



SIGNIFICANCE OF *Halobacterium cutirubrum* IN POLLUTION CONTROL – AN ATTEMPT TOWARDS SUSTAINABLE DEVELOPMENT GOALS

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ARTICLE INFO

Article History:

Received: 16 December 2025

Revised: 10 April 2026

Accepted: 21 April 2026

Published: 15 July 2026

Keywords:

Pollution control, inter-linkage of sustainable development goals, *Halobacterium cutirubrum*, biosorption and biodegradation, influencing factors

ABSTRACT

Clean water and climate change are two important interlinking sustainable development goals. Water crises are expected to be primarily caused by climate change through worsening floods, sea level rise and shrinkage of ice fields, wildfires and severe droughts not confined to a particular season. Sustainable management of water is in building the resilience of societies apart from reduction of carbon emission. It is vital that effective water pollution mitigation measures are developed to ensure clean water supply. Heavy metals and organic residues from industrial and other related processes are dangerous water contaminants. Several conventional treatment technologies are satisfactory but non-economical. Green techniques like biosorption, biodegradation and biotransformation have been favoured in recent years. Several microbial contents have proven effective in entrapping heavy metallic moieties and other toxic compounds. The objective of the present work is to investigate the capability of *Halobacterium cutirubrum* in removing selected toxic metals, phenolic moieties and dyes present in aqua-media with involved mechanisms. The influence of various factors are observed to optimise the process. The findings highlight that the process is both metabolic and non-metabolic dependent and the entrapment found to be drastic under immobilised conditions. Relative efficacy of biomass in sorption and degradation with suitable mechanisms was discussed. The process was found to be impactful, green, low-cost and effective for remediation of liquid waste which supports sustainable management of water and climate-resilient clean water augmentation.

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Introduction

Climate change can worsen floods, increase sea levels, melt ice fields, create more intense wildfires and severe droughts. These phenomena impact water sustainability in different areas of the world. Sustainable water management is very important to build the resilience of societies and ecosystems, and in carbon emissions reduction. Effective water pollution mitigation measures are important as the water is important to sustain all life.

Several organic residues that typically contaminate water viz., phenolic compounds,

dyestuffs, and heavy and toxic metals, originated from different industrial and other related processes. The removal of contaminants and the efficient treatment of waste is of high importance. Several solutions developed through biotechnology hold promise, including biohazard identification, and bioremediation of industrial, agricultural and household discharge.

Examples of environmental biotechnology include the involvement of microorganisms in metal entrapment from ores and bio-based fertilisers. Metabolic path ways of microbes

have been found to play key roles in many of these aspects (Fourest *et al.*, 1992; Ahalya *et al.*, 2003; Muhmad *et al.*, 2007). Biosorption, biodegradation and biotransformation have been intensely researched for the safe removal of contaminants which includes carbon capturing from the water habitats. Toxic metal and radio nuclides removal at the source are of special interest to environmental protection researchers (Atkinson *et al.*, 1998; Bailey *et al.*, 1999). Apart from being economically valuable, heavy metals are also toxic and non-degradable pollutants. Metals like lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper, and nickel typically enter the environment through industrial, mining and agricultural activities. Operations associated with mining, refining ores, disposal of sludge, incinerator releases like fly ash, radioactive process, metal plating units or the manufacturers of electrical equipment, paint industries, alloy manufactures, batteries production units, pesticide industries are also major sources of contamination (Ahalya *et al.*, 2003). Toxic metals contamination leads to several temporary or permanent health risks in humans (Michael *et al.*, 1992; Tsuritani *et al.*, 1996; Hellstrom *et al.*, 2001).

Conventional techniques viz., precipitation, coagulation, membrane process, reduction, deionisation, and surface adsorption are recognised processes in the treatment of waste water (Rich & Cherry, 1987). However, these methods have several technical/economic constraints. Eco-friendly techniques like biosorption, biotransformation and biodegradation, which involves the utilisation of appropriate microorganisms, are now favoured in pollution control.

