ISOLATION OF CYANOBACTERIA FROM DIFFERENT POND WATER OF VALSAD DISTRICT AND ITS POTENTIAL USE OF ITS BIOREMEDIAL ACIVITY ON REACTIVE RED-31 AND UNTREATED INDUSTRIAL EFFLUENT

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Abstract: This study was carried out to determine effectiveness of the isolated cyanobacteria for its bioremedial activity on decolourization of azo dye, and treatment of industrial effluent. Total five different cyanobacterial isolates were isolated and were studied for the above mentioned experiment and got promising results. Among all the isolates the highest percentage of decolorization of dye was given by isolate S2C3- 95.97% and lowest percentage of decolorization of dye was given by mixed consortium – 75.74%. Reduction in acidity was observed from 623.6mg/l to 0.00mg/l. pH value of the untreated sample was acidic i.e. 3.4 and after treatment the pH value was increased to alkaline 8.7.Calcium hardness and total hardness of the untreated sample was 135mg/l and 410mg/l respectively and was reduced to 81 mg/l by mixed consortium and total hardness was reduced to 95mg/l by S4C3respectively. The reduction of chloride ion was reduced to 236.3mg/l from 987.4mg/l by mixed consortium. The highest reduction of chemical oxygen demand was reduced to 40% .The reduction of biochemical oxygen demand was reduced to 7.489mg/l. The reduction of total dissolve solids was reduced to 470mg/l from 5200mg/l by isolate S2C3.The reduction of total dissolve solids was reduced to 50mg/l by S2C3.

Keywords: Cyanobacteria, Azo dye, Industrial effluent, Bioremediation.

1. INTRODUCTION

As the population is increasing in the world and daily life demands supplied through industries and modern industrialized systems ,the amount of wastes produced are increasing rapidly so the need for preservation of ecosystem is quickly revealed. To prevent these changes only by natural processes is not sufficient .Industrial plants has become the major factor that release a vast variety of water pollutants that are expensive and difficult to degrade. For example, the treatment of contaminated water from colored compounds like dyes and industrial effluent of chemicals are the most difficult environmental problems now a day. To treat such contamination we need to discover some quick, inexpensive and easy methods. According to the survey, there are over 1 lakh different dyes are used and synthesized on industrial level and most of them are mainly used in textile industries all over the world. At the time of processing, textile and dyeing industries uses huge amount of water and such type of effluent contains about 10-40% of unused dyes tuff which upon released into the environment causes serious pollution problem and negative impact on aquatic or terrestrial environment and its ecological functions. One of the most economical and stable approaches to cope with this vital task is the use of the technique–"BIOREMEDIATION", exploiting BLUE GREEN ALGAE (Cyanobacteria) is essential to remediate contaminated ecosystem. They goal of bioremediation is to reduce pollutant level to undetectable, nontoxic or acceptable levels. Textile waste water effluent is directly released to the ponds and river without treated so the application of cyanobacteria to these

locations will be more beneficial as chances of Cyanobacteria to grow in such condition is so predictable. Since, externally added bacteria may have some deleterious effects on ecosystem. The application of cyanobacteria has showed immense potential in waste water and industrial effluent treatment, bioremediation of aquatic and terrestrial habitats, chemical industries, biofertilizers, food, feed, fuel, etc. In addition, pH, carbon dioxide, organic matter, alkalinity, nitrates and phosphates are factors important in determining the distribution of cyanobacteria. Cyanobacteria have a great deal of potential as a source of fine chemicals, as a bio-fertilizer and as a source of renewable fuel. Recently, there has been increasing awareness about using cyanobacteria as bioremediation and pollution control agents, either as wild-type, mutant or genetically engineered forms. Blue greens have been shown to be highly effective as accumulators and degraders of different kinds of environmental pollutants, including pesticides. Worldwide, cyanobacteria have been used efficiently as a low-cost method for remediating dairy wastewater by converting the dissolved nutrients into biomass and for biotreatment (removal) of dissolved inorganic nutrients from fish farms, to allow them to be used as economic and low- maintenance remediation technology for contaminated systems.

2. METHODOLOGY

A. Sample collection:

Water sample was collected from Marine water sample, RTO pond water, Lilaporpond water, Kalamtha pond water and industrial effluent were stored in a plastic container and labeled properly.(N Karthika, A Muruganandam, 2019).

B. Enrichment :

10ml of sample were enriched in 100 ml of sterile Algal culture medium and incubated for up to15 days at room temperature in sunlight and observed turbidity.(Gahlout and prajapati*et.al* 2017) with modification.

C. Isolation and Screening of Cyanobacteria :-

Water sample was inoculated in Erlenmeyer flask having algal culture medium and incubated at room temperature under continuous dark and sunlight period for 15-20 days, growth from the incubated flask were spreaded on algal culture plate and incubated at room temperature under continuous dark and sunlight period for 15-20 days. Isolated colonies were observed in microscope for morphological characterization.(Gahlout and prajapati*et.al* 2017) with modification.

D. Identification of cyanobacteria:-

Microscopic observation was done by spreading isolated culture on glass slide using forceps. Culture were covered with glass cover slips and observed under low (10X) and high power (45X) objective lens of compound light microscope. .(Gahlout and prajapati et.al 2017).

E. Degradation of azo dye :-

Degradation of azo dye was tested by providing 0.005 ppm of azo dye in 50 ml of Algal culture medium which was already inoculated with 5 different cyanobacterial isolates individually in each flask and same was proceed with mixed consortium. Incubate all the flask in sunlight and were studied for dye decolorization at almost every 24 hrs of time interval and observation were noted down . A control flask was also maintained in same way. After every time interval of 24 hours 5 ml of decolorized samples were taken for degradation analysis and centrifuged at 1000 rpm for 15 minutes. The supernatant was taken and optical density was measured spectrophotometrically at 520 nm.(Tarun Agarwal and Rachana singh, 2012).

$$\% \text{ Decolorization per day} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

Percentage dye degradation was calculated after 24 hour using above described formula.

