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Isolation of Microbes from Marine Water and Screening for Potent Microbefor Dye Degradation by Immobilization

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ABSTRACT

Colors have been dazzling humanity since ages and ended up being perfectly key unit of individuals advancing groundbreaking circumstance. Due to speedy extension in people's plan resolved assortment demands, there is massive development in material ventures which has shown a tremendous use of produced complex regular tones as the Coloring material. A part of these tones, their precursors, or their biotransformation things like sweet-smelling amines, have been exhibited to be mutagenic and malignant growth causing and consequently this implies why their removal is a hot conversation as of quite recently. Wide creative work has focused in on regular procedures as an eco-obliging choice for remediation of shadings. Most assessments on azo shading biodegradation have focused in on infinitesimal life forms and developments, in which microorganisms were by and large used for azo tones decolorization in view of their high development, wide transport and strong adaptability. This study was finished with a place of disengagement of microorganisms from marine water since marine water environment is an inconceivably mentioning situation for perseverance and accepting minute life forms from this environment creates life with thrashing survival skills,and couple of physically productive properties as well, as there is some incredible in each life coming from this environment, this study focused on division and depiction of organisms prepared for defiling azo shadings and degradation of azo tones by the compelling microbial cell mass in a collaboration called as immobilization.

Key Words: Azo Dyes, Microbes, Dye degradation, Immobilization, Marine Water.

INTRODUCTION

The material business is a giant water client and conveys huge volumes of ruined water. The material business everything considered experiences issues in party squander water release limits, especially as to isolated solids, ionic salt, pH, COD, hiding and critical metal. Treatment of concealing degraded squander water set liberated from the material and other concealing stuff associations is basic to forestall pollution of soil, surface and ground water. Planned colors and colorants are dynamic consistently utilized these days by paper, material, food, greatness care things, and prescription associations. Among these, material undertakings are the best client of tones and shades, tending to 80 % of full-scale creation (Jyoti Kumar Thakur et al., 2014). They are comprehensively used in the material, paper, food, calfskin, magnificence care items and medication organizations (*Telke et al., 2008*). Less than ideal arrival of material tone spouting containing azo tones and their metabolites in watery organic frameworks is beautifully unpleasant and prompts an abatement in sunshine entrance, which in this manner reduces photosynthetic activity, separated oxygen concentration, and water quality, and hurtfully affected maritime verdure, making outrageous normal issues all over the planet (*Vandevivere et al., 1998*). Additionally, azo shadings in like manner have an ominous impact similar to hard and fast total organic carbon (TOC), biological oxygen demand (BOD) and chemical oxygen demand (COD) (*Saratale et al., 2009b*). This insight has incited the suggestion that anaerobic/oxygen consuming structures might be convincing in achieving the all-out biodegradation of azo shadings. Furthermore, bacterial decolourization is regularly faster appeared differently in relation to fungi with respect to the decolourization and mineralization of azo colors (Banat et al., 1996). Since different bacterial species including Bacillus, Pseudomonas, Enterobacter, Halobacterium, and Aeromonas have been to decolorized and detoxify a wide extent of azo tones took a gander at of phenylamine, benzenediazonium chloride or phenol.(Telkeet al., 2008; Mendes t al.,2011; Feng et al.,2012).

Presentedwork here, based on screening and disengagement of shading spoiling microorganisms from marine environment since creatures of this environment have novel bioactive compound that they produce as a result of strain threw via ocean environment.

MATERIALS & METHOD

Sample Collection:

The water sample was taken at Rangaon Beach in Vasai. Samples were collected in sterilised BOD bottles, transported to the lab in an ice-cube-filled box, and processed within hours.

Enrichment and Isolation of organism:

The marine microorganisms in the acquired water sample were increased in Sterile Zobell Marine (ZM) Broth. The cells were then dispersed plate grown on Sterile ZM Agar Plates with an inoculum size of 0.1mL. In addition, pure cultures of the various colonies collected were maintained on the same medium.