Biosorption involves the accumulation of heavy metals from solid or liquid wastes either via metabolic mediation or through some physical-chemical approach (Fourest & Roux, 1992). Many kinds of biomass are useful in the sorption of metals (Gupta *et al.*, 2000; Pal *et al.*, 2005). The advantages of these processes include the removal of heavy

metals from aqueous media even at very dilute concentrations. Several mineral oil fragments or derivatives, viz., phenolic compounds, dye beads and halogenated products of petrochemicals, are severe organic pollutants in the environment which cannot get entrapped by sorption (Semple & Cain, 1996; Delille *et al.*, 1998; Banat *et al.*, 2000). Biodegradation is the microbially catalysed breakdown of complex organic pollutants into simpler compounds, such as water and carbon dioxide, through oxidation (Nortermann *et al.*, 1986). Carbon and energy will be produced during this process, which helps in the growth and reproduction of microbial cells that are important to mitigating environmental pollution. However, the degradation of pollutants is mostly dependent on the structural and sustainability aspects of microbes in aerobic and anaerobic conditions (Haug W *et al.*, 1991; Fourest E & Volesky B, 1996; Satyanaryana U, 2005;).

This investigation examines the sorption and degradable capacity of *Halobacterium cutirubrum* (scientific name is *Halobacterium salinarum*, a rod-shaped bacterium, less than a micron long. This microorganism has been chosen for its resistance to light and oxidation as a result of its pigmentation. Its protein-rich cell walls also make it suited for the treatment of saline or industrial waste waters containing toxic pollutants (Sharma N *et al.*, 1984). Free and immobilised cell conditions under the influence of different environmental factors were studied in order to determine the most efficient mechanism.

Materials

Microorganisms and Cultures Conditions

The biomass used for the sorption and degradation studies is *Halobacterium cutirubrum* (MTCC 1599) was procured from the Institute of Microbial Technology, Chandigarh. Collected microbial mass was placed in 250 ml Erlenmeyer flasks to grow in 100 ml medium containing 25 g sodium chloride, 2 g magnesium sulphate, 0.2 g potassium chloride, 0.3 g peptone, and 0.5g sodium citrate at pH 7.4.

The culture suspensions in homogeneous condition were prepared fresh and inoculated in an Erlenmeyer flask. It was placed in an incubator at 37°C for 7 days at 120 rpm. The biomass was centrifuged at 12,000 x g for 10 minutes, thoroughly washed and dried at room temperature.

Solutions of Metals

Analytical Reagent (AR) grade chemicals, either from S.D. Fine Chemicals Ltd. or Loba Chemie Pvt. Ltd, were used. The solution stocks of zinc, barium, lead, copper, cadmium, and magnesium were prepared by dissolving 1 g/l of ZnCl₂, BaCl₂, PbCl₂, CuCl₂, CdCl₂, and MgCl₂ in 15% NaCl solution prepared in fresh double distilled water. Exact dilution quantities were prepared from the corresponding solutions of stock for the purpose of sorption studies.

Phenolic Compounds

Phenol, orcinol, p-cresol, and resorcinol for degradation studies were obtained from the sources mentioned above. The stock solutions of the respective phenolic compounds were prepared with the dissolution of 100 mg in the 100 ml solution of 1% Na₂CO₃ and 15% NaCl. Required diluted solutions were prepared from the basic stock and used for biodegradation studies.

Azo Compounds

AR grade chemicals Methyl Red (MR), Methyl Orange (MO) and Congo Red (CR) were selected for degradation studies. 100 ml of the azo compound was dissolved in 100 ml of 1% NaCl solution. The required diluted solutions of the stocks were utilised for degradation studies.

Immobilised Materials

For immobilisation of microbial cells, calcium chloride and sodium alginates were used.

Methods

Biomass Characterisation

Phosphate presence on *Halobacterium cutirubrum* cell walls was determined by spectrophotometer using the Molybdenum Blue Method (Bassett., J. 1978).

Halobacterium cutirubrum Immobilisation

6 g of sodium alginate was diluted in 100 ml distilled water for preparation of 6% sodium alginate solution. The solution was autoclaved for 20 minutes at 120°C and pressure of 15 lb. 100 mg of biomass was macerated in the sodium alginate (6%) solution, the resultant slurry was transferred to a 100 ml flask. 2% CaCl₂ solution was added drop by drop into this slurry to obtain around 2,000 immobilised cell composites, approximately equivalent with biomass of 10 mg.

Biosorption Studies of Metals

In a 100 ml Erlenmeyer flask, 10 ml of metal solution, and 10 mg of dried *Halobacterium cutirubrum* biomass were suspended. The flasks were then agitated at 37°C on a rotary shaker (120 rpm). The samples were then withdrawn at fixed time intervals and centrifuged for five minutes. The supernatant was then analysed for residual metal content, using titrimetric and gravimetric techniques. Atomic Absorption Spectroscopy was used for confirmation studies. The maximum error considered is 2%.