F. Analysis of Physio-chemical properties of untreated industrial effluent treatment by isolated species :-

Physical parameter of untreated industrial effluent included color, temperature, pH, odour. Chemical parameter of untreated industrial effluent included acidity, alkalinity biochemical oxygen demand (BOD),chemical oxygen demand (COD),Total dissolve solids(TDS), Total suspended solids(TSS),calcium ,chloride, magnesium were characterized before and after treatment to determine the effectiveness of the remediation process.(Dubey.et.al.,2011)

a) pH meter was used to measure the pH of the sample. Color and odour of the sample were noted with the help of human sense organ. Temperature of the sample was checked by thermometer after bringing it to the laboratory.

b) Acidity

Acidity of the collected untreated industrial effluent was checked before and after the treatment, according to the method described in American Public Health Association. (APHA, 2005).

c) Alkalinity

Alkalinity of the collected untreated industrial effluent was checked before and after the treatment, according to the method described in American Public Health Association. (APHA, 2005).

d) Total dissolved solids (TDS)

Acidity of the collected untreated industrial effluent was checked before and after the treatment, according to the method described in American Public Health Association. (APHA, 2005).

e) Total suspended solids (TSS)

Acidity of the collected untreated industrial effluent was checked before and after the treatment, according to the method described in American Public Health Association. (APHA, 2005).

f) Hardness

Acidity of the collected untreated industrial effluent was checked before and after the treatment, according to the method described in American Public Health Association. (APHA, 2005).

g) Chloride

Acidity of the collected untreated industrial effluent was checked before and after the treatment, according to the method described in American Public Health Association. (APHA, 2005).

h) Chemical oxygen demand

Acidity of the collected untreated industrial effluent was checked before and after the treatment, according to the method described in American Public Health Association. (APHA, 2005).

i) Biochemical oxygen demand (BOD)

Acidity of the collected untreated industrial effluent was checked before and after the treatment, according to the method described in American Public Health Association. (APHA, 2005).

j) Study on COD reduction :-

This test were carried out in 6 Erlenmeyer flasks of 250 ml containing 100 ml of algal culture medium in each . All the flask were sterilized at 121^0C for 15 min at 15 lbs. All the sterilized flasks were cooled and inoculate with 5 different cyanobacterial isolates individually in each flask along with mixed consortium. 10% v/v of industrial effluent and 10% v/v algal culture were inoculated in all the experimental flask. The inoculated flasks were gently agitated on a shaker with a constant shaking rate at 120 rpm for 15 days. Samples were taken from the flask at regular time intervals of 3 days for the determination of residual COD in the experimental flask.

3. RESULT AND DISCUSSION

1. Result of sample collection:

Water sample were collected from different sources in Valsad region. Total four water sample were collected from which three water sample were taken from different ponds and one sample was collected from marine. Marine sample was collected from Tithal beach, and the three ponds were RTO pond ,Lilapor pond and kalamtha pond of valsad region.

**2. Result of physical analysis of collected water sample:**

Parameter like color, temperature, pH, and odour done to study physical analysis of collected water samples.

**Table 1: Result of physical analysis of collected water sample**

Parameter	Marine	RTO pond	Lilapor pond	Kalamtha pond
Color	Brown	Green	Pale yellow	Green
pH	8.1	7.4	7.5	8.3
Odour	Fishy	Rotten egg smell	Putrid	Putrid
Temperature	25	28	28	30

3. Enrichment:

For, enrichment 10 ml of sample were enriched in 100 ml of sterile Algal culture medium and incubated for upto 15 days at room temperature in sunlight and turbidity was observed.
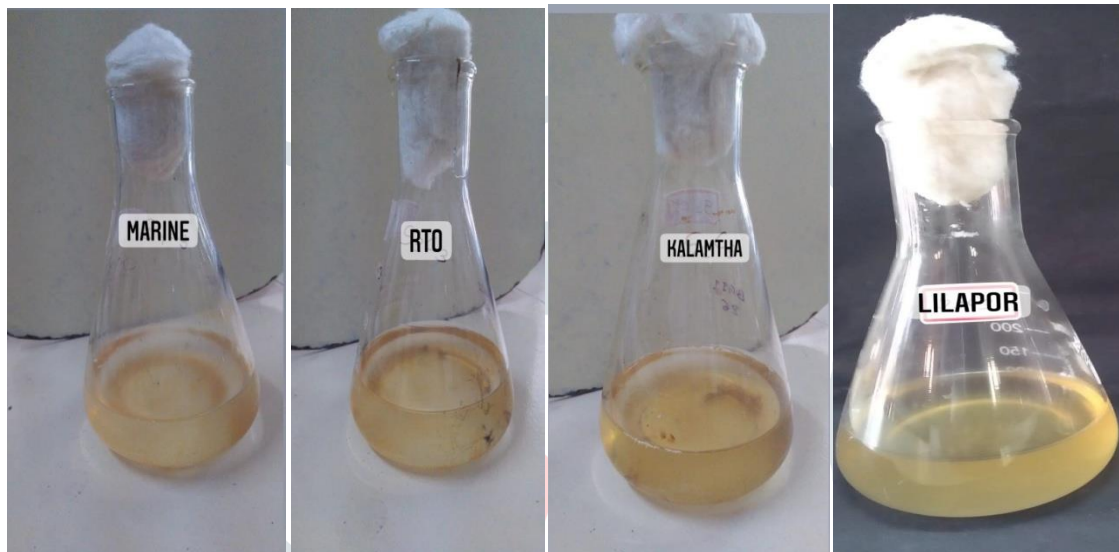
Figure 1: Enrichment of collected water sample in Algal culture medium for 15 days