Morphological characterization:

Characterization of colonies Gram staining, sugar fermentation, and biochemical analysis according to Bargey's protocol were used to identify probable bacterium isolates. The colony's cultural properties, such as margin, size, form, colony type, and nature (mucous, rough, smooth, translucent, etc.) were noticed.

Screening of dye degrading bacteria:

In 100ml dw, 0.5gm Congo red was made. The dye was inoculated with the bacterial strains that had been obtained. For the degrading activity, it was incubated for two days. O.D. was measured after two days.

Preparation intracellular materials:

The bacterial isolate that showed highest dye degrading activity following primary screening was centrifuged for 3 hours at 3000rpm, with the supernatant containing intracellular components recovered and the pellet discarded.

Immobilization of Cellular components:

To carry out the process of immobilization of the cells, the 4% sodium alginate solution and 6% calcium chloride solution was prepared and then autoclaved to make them sterile. Then 5ml of cell supernatant was added to the 25 ml 4% Na Agsolution aseptically and stirred well. For the beads, drew this solution in 10ml of syringe and added it drop wise manner to 100ml of 6% $CaCl_2$ solution. The beads were kept in $CaCl_2$ solution for 24 hrs. The alginate will be cross linked by calcium ions. After 24 hrs. of incubation the beads were taken out of the $CaCl_2$ solution and washed with distilled water several time. For the activation of beads activation media used was glucose at 15% concentration. After washing of beads was done, the beads were transferred to the activation media for 24 hrs. The cells were immobilized using a 4 percent sodium alginate solution and a 6 percent calcium chloride solution that had been prepared and autoclaved to make them sterile. The cell supernatant was then added aseptically to the 25 ml 4 percent NaAg solution and well mixed. For the beads, draw this solution into a 10ml syringe and drop it into a 100ml 6 percent CaCl2 solution drop by drop. For 24 hours, the beads were immersed in CaCl2 solution. Calcium ions will crosslink the alginate molecules. After 24 hours of incubation, the beads were removed from the CaCl2 solution and washed numerous times with distilled water. Glucose at a concentration of 15% was employed as the activation medium for the beads, after a thorough cleaning beads were transferred to this activation media.

Treatment of Synthetic Dyes:

The colours were made by combining 1 gramme, 0.5 gramme, and 0.1 gramme of each dye (Congo red, Crystal violet) in 100 milliliters of distilled water. The beads were then incubated with the appropriate colors and concentrations (1 percent, 0.5 percent, 0.1 percent). It was subsequently incubated for 7 days, with the O.D. being monitored every day.

Decolorization Study:

Percent decolorization ability was used to represent the degree of decolorization capacity. At A_{660}, and A_{400} nm, the reduction in absorbance was measured. The activity of decolorization was computed using the formula,

$$\% \text{ Decolourization} = \frac{\text{Initial OD - Final OD x 100}}{\text{Initial OD}}$$

RESULTS & DICUSSION

Isolates:(Figure1& 2)