Biodegradation Studies of Phenolic Compounds

100 mg of selected phenolic compound was taken in a 100 ml Erlenmeyer flask to which the dried bacterial biomass of 100 mg was added. The solution pH was adjusted to 7.4, the optimal pH for the *Halobacterium cutirubrum*. Samples were agitated on a rotatory shaker and withdrawn for sorption studies. Supernatant of 0.1 ml were collected in test tubes, to which 0.2

ml of Folin-Ciocalteu (FC) reagent, 0.2 ml of sodium carbonate, and 2 ml distilled water were added. The samples were incubated at 37°C for two hours. The concentrations of phenolic compounds were estimated by measuring the absorbance at 650 nm. The percentage of phenolic compound degradation was determined by the FC reagent standard curve method. The experiments were conducted three times for the confirmation of result with a maximum error of 2%.

Biodegradation Studies of Azo Compounds

The azo solutions were taken in flasks and 100 mg of dried bacterial mass was added and the pH of 7.4 was maintained. The solutions were agitated in a rotatory shaker for agitation at 120 rpm and 35°C. The samples were centrifuged at incubation period intervals and the concentrations were estimated by using their corresponding $\lambda_{\max}$ values. The percentage of degradation of the azo compounds was calculated by using the equation, $[C_0 - C_t/C_0] \times 100$, where C_0 and C_t are the concentration of the azo compound at the initial time and at a particular time of incubation, respectively (Wijetunga *et al.*, 2007). The mean values of three independent experiments with a maximum error of 2% were reported.

Effect of Biomass Concentration

To verify the influence of biomass concentration, 2 mg, 4 mg, 6 mg, 8 mg, 10 mg, 12 mg, and 14 mg of dried pellets were added to 10 ml of stock solutions of metals, azo compounds and phenolic compounds in 100 ml flasks. Then the solutions were placed in an incubator along with their corresponding reference solutions, and incubated for 24 hours.

pH Effect

10 ml of each of the metals, azo stuffs and phenolic compound stock solution were added to 10 mg of bacterial mass in 50 ml Erlenmeyer flasks at 3, 4, 5, 6, 7 and 8 pH. Solutions were thoroughly mixed and incubated for 24 hours.

Temperature and incubation periods were maintained.

Contact Time Effect

10 mg of dried bacterial biomass was added to 10 ml of each targeted solution in 100 ml Erlenmeyer flasks. The solutions were then agitated at 120 rpm in an incubator shaker. At required time intervals, studies were carried out at the optimal concentration of biomass, temperature and pH of the solutions.

Temperature Effect

The impact of temperature on the efficacy of bioremediation was examined at corresponding pHs, optimal biomass concentrations and optimal times. At 25°C, 30°C, 35°C, 40°C, and 45°C, the reaction mixtures were incubated for 24 hours and the influence of temperature on biosorption and biodegradation was verified.

Instruments

UV-Vis Spectrophotometer, Systronics, Model No. 117, U.A Trace Metal Analyzer Model No. 746, Cooling Centrifuge REMI, C24, Orbital Incubator Shaker, KEMI, K091, were used.

Results and Discussion

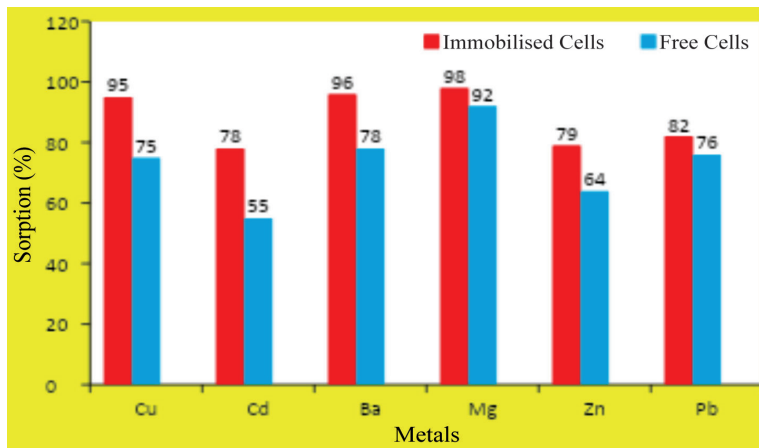
Biosorption of Heavy Metals by *Halobacterium cutirubrum*

After incubation for 24 hours, the reaction mixtures were used to verify the sorption capacity of *Halobacterium cutirubrum*, for all the selected metals from their aqueous solutions. As per the studies, the biomass was found to be effective in entrapping Ba, Mg, Cu, Cd, Zn, and Pb in free and as well as in immobilised conditions while maintaining similar experimental conditions. The results are depicted in Table 1 and Figure 1.