4. Result of isolation and screening of cyanobacteria:

Collected water sample were inoculated in algal culture medium for upto 15 days for enrichment. From different water samples collected from different region, total 5 isolates were isolated and were studied for Gram reaction, colony characteristics and wet mount of isolates. **In Figure 2: Images of plates showing isolated colonies is shown below.**
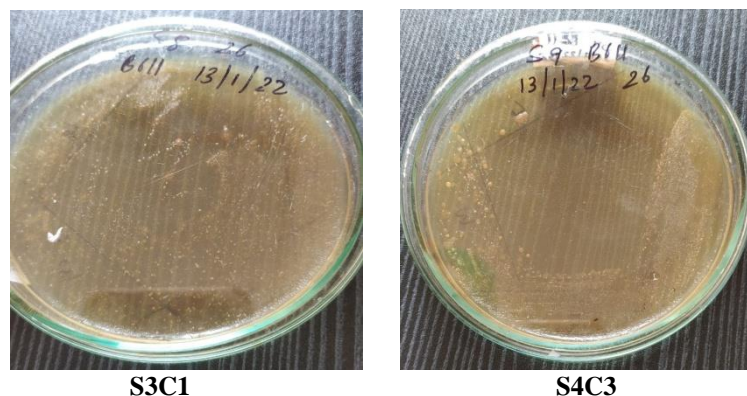

S2C1   S3C3   S2C3
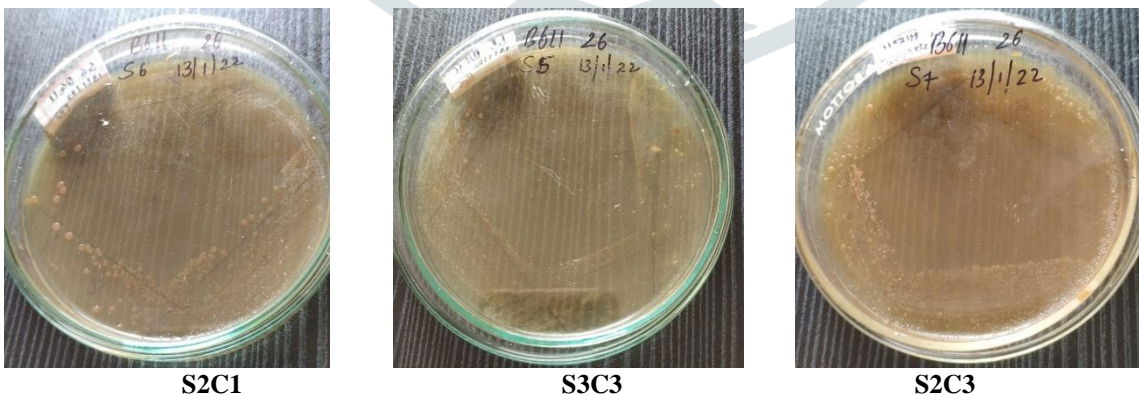
Figure 2: Images of plates with isolated colonies.

5. Results of identification of isolates

Microscopic observation was done by spreading isolated culture on glass slide using forceps. Culture was covered with glass cover slips and observed under low (10X) and high power (45X) objective lens of compound light microscope. (Gahlout and prajapati et.al 2017). Total five isolates were identified to be cyanobacteria. The isolate S2C1 was having similar morphological characteristics to *Oscillatoria sp.*of cyanobacteria which was Long, cylindrical, green filamentous slightly tapering, unbranched, non heterocystous. The isolate S3C3 was having similar morphological characteristics to *Microcystis sp.*of cyanobacteria which was Small, Round, Green, found in large group, heterocystous and non-filamentous. The isolate S2C3 was having similar morphological characteristics to *Synecococcus sp.*of cyanobacteria which was Spherical or ellipsoidal, Green, single or in pair, non- filamentous, non heterocystous. The isolate S3C1 was having similar morphological characteristics to *Anabaena sp.*of cyanobacteria which was Straight, Curved or coiled, long cylindrical, yellowish green in color, filamentous and heterocystous. The isolate S4C3 was having similar morphological characteristics to *Nostoc sp..*of cyanobacteria which was Large, Round, found in chain and singly, Green, mucilaginous, heterocystous. (Gahlout and prajapati *et.al,*2017).

6. Result of degradation of azo dye:

All the five isolates were tested for their dye degradative efficiency from which S2C1 has shown 78.17% of decolorization of dye, S3C3 has shown percentage 93.66% of decolorization of dye S2C3 has shown percentage 95.97% of decolorization of dye, S3C1 has shown percentage 84.42% of decolorization of dye, S4C3 has shown percentage 91.90% of decolorization of dye and Mixed consortium has shown percentage 75.74% of decolorization of dye. In Table Percentage (%) of decolorization of dye is shown.

Table 2: Result of percentage (%) degradation of Reactive Red azo dye by different isolates and mixed consortium at every 24 hours of time interval up to 6 days.