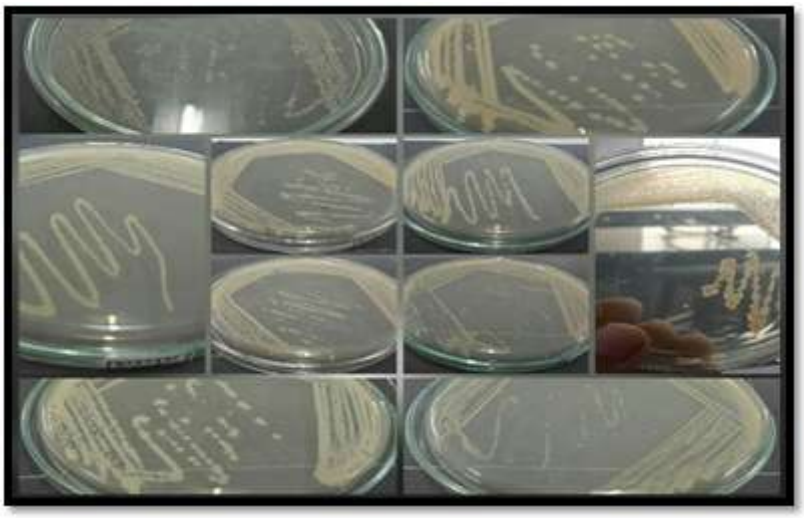


Figure 1 Marine Isolates

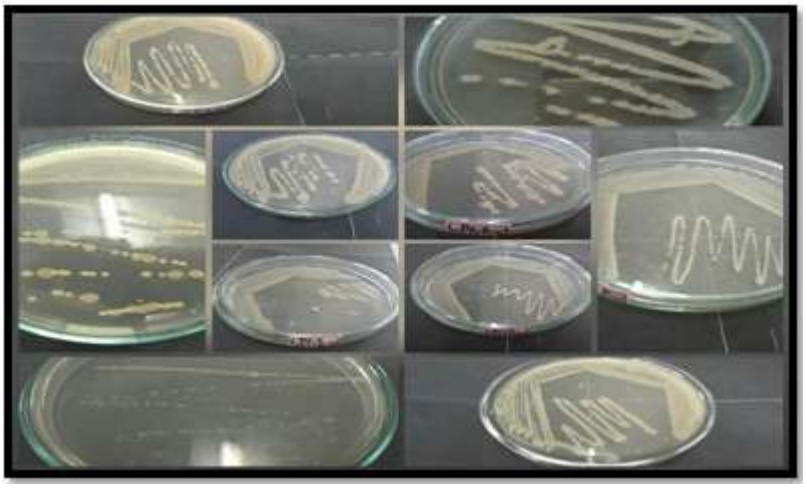


Figure 2 Marine Isolates

Colony Characteristics: (Table 1)

Table 1 Colony Characteristics

Sr. No.	Orgm.	Size (mm)	Shape	Margin	Elevation	Colour	Opacity	Gram Nature
1	PC1	5	Irregular	Entire	Concave	Cream	Opaque	+ve cocci
2	PC2	3	Circular	Irregular	Flat	Off White	Opaque	-ve rod
3	PC3	4	Circular	Undulated	Raised	Cream	Opaque	-ve cocci
4	PC4	3	Irregular	Entire	Raised	Cream	Opaque	-ve rod
5	PC5	3	Circular	Irregular	Concave	Cream	Opaque	-ve rod
6	PC6	2	Circular	Entire	Concave	Cream	Opaque	+ve rod
7	PC7	3	Irregular	Irregular	Raised	Cream	Opaque	-ve rod
8	PC8	2	Circular	Irregular	Concave	Cream	Opaque	-ve rod
9	PC9	2	Circular	Irregular	Concave	Cream	Opaque	-ve rod
10	PC10	2	Irregular	Irregular	Flat	OffWhite	Opaque	-vecb
11	PC11	3	Circular	Irregular	Concave	Cream	Opaque	-ve rod
12	PC12	4	Irregular	Irregular	Concave	Cream	Opaque	-ve rod
13	PC13	2	Circular	Entire	Concave	Cream	Opaque	+verod
14	PC14	4	Circular	Irregular	Concave	Cream	Opaque	-verod
15	PC15	4	Circular	Irregular	Raised	Cream	Opaque	-ve rod
16	PC16	3	Circular	Entire	Raised	Cream	Translucent	+ rod
17	PC17	4	Circular	Entire	Concave	Cream	Translucent	+verod
18	PC18	2	Circular	Irregular	Raised	Cream	Opaque	+verod
19	PC19	3	Circular	Irregular	Flat	Cream	Opaque	-vecocci

20	PC20	2	Circular	Entire	Raised	Cream	Opaque	-verod

Gram's Staining:(Figure 3)