Table 1 and Figure 1 show that immobilised and free cells of *Halobacterium cutirubrum* exhibited sorption activity in all conditions. However, the sorption was found to be highest in immobilised conditions, which are attributed to

Table 1: Biosorption (%) of metals at 24 hours

	Cu	Cd	Ba	Mg	Zn	Pb
Sorption in immobilised conditions	95	78	96	98	79	92
Sorption in free cell conditions	75	55	78	92	64	76

Figure1: Biosorption of metals by *Halobacterium cutirubrum*

the larger surface area (Costa *et al.*, 1993; Lopez *et al.*, 1997). Mg was entrapped to a maximum of 92%, and 98% in free and immobilised cell conditions, respectively. For Cd, only 55% and 78% were entrapped in free and immobilised cell conditions, respectively. We attribute the poor entrapment of Cd to the bigger size of the metal, and the highly toxic nature of Cd. The toxicity is hypothesised to deactivate the microorganism. The higher sorption of Mg is due to the fact that Mg is a key metabolite for the microorganism under study.

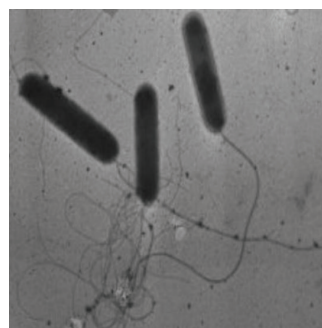
Biosorption Mechanism

The *Halobacterium* species belongs to the domain of Archaea. They are less than a micron long and closely one-thousandth millimetre in size. Its structure is shown in Figures 2 (a) and (b).

Halobacterium cutirubrum contain phospho lipids, which have several metal-binding phosphate groups. Hence, it is expected that microbial cells may take up the metal ions in different possible ways. Our results confirm



(a)



(b)

Figure 2: *Halobacterium cutirubrum* species

that in case of Mg, the biosorption is metabolism dependent. However, the sorption of Ba, Cu, Cd, Zn, and Pb is not dependent on metabolism, and instead rely on physico-chemical interaction, where coordination bonds are being formed between the metals and phosphate groups in the cell wall (Kuyucak *et al.*, 1998). Since Mg is a key metabolite for *Halobacterium cutirubrum* (Costa *et al.*, 1991), its microbial cell membranes are believed to be responsible for its sorption. To confirm this, the phosphate content in the cell wall was estimated after the stipulated period of agitation of all the metals along with the reference (Bassett *et al.*, 1978). In the case of Mg, it was observed that the variation in the concentration of phosphate groups is less whereas the phosphate concentration was found to be considerably suppressed in other metals, which confirms that the phosphate groups are involved in complexation.

Biodegradation of Phenolic Compounds

Unlike heavy metals, the phenolic compounds resorcinol, orcinol, phenol, and p-cresol undergo degradation instead of sorption by *Halobacterium cutirubrum* in its free cell condition. To identify the relative structural influence of the molecules on the degradation process, investigations were carried out at similar experimental conditions of optimal pH and concentration of biomass. The UV-Visible spectral profiles of these phenolic compounds are presented in Figure 3.

Figure 3 reveals that the phenolic compounds have typical variations in their respective λ_{max} values, this is attributed to the substituent influence on phenolic moieties (Suryanarayana Raju & Ramachandraiah, 1996). Typical quick variations were observed in the spectral profiles of all the compounds that had the addition of biomass. We propose that this variation is due to phenolic compounds degradation. To confirm this phenomenon, the spectral changes were verified after 24 hours. This observation for phenol is shown in Figure 3. The studies were continued for 10 days for verification of degradation trends of the phenolic compounds. From Figures 4 and 5, it is shown that all the phenolic compounds were degraded by the free cells of *Halobacterium cutirubrum* in the typical period of experimentation. The estimation of degradation percentage of phenolic compounds by the FC reagent method is shown in Table 2 and Figure 5.