ISOLATES	24 HOUR	48 HOUR	72 HOUR	96 HOUR	120HOUR	144HOUR
S2C1	10.00%	56.77%	64.62%	64.62%	70.53%	78.17%
S3C3	38.29%	40.175	52.53%	78.21%	93.56%	93.66%
S2C3	16.68%	33.86%	64.52%	84.42%	95.67%	95.97%
S3C1	20.37%	55.61%	58.91%	70.04%	79.20%	84.42%
S4C3	27.68%	29.06%	56.05%	70.58%	85.12%	91.90%
MIX	28.51%	30.07%	51.34%	68.51%	72.34%	75.74%

Graph 1: percentage (%) degradation of Reactive Red azo dye by different isolates and mixed consortium at every 24 hours of time interval up to 7 days.
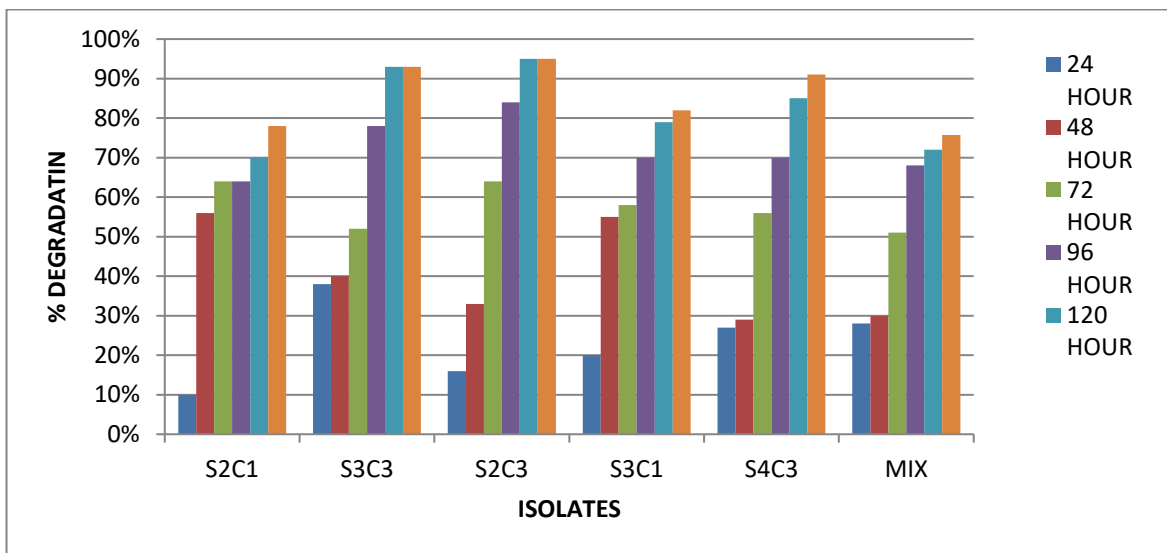
Graph 2:% degradation of Reactive Red 31 azo dye by isolates and mixed consortium.
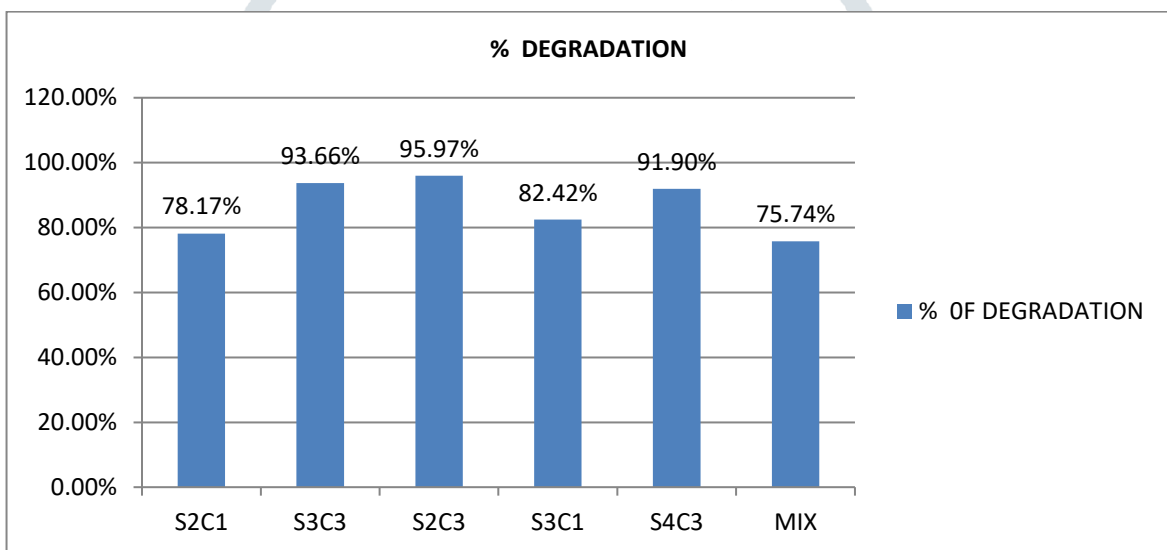
From the Table 2 and graphical representation of 1 and 2 it has seen that among all the isolates the highest percentage of decolorization of dye is given by isolate S2C3- 95.97% and lowest percentage of decolorization of dye is given by mixed consortium – 75.74% Decolorization of dye can be seen in image mention below.

7. Result of before and after physio-chemical parameter of the industrial effluent by isolates:

7(a). Acidity:

Acidity (Before): Acidity of fresh industrial effluent was done before bioremediation of industrial effluent.

Acidity (After): Acidity was tested after bioremediation of industrial effluent by all 5 cyanobacterial isolates and mixed consortium.

