6	TSS	320	281	277	235	225	210

Table: 4Treatment of pulp and paper effluent by*Pseudomonas* sp

S.No	Parameters	Initial	Day 5	Day10	Day15	Day20	Day25
1	pH	7.8	7.60	7.54	7.32	7.20	7.15
2	BOD	318	310	290	270	265	250
3	COD	1256	1240	1164	1137	1095	980
4	TS	3320	3100	3840	2825	2793	2873
5	TDS	3000	2840	3570	2605	2521	2580
6	TSS	320	260	270	220	272	115

Fig. 1 Effluent from pulp and paper industry Fig.2 Isolation of bacteria

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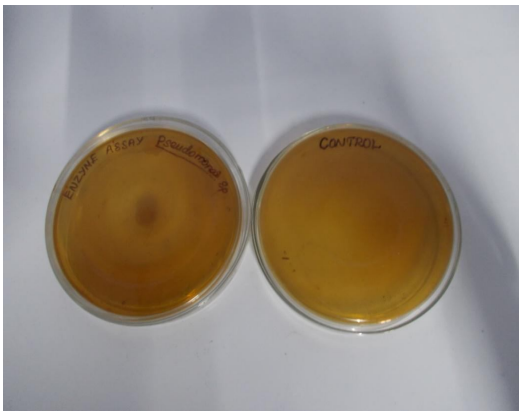


Fig.3 Enzyme assay *Bacillus* spFig.4 Enzyme assay *Pseudomonas* sp

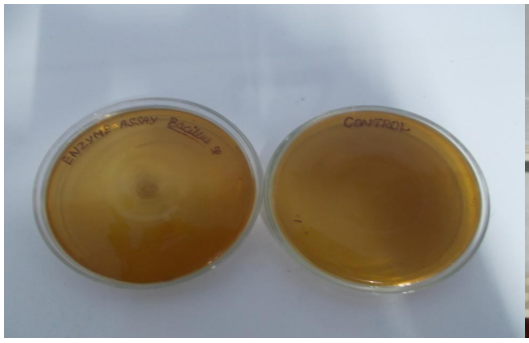




Fig.5 Biochemical test for *Bacillus* spFig.6 Biochemical test for

*Pseudomonas*sp



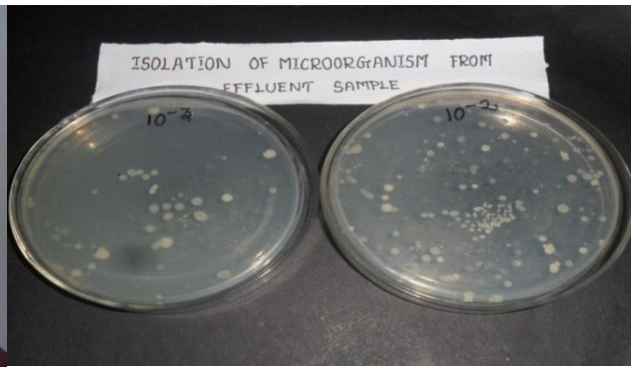


Fig. 7 Estimation of biological oxygen demand Fig.8 COD



Fig. 9 Biodegradation byBacillussp

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Fig. 10 Biodegradation by Pseudomonas

IV. DISCUSSION

Treated effluent showed variation in the Physico-chemical parameters when compared with the untreated effluent. Pseudomonas sp. was found efficient in degrading the industrial effluent of pulp and paper than Bacillus sp. The present study finding is similar to the findings of Ajao et al.[7] suggesting that Pseusodomonasaeruginosa and Bacillus subtilis proved to degrade mill waste effectively. Furthermore Pseusodomonasaeruginosakept higher degradation rate than other isolates.