All the selected compounds were susceptible to degradation by *Halobacterium cutirubrum*. However, the degradation differed from one phenolic compound to another. We propose that the presence of substituents on the compound has significant influence and hence the degradation is not just dependent on conditions of experiment but mostly on structural factors (Rosa & Franz, 2001). Further, it is understood that the more the conjugation, the higher the degradation.

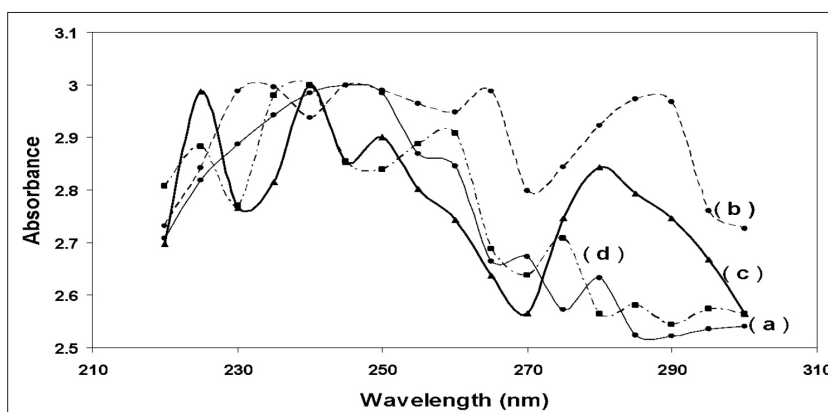


Figure 3: UV-Visible spectral profiles of (a) phenol, (b) resorcinol, (c), orcinol, and (d) p-cresol

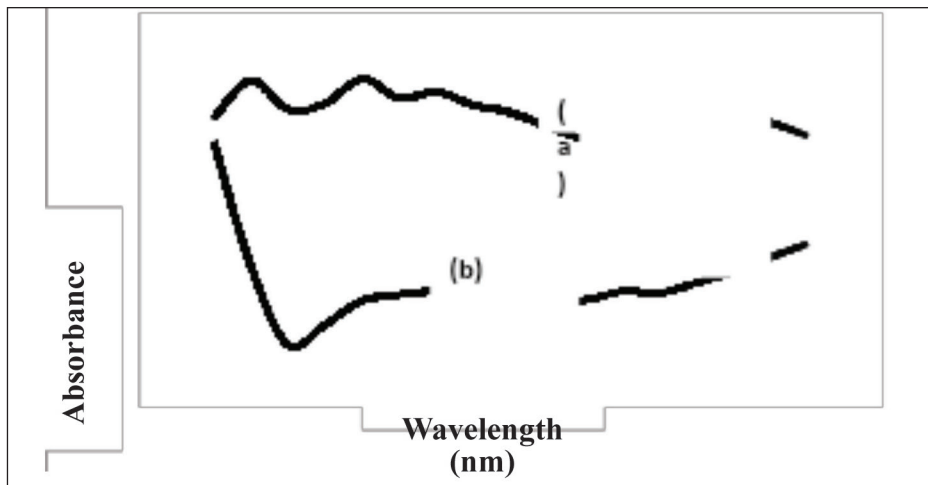


Figure 4: UV-Visible spectra of phenol for (a) after 30 minutes and (b) after 24 hours

Table 2: Biodegradation (%) of phenolic compounds

	Phenol	Resorcinol	Orcinol	p-Cresol
Degradation in free cell conditions	52	78	90	86