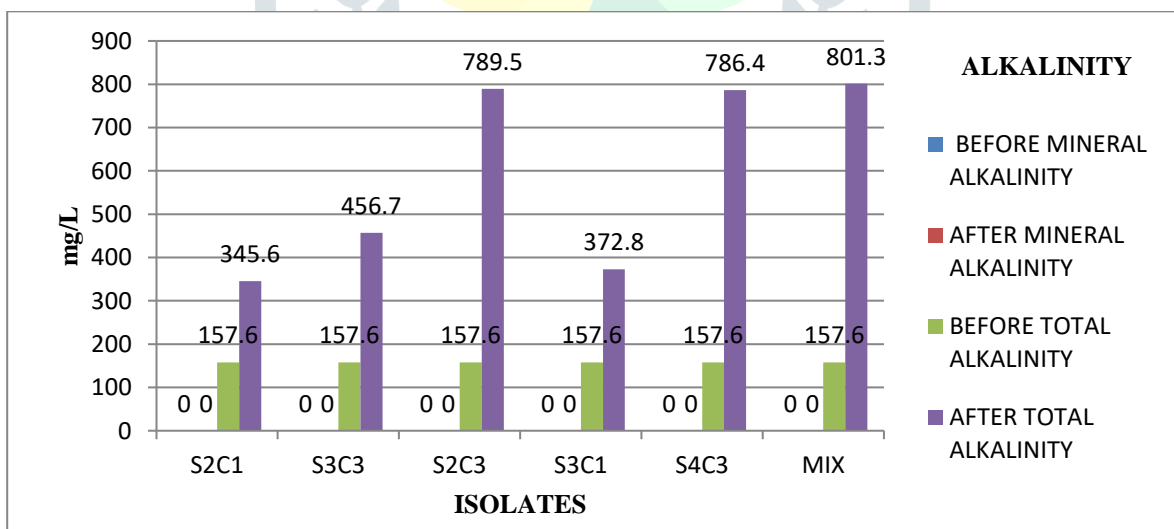**Graph 3:** Before and after result of mineral and total acidity.
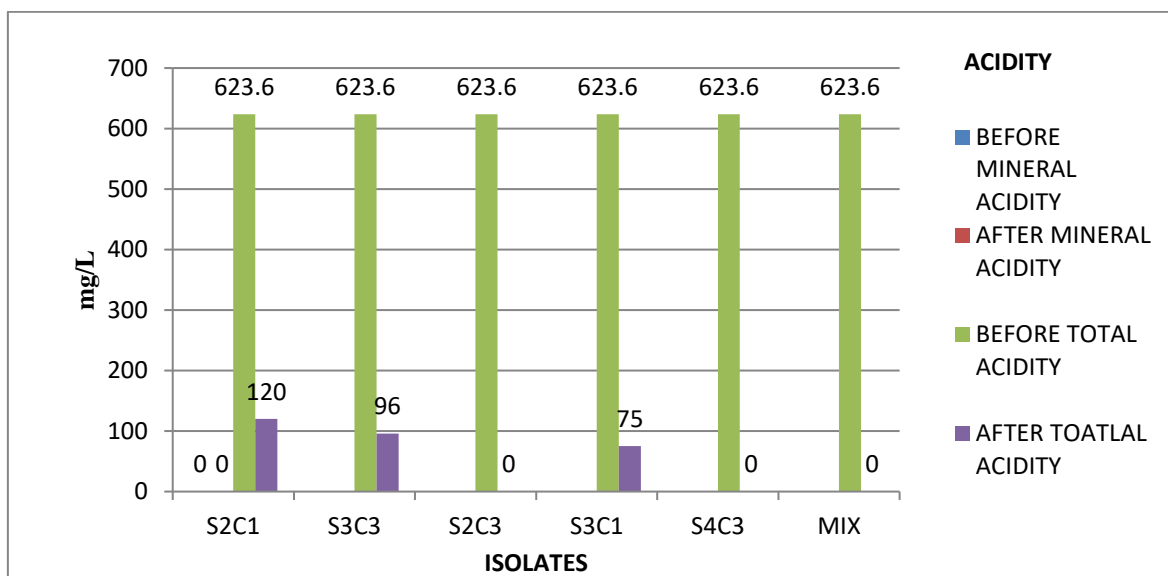
From the above result of graphical representation 3, it can be said that the total acidity of the untreated sample is reduced from 623.6mg/l to 0.0mg/l after treating it. The isolates S2C3, S4C3 and mixed consortium gave total acidity 0.0 mg/l whereas S2C1 gave 120 mg/l, S3S3 gave 96 mg/l and S3C1 gave 75 mg/l.

7(b). Alkalinity:

Alkalinity (Before): Alkalinity of fresh industrial effluent was done before bioremediation of industrial effluent.

Alkalinity (After): Alkalinity was tested after bioremediation of industrial effluent by all 5 cyanobacterial isolates and mixed consortium.

Graph 4: Before and after result of mineral and total alkalinity.
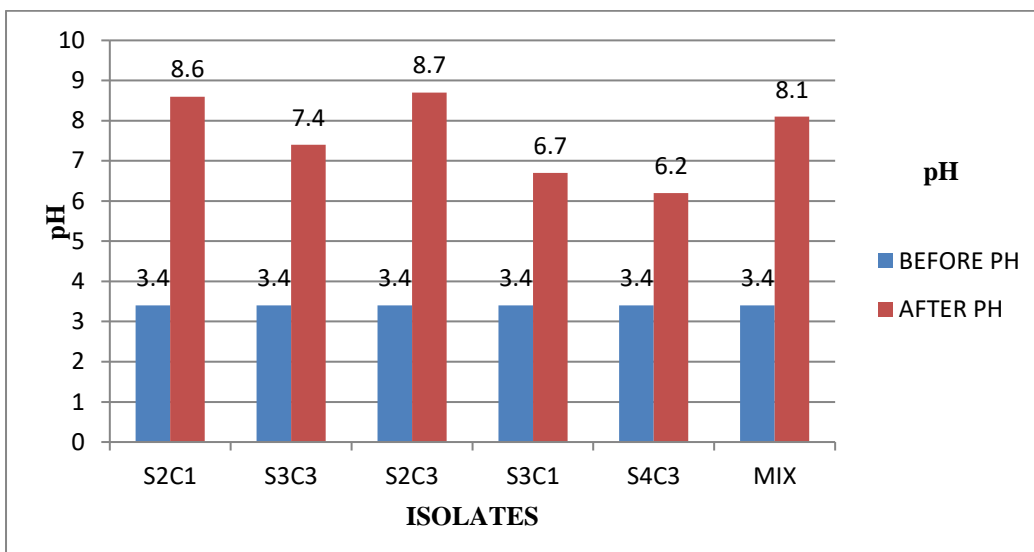

From the above result of graphical representation 4, it can be said that the total alkalinity of the untreated sample is increased from 157.6mg/l to 801.3 mg/l after treating it with cyanobacterial isolates. Mineral acidity was not found in untreated as well as treated sample. According to the results of the experiment we can say that as the alkalinity is increased after inoculation of cyanobacterial isolates so acidity was not found after inoculation of cyanobacterial isolates in the experiments.