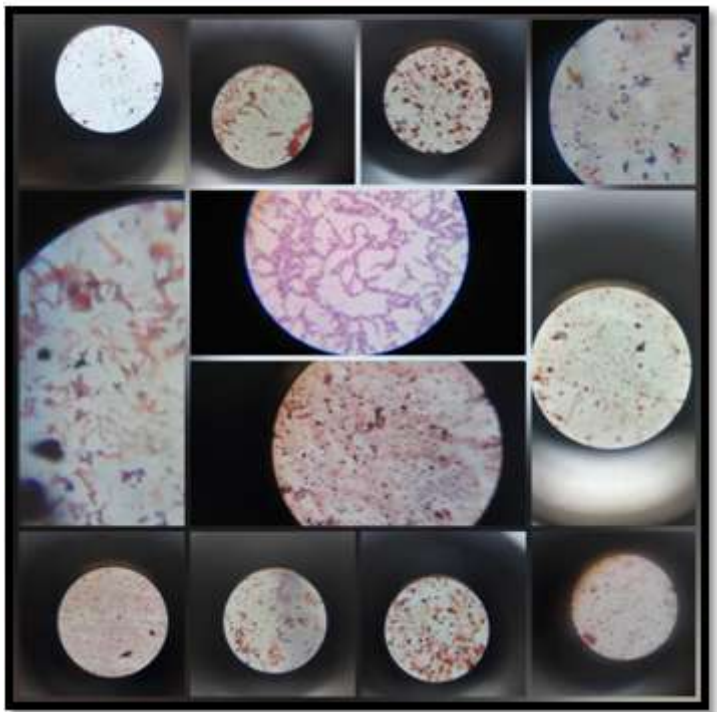


Figure 3 Gram's Staining of bacterial cells

Biochemical Tests: (Table 2)

Table 2 Biochemical Characterization of Isolates

Organism	Indole	Methyl Red	Voges-Proskauer	Citrate	TSI	Catalase	MSB
PC1	-	++	±	-	k/A/-/-	++	+
PC2	-	-	+	-	k/A/-/-	±	±
PC3	-	++	+	±	k/A/-/-	±	±
PC4	-	++	+	-	k/A/-/-	++	+
PC5	-	-	+	-	k/A/-/-	++	±
PC6	-	-	+	+	k/A/-/-	++	+
PC7	-	++	+	+	k/k/-/-	++	+

PC8	-	++	±	-	k/k/-/-	++	+
PC9	-	++	+	-	k/k/-/-	++	+
PC10	-	±	+	-	k/k/-/-	++	-
PC11	-	+	+	-	A/A/-/-	++	+
PC12	-	+	+	-	A/A/-/-	±	±
PC13	-	+	±	-	A/A/-/-	+	+
PC14	-	++	+	-	k/A/-/-	++	±
PC15	-	++	±	-	A/A/-/-	++	-
PC16	-	+	+	-	k/A/-/-	±	±
PC17	-	±	+	-	k/A/-/-	++	++
PC18	-	±	+	-	k/k/-/-	+	-
PC19	-	++	+	-	k/A/-/-	++	±
PC20	-	++	+	-	k/k/-/-	±	±

KEY:
±: Positive/Negative
- : Negative
+: Moderately Positive
++: Strongly Positive
k/k: Alkaline Slant/Butt
A/A: Acidic Slant/Butt
-/-: No gas/No H_2S

Citrate Utilization Test: (Figure 4)

Fig 4

Figure 5 Immobilization of PC18 Cell Suspension inNaAg-CaCl2 Beads

Primary Screening for Dye Degrading Bacteria:
13 of the 20 isolates showed some dye degrading activity, with PC18 exhibiting the greatest, i.e., 25% degradation activity, and was then integrated for immobilization and subjected to several dyes of varying concentrations.
Immobilization and Dye Degradation Activity by PC18 Isolate:

Figure 6 Control Beads From day 1 to Day 7 Remained Unchanged

Figure 7 Day1 of Dye Inoculation of Congo Red

Figure 8 Day 1 of Dye Incubation of Crystal Violet

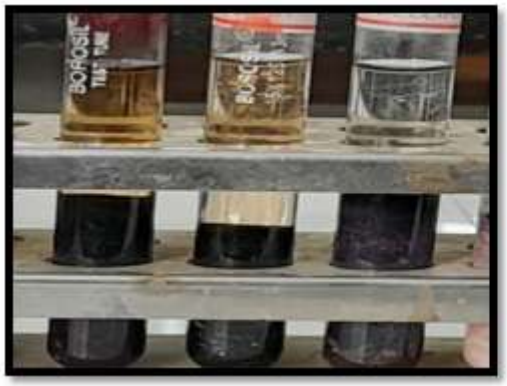


Figure 9 day 7 of Dye degradation by Congo Red

Figure 10 Day 7 of Dye Degradation by Crystal Violet

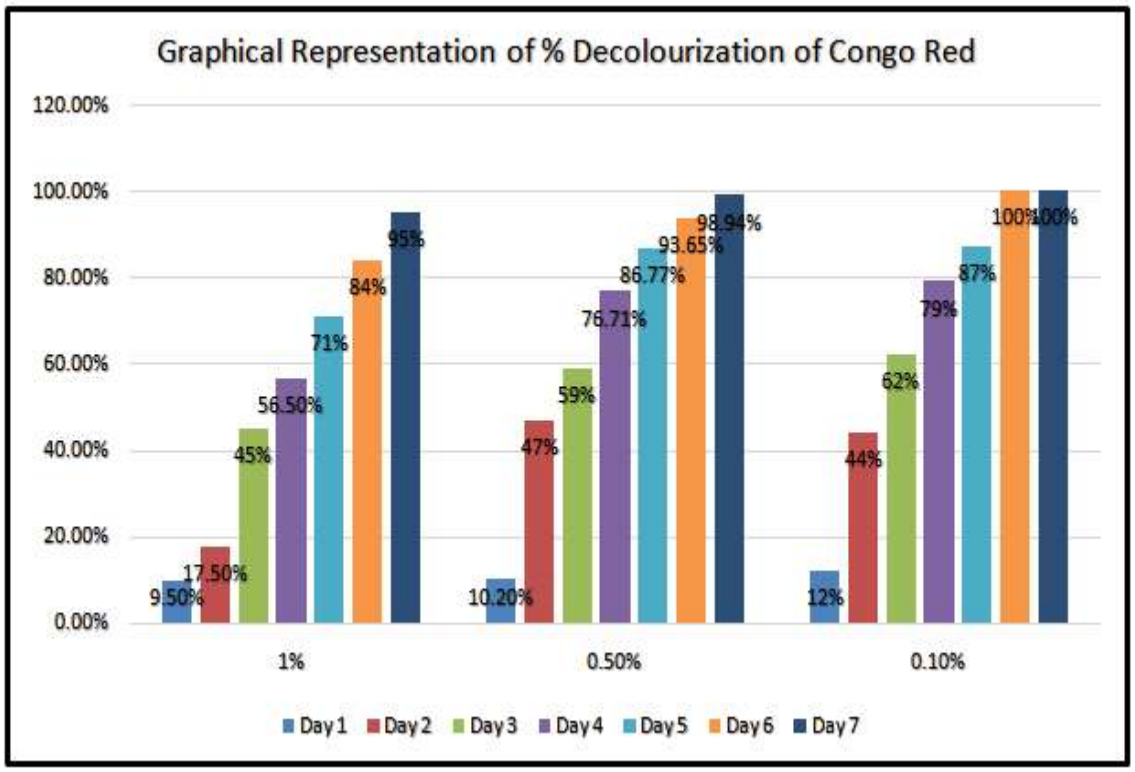
Table 3 Day wise O.D of PC 18 + Dyes containing tubes