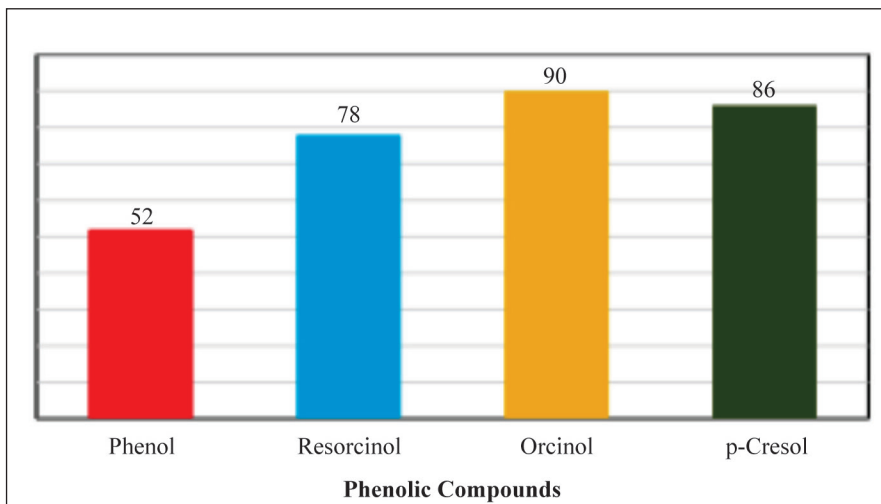


Figure 5: Degradation of phenols by *Halobacterium cutirubrum*

Biodegradation Mechanism - Phenols

The degradation of phenolic compounds by biological species is expected to take place in different ways due to structural variations of microorganisms (Dagley *et al.*, 1964). In view of the typical structure of *Halobacterium cutirubrum*, we suggest that phenolic compounds

degrade in different ways. Dihydroxylation is a key step in aromatic ring cleavage in phenols (Weijie Fu, 1999; Rebecca *et al.*, 2000; Sharma, 2005). In the case of cresol, the fission may start either at the aromatic ring or at the aliphatic chain (Emerson *et al.*, 1994). Catechol is an important

intermediate compound in degradation. It facilitates fast cleavages at the *ortho/meta* positions on the basis of neighbouring group presence in molecules. Our studies confirm by observing the original spectra of catechol. With increased incubation period, absorbance at ≈ 240 nm falls slowly and disappears afterwards. We had also observed that substituents on phenolic moieties highly influence the degradation. From phenol to orcinol, gradual increment in degradation was observed, with the increase in the number of hydroxyl groups leading to the fast generation of the catechol which subsequently collapses to CO_2 and H_2O . Exceptional increase in degradation is observed in the case of p-cresol, due to the presence of methyl groups, which are more electron-donating in nature. It is confirmed by cross verification of degradation studies of all the selected compounds at continuous spectral mode.

Biodegradation of Azo Compounds

Textile industries are one of the largest water consumers, their waste effluents contain various dyes etc. (Heinflieg *et al.*, 1998). These colouring agents in water sources have

a huge impact on aquatic life, as they suppress dissolved oxygen and sunlight, (Kalyuzhnyi & Sklyar, 2000) severely degrading water quality. Azo stuffs methyl red, methyl orange, and congo red were tested for degradation with a view of developing effective water treatment methods.

Biomass in sufficient amounts was added separately to the solutions of the azo compounds to investigate the degradation capability after incubation of reaction mixtures for a maximum three hours of incubation, and observed no significant change afterwards. The studies were conducted at optimal pH, temperature and biomass concentrations. For each of the compounds, the UV-Visible spectral profiles and maximum absorbance wavelength were scanned at the beginning and end of incubation. Scans from the end stage showed about 90% colour degradation.

The UV-Visible spectral profiles of three azo compounds along with the biomass culture before incubation and after three hours of incubation are presented in Figures 6 to 8, which show the remarkable changes before and after the incubation as a result of the degradation of azo compounds.

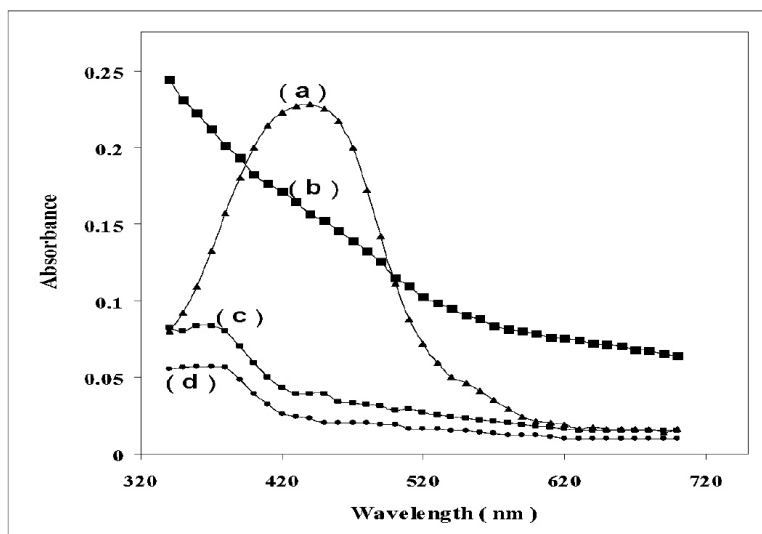


Figure 6: Spectral variations (UV-Visible) of methyl red along with microbial culture at 35°C with (a) zero time, (b) 30 minutes, (c) 60 minutes, and (d) 120 minutes