7(c). pH.

pH was tested before and after bioremediation of industrial effluent by all 5 cyanobacterial isolates and mixed consortium.

**Graph 5:** Before and after results of pH.

From the above result of graphical representation 5, it can be stated that the pH of the sample has been changed from acidic value to alkaline value. The pH value of the untreated sample was acidic i.e.,3.4 and after inoculation of cyanobacterial isolates the pH value was increased to alkaline 8.7 after 15 days.

7(d). Hardness

Calcium and Total Hardness was tested before and after bioremediation of industrial effluent by all 5 cyanobacterial isolates and mixed consortium.

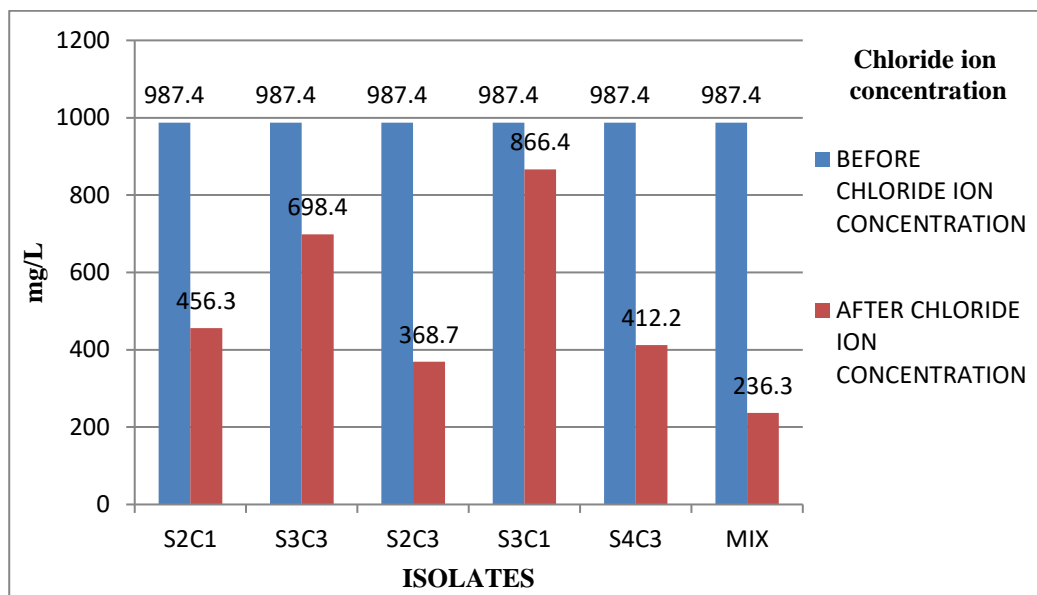**Graph 6:** Before and after results of hardness.
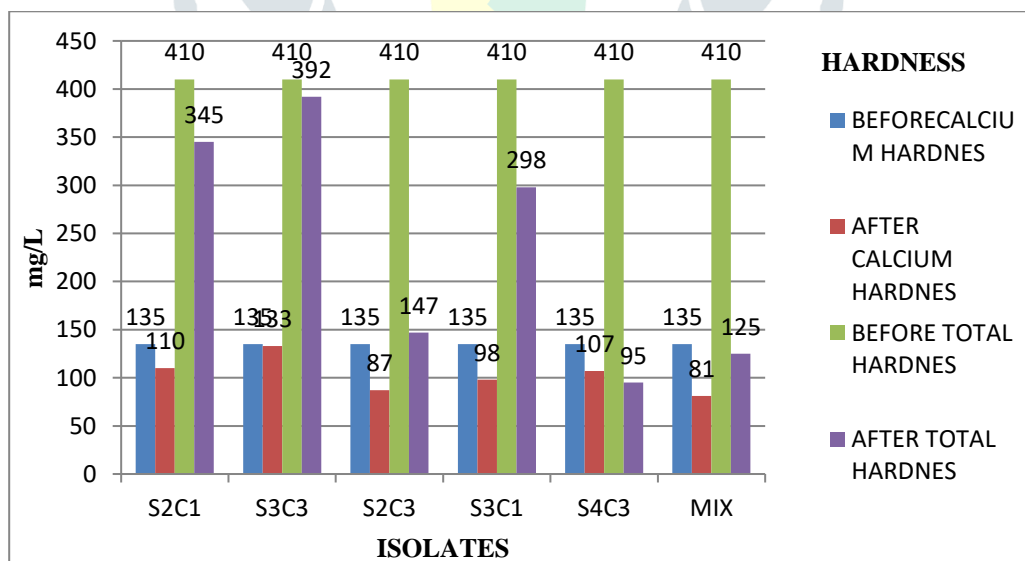
From the above result of graphical representation 6, it can be said that the reduction in hardness has been given by cyanobacterial isolates. Calcium hardness and total hardness of the untreated sample was 135mg/l and 410mg/l respectively. After treating it with cyanobacterial isolates the calcium and total hardness was reduced to 81 mg/l by mixed consortium and total hardness was reduced to 95 mg/l by S4C3 respectively.

7(e). Chloride

Chloride was tested before and after bioremediation of industrial effluent by all 5 cyanobacterial isolates and mixed consortium.