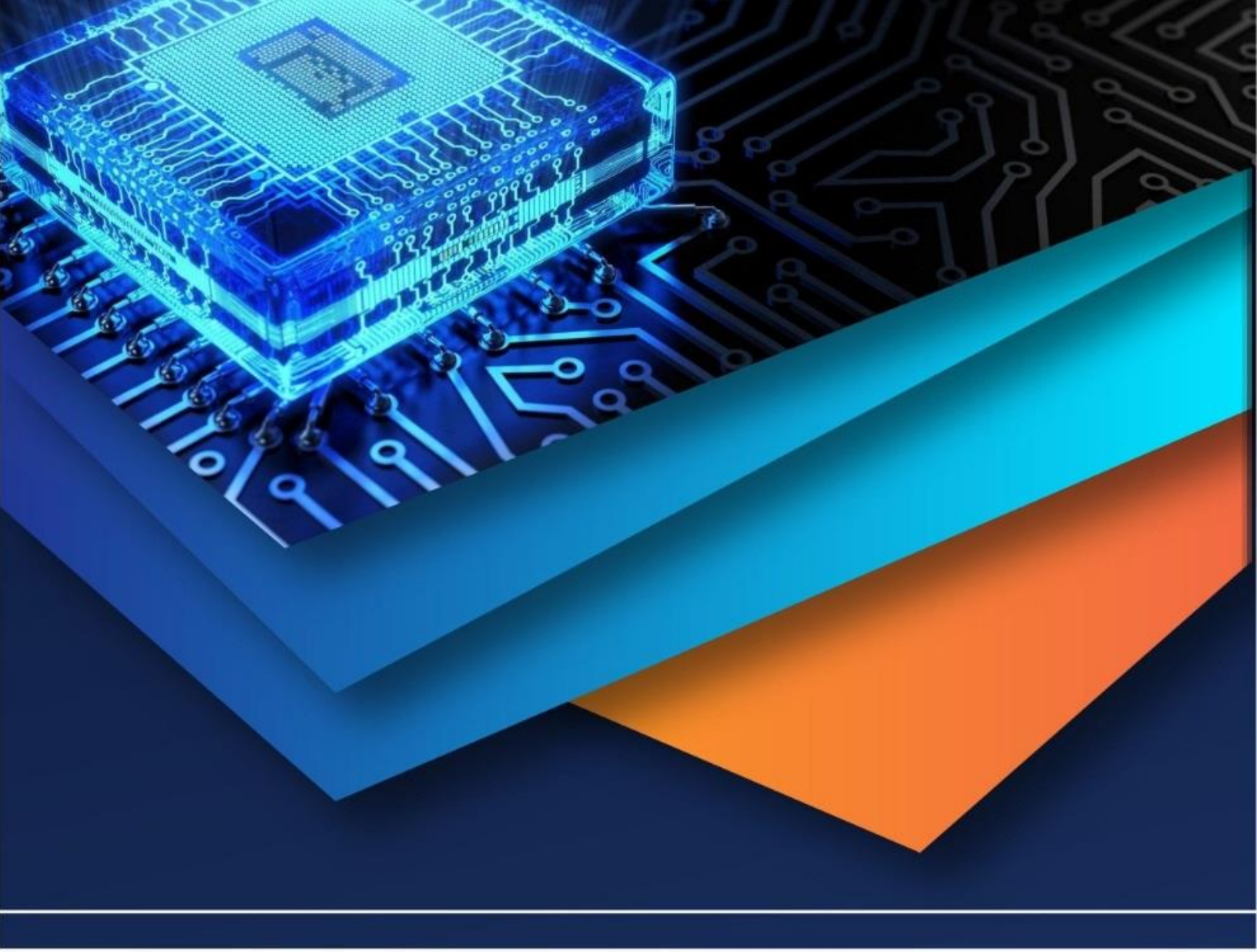
V. ONCLUSIONS

The results indicate that the effluent affects the water quality which leads to significant environmental and health risk to the rural communities who rely on the receiving water as their source of domestic water purpose without treatment. Bioremediation is taken to be an attractive option for reducing the pollution load from contaminated water because of its high efficiency and economical impact than the chemical remediation.

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