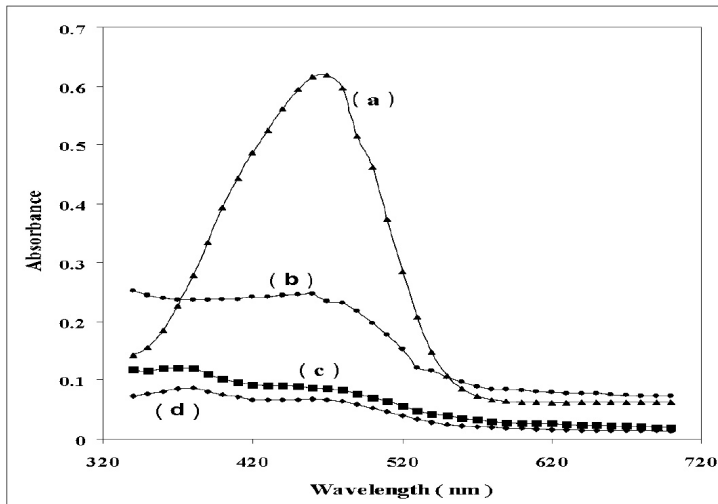


Figure 7: Spectral variations (UV-Vis) of methyl orange along with microbial culture at 35°C with (a) zero time, (b) 30 minutes, (c) 60 minutes, and (d) 120 minutes

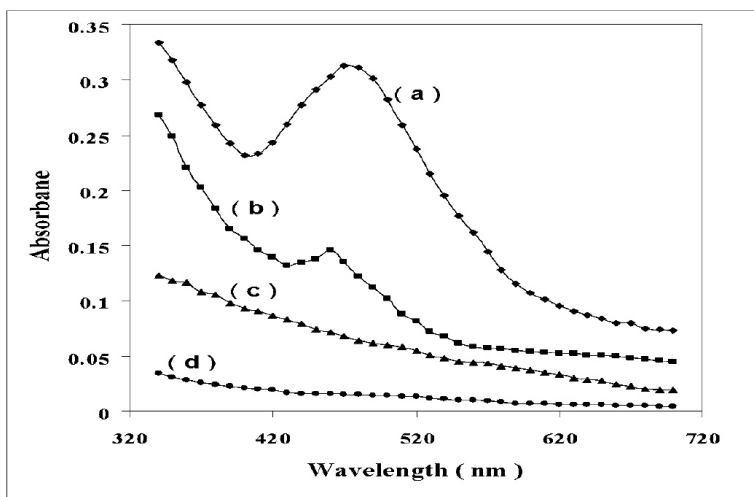


Figure 8: Spectral variations (UV-Vis) of congo red along with microbial culture at 35°C with (a) zero time, (b) 30 minutes, (c) 60 minutes, and (d) 120 minutes

Due to degradation by aerobic microorganisms, the structural modifications in azo compounds are reflected through variations in their spectral profiles. The typical variations observed in the far UV region and near visible region are due to the degradation of azo compounds via the use of carbon as a source of energy (Rui B *et al.*, 2001; Manu B & Sanjeev C, 2003). This is further confirmed

by the observation of fast and sudden fall in absorbance in the visible region at a typical wavelength and subsequent new peak generation in the UV region. This is further evidence of our assumption that degradation is taking place instead of absorption. During these changes, the azo compounds lose colour. These results are in Table 3 and Figure 9.

Table 3: Biodegradation (%) of azo compounds

	MR	MO	CR
Degradation in free cell conditions	89.92	89.45	95.21

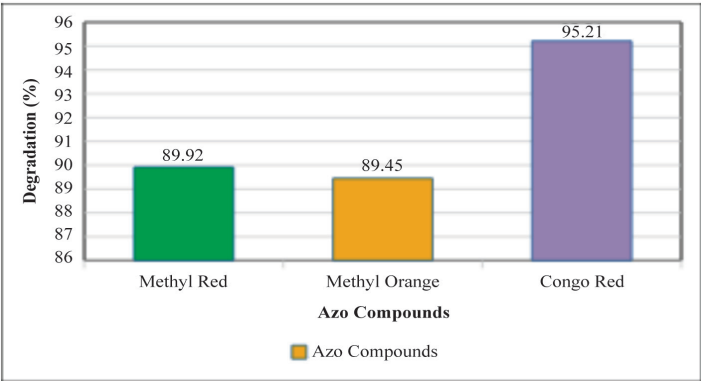


Figure 9: Biodegradation of azo compounds by *Halobacterium cutirubrum*

Hence, from the above, one can observe that the *Halobacterium cutirubrum* is active in degrading azo compounds with the consumption of carbon as a source of energy.