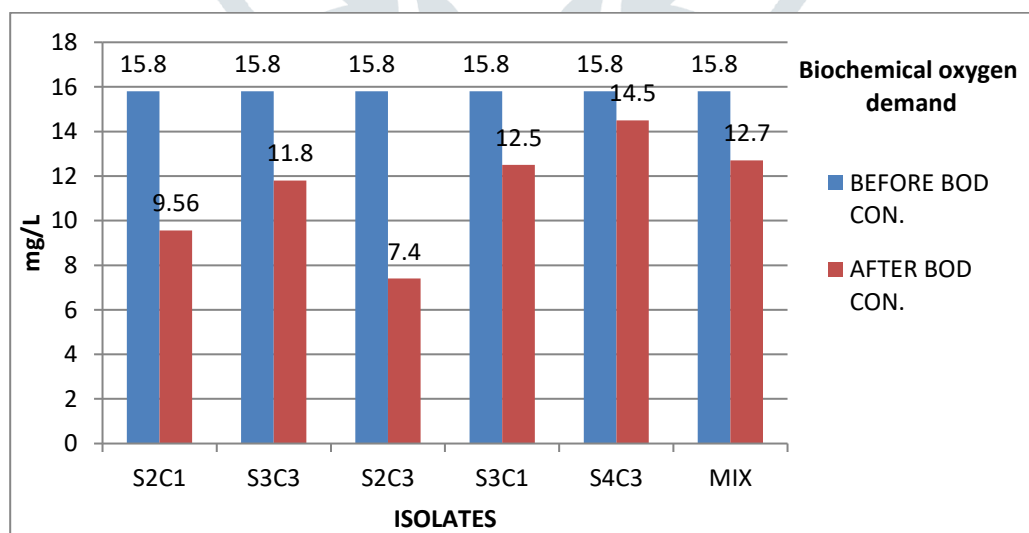**Graph 7:** Before and after results of chloride ion concentration.

From the above result of graphical representation 7, it can be said that the reduction in chloride ion has been given by cyanobacterial isolates. Chloride ion concentration of the untreated sample was 987.4mg/l. After treating it with cyanobacterial isolates the highest reduction of chloride ion was reduced to 236.3 mg/l by consortium and total lowest reduction was 866.4 mg/l by isolate S3C1.

7(f). Biochemical oxygen demand.

Biochemical oxygen demand was tested before and after bioremediation of industrial effluent by all 5 cyanobacterial isolates and mixed consortium.

Graph 8: Before and after results of biochemical oxygen demand.

From the above result of graphical representation 8, it can be said that the reduction in biochemical oxygen demand has been given by cyanobacterial isolates. Biochemical oxygen demand of the untreated sample was 15.89mg/l. After treating it with cyanobacterial isolates the highest reduction of biochemical oxygen demand was reduced to 7.489mg/l by isolate S2C3 and total lowest reduction was 12.75mg/l by isolate consortium.
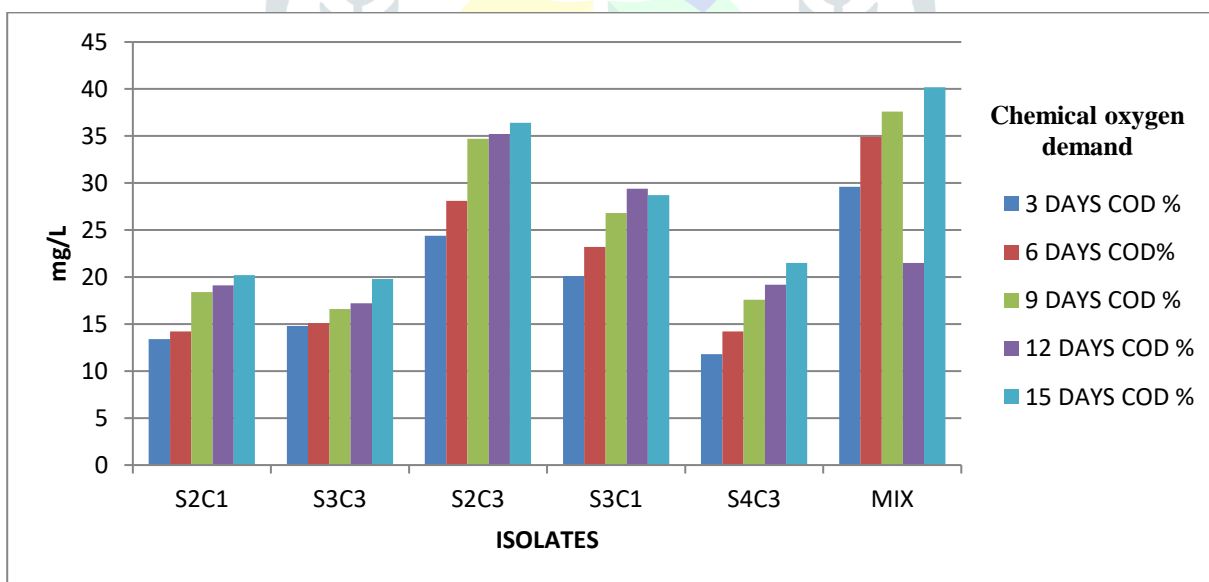
7(g). Chemical oxygen demand.

Chemical oxygen demand was tested before and after bioremediation of industrial effluent by all 5 cyanobacterial isolates and mixed consortium.

Table 3: Before and after results of Biochemical oxygen demand.