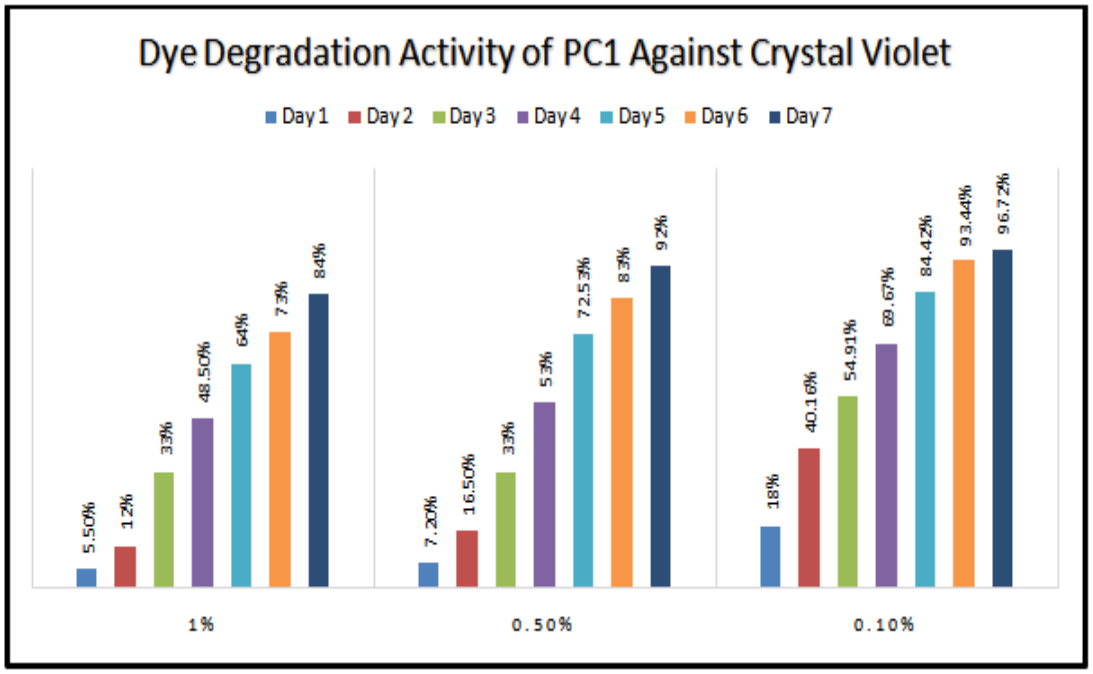
Dyes	Days						
	1	2	3	4	5	6	7
Congo Red (660nm)							
1%	1.81	1.65	1.10	0.87	0.58	0.32	0.09
0.5%	1.69	1.00	0.76	0.44	0.25	0.12	0.02
0.1%	0.88	0.56	0.38	0.21	0.13	0.00	0.00
Crystal							

Violet (400nm)							
1%	1.89	1.76	1.34	1.03	0.72	0.54	0.32
0.5%	1.79	1.61	1.28	0.89	0.53	0.31	0.15
0.1%	1.00	0.73	0.55	0.37	0.19	0.08	0.04

Table 4 Day wise % Decolorization

Dyes	**Days (% Decolourization)**						
	1	2	3	4	5	6	7
Congo Red 1%	9.5%	17.5%	45%	56.5%	71%	84%	95%
0.5%	10.2%	47%	59%	76.71%	86.77%	93.65%	98.94%
0.1%	12%	44%	62%	79%	87%	100%	100%
Crystal Violet 1%	5.5%	12%	33%	48.5%	64%	73%	84%%
0.5%	7.2%	16.5%	33%	53%	72.53%	83%	92%
0.1%	18%	40.16%	54.91%	69.67%	84.42%	93.44%	96.72%