% Chemical oxygen demand (COD)					
Samples	3 Days	6 Days	9 Days	12 Days	15 Days
S2C1	13.4	14.2	18.4	19.1	20.2
S3C3	14.8	15.1	16.6	17.2	19.8
S2C3	24.4	28.1	34.7	35.2	36.4
S3C1	20.1	23.2	26.8	29.4	28.7
S4C3	11.8	14.2	17.6	19.9	21.5
Consortium	29.6	34.9	37.6	38.5	40.2

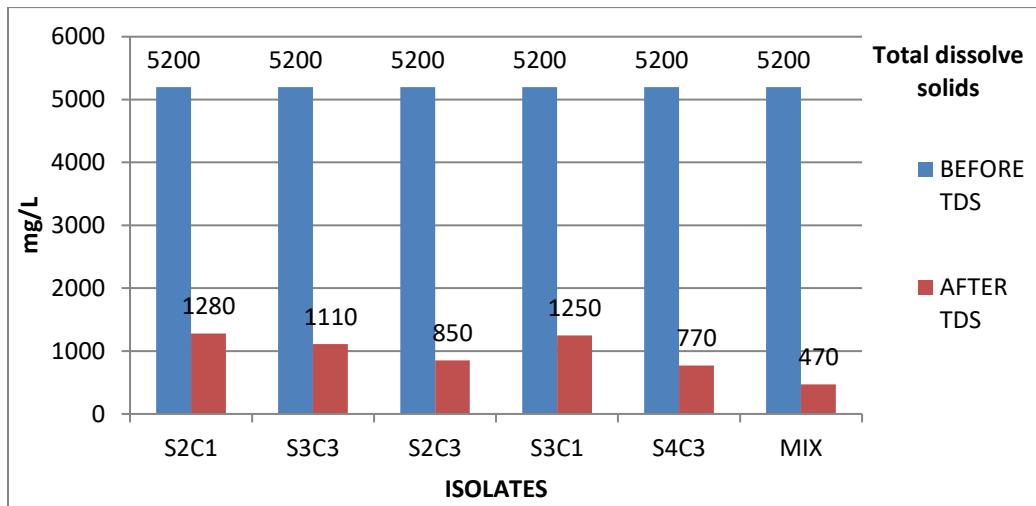**Graph 9:** Before and after results of chemical oxygen demand

From the above result of graphical representation 9, it can be said that the reduction in chemical oxygen demand has been given by cyanobacterial isolates. Chemical oxygen demand of the untreated sample was 846.6.After treating it with cyanobacterial isolates the highest reduction of chemical oxygen demand was reduced to 582.3mg/l(40%) by isolate Mixed consortium and total lowest reduction was745.3mg/l(19.8%)by isolate S3C3 after 15 days of experiment.

7(h). Total dissolve solids (TDS).

Total dissolve solids (TDS) was tested before and after bioremediation of industrial effluent by all 5 cyanobacterial isolates and mixed consortium.

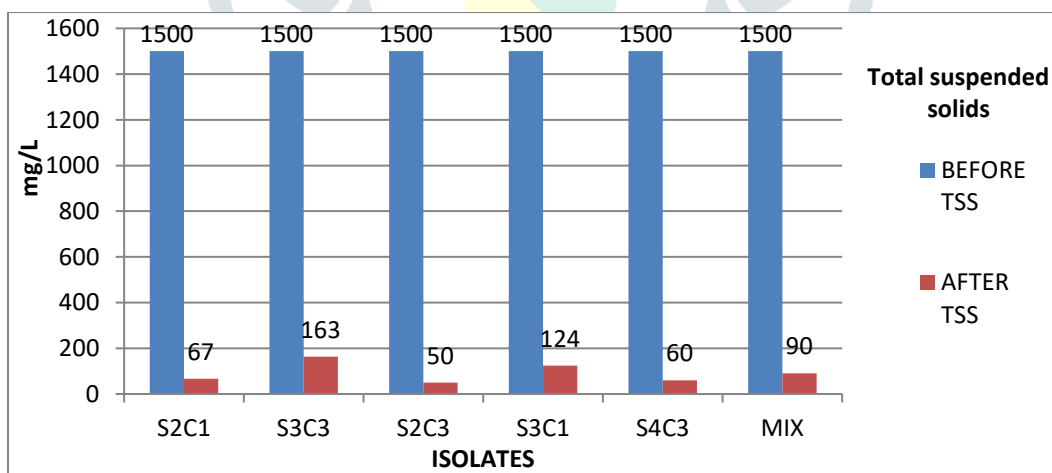**Graph 10:** Before and after results of total dissolve solids.

From the above result of graphical representation10, it can be said that the reduction in total dissolve solids has been given by cyanobacterial isolates. Total dissolve solids of the untreated sample was9000mg/l. After treating it with cyanobacterial isolates the highest reduction of total dissolve solids was reduced to 470mg/l by consortium and total lowest reduction was 1280 mg/l by isolate S2C1.

7(i). Total suspended solids (TSS).

Total suspended solids (TSS) were tested before and after bioremediation of industrial effluent by all 5 cyanobacterial isolates and mixed consortium.

Graph 11: Before and after results of total suspended solids.

From the above result of graphical representation 11, it can be said that the reduction in total suspended solids has been given by cyanobacterial isolates. Total suspended solids of the untreated sample were 1500mg/l. After treating it with cyanobacterial isolates the highest reduction of total dissolve solids was reduced to 50 mg/l by isolate S2C3 and total lowest reduction was 160 mg/l by isolate S3C3.

4. CONCLUSION

All the five cyanobacterial isolates were isolated from the collected water sample of different pond water from valsad region using Algal culture medium. All the five isolates were studied for their dye degradative efficiency and for the treatment of industrial effluent. Among all the isolates the highest degradation of reactive red 31 was given by isolates S2C3 and the lowest degradation was given by mixed consortium. From the above obtained results, it has seen that the isolate S2C3, S4C3 and mixed consortium has given more promising results for the treatment of industrial effluent. From the results of all the experiments, it can be concluded that the cyanobacterial isolates gave the good results in dye decolorization and treatment of industrial effluent, so can be used to solve the future problems of environment as a natural bioremediation source.

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