DISCUSSION

Azo colors are the biggest gathering of colors. Various assortments of azo colors are widely utilized in the material, paper, food, beauty care products and drug ventures. They are the biggest and most adaptable class of color, yet have underlying properties that are not normally eliminated from water by ordinary waste water framework. Azo colors are intended to oppose compound and microbial assaults and to be steady in light and washing. The expulsion of azo colors from profluent is significant because of their mutagenicity and cancer-causing nature along with their extraordinary shading. Both physicochemical and natural strategies for the expulsion of colors have been researched broadly. The separation of good color decolorizing species requires screening, and these disengaged strains ought to have capacity to corrupt and detoxify material colors (Silveira et al., 2009). The current review was centered around decolourization of material azo colors and biodegradation of material color emanating by utilizing microbes separated from marine water test. Biodegradation of monetarily accessible material colors Congo red, Crystal Violet, was contemplated against PC18 which has been disengaged from the marine water by spread plate strategy and % decolourization was displayed in the figures and Tables going with the outcomes. Twenty unique microbes were secluded from the material color defiled water. In view of primer test, plating on particular media and biochemical test, the strong color debasing microbes with greatest decolorization movement can be accepted that is from Bacillus species for additional explanation of the species and variety of segregates explicitly PC18 Identification utilizing progressed sub-atomic devices like 16S rRNA Sequencing and, bioinformatics is under review.

Sriram et al., 2013 separated three unique bacterial, for example, Bacillus subtilis, E.coli, Pseudomonas fluorescens for the corruption study. Saranraj et al., 2010 segregated 5 unique bacterial from the material color profluent and distinguished as Bacillus subtilis, Proteus mirabilis, Pseudomonas fluorescens, and Staphylococcus aureus. In view of starter tests, plating on particular media and biochemical tests, they were distinguished as Bacillus subtilis, Pseudomonas fluorescens, and E. coli. The reduction in the decolorization productivity apparently was diminished with expansion in the grouping of the colors. Comparable perceptions have been recorded before for decolourization of Turquoise Blue color by Bhoomi Joshi et al., 2013.

Lately, the utilization of immobilized cell has been getting expanded consideration in the field of wastewater decolorization since this technique not just works on detachment and recuperation of immobilized microbes and the limiting specialist yet in addition makes the application reusable, which diminishes the general expense. All in all, immobilized cells are more lenient to neighborhood bothers like changes in temperature, pH and presence of inhibitor compounds. It has been expressed that sodium alginate is an appropriate network material since it is non-harmful and the strategy utilized for its gelation is gentle towards the microorganisms.The outer carbon source utilized for this study was glucose. The decision of glucose was conscious. During decrease of azo colors, it is by and large detailed that the presence of promptly accessible substrates that go about as electron benefactors for azo security decrease is crucial. a few investigations have announced the

utilization of glucose as an optimal wellspring of carbon and energy for Decolouration of azo colors. In this concentrate on the glucose fixation utilized was 15% of glucose and most extreme decolorization was acquired for Congo Red with 95% decoloration in 1%, 98.94% in 0.5%, and 100 percent decolorization was seen in 0.1% as contrasted and Crystal violet whose % decoloration for 1%, 0.5% and 0.1% are 84%, 92%, 96% individually.sss.

CONCLUSION

In ends, the material, coloring and completing industry utilize wide assortment of dyestuffs because of the quick changes in the client's requests. Accordingly, by the utilization of the above confine economical biodegradation of the hurtful azo colors used by the color, material, paper ink and so forth ventures can be conceivable. These techniques are eco-accommodating as well as industrially reasonable in any event, for the limited scale ventures. An intensive examination, thinking about of specific boundaries, for example, streamlining of the color focus for the disengages as well with respect to the color to be corrupted, impact of physicochemical boundaries on debasement and so on the loose scale is important to give unequivocal proof to the value of these confine in supporting color corruption ability. Further sub-atomic review on their enzymatic property and debasement cycle could uncover them as a significant material color degrader. Hence, by this current it is inferred that the bacterial disconnects like Bacillus sp., can utilized as a decent microbial hotspot for treatment.

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