Biodegradation of Azo Compounds - Mechanism

Anaerobic consumption generally produces amines, an initial step of the process (Nortemann *et al.*, 1986; Haug *et al.*, 1991; Pourbabee *et al.*, 2005). It was found that the new peaks generated in the UV region as time proceeded were not observed at the beginning. This is due to the generation of amine derivatives due to aerobic

degradation, leading to pyruvate and fumarate, energy sources for bacterial species.

Factors Influencing the Activity of *Halobacterium cutirubrum*

Effect of Biomass Concentration

The reaction mixtures with different initial concentrations of biomass were incubated and used for identifying variations in degradation as well as sorption trends by the biomass. The results are shown in Tables 4 to 6, respectively, for sorption and degradation. A pictorial representation for the sorption of metals as a representative case is shown in Figure 10.

Table 4: Biomass initial concentration effect on sorption (%) of metals

Concentration	Cu	Cd	Ba	Mg	Zn	Pb
0.2	28	15	39	37	17	13
0.4	46	29	52	70	32	33
0.6	64	37	61	89	52	52
0.8	72	45	71	90	60	70
1.0	75	54	78	92	64	76
1.2	73	53	76	91	62	75
1.4	72	49	76	91	62	75

Table 5: Biomass initial concentration effect on degradation (%) of phenols

Concentration	Phenol	Resorcinol	Orcinol	p-Cresol
0.2	0	0	0	0
0.4	26	29	31	32
0.6	39	46	52	48
0.8	46	66	77	69
1.0	52	78	90	86
1.2	50	76	88	84
1.4	44	76	87	82

Table 6: Biomass initial concentration effect on degradation (%) of azo compounds

Concentration	Methyl Red	Methyl Orange	Congo Red
0.5	65.35	71.10	72.20
1.0	89.91	89.29	95.21
1.5	78.51	85.06	87.86
2.0	70.18	77.76	82.43

As it is revealed, the initial concentration of biomass has a significant influence on sorption or degradation. It was observed that the sorption or degradation increased with the increase in the initial concentration and continues until it reaches equilibrium due to increase in surface area (Travieso, *et al.*, 2002; Bhatnagar, *et al.*, 2006). 1 mg/ml was found to be the optimum biomass concentration.

Effect of pH

In general, the pH of the media/system is expected to be an important factor in most biological processes, with a considerable influence on the presence of functional groups in biomass (Lezcana *et al.*, 2001; Alaa *et al.*, 2006). Due to the survival problem of our biomass, the studies could not conduct in higher acidic and basic conditions. The results obtained are shown through Table 7 and Figure 11. It is understood

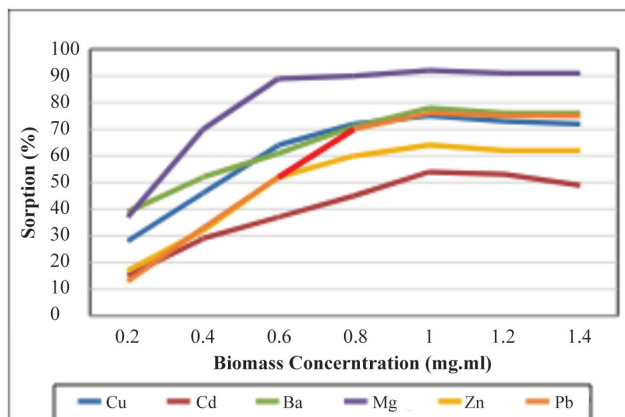
Figure 10: Initial concentration effect on biosorption of metals by *Halobacterium cutirubrum*

Table 7: Effect of pH on sorption (%) and degradation (%)

pH	Cu	Cd	Ba	Mg	Zn	Pb	Phenol	Resorcinol	Orcinol	p-Cresol	MR	MO	CR
3	12	8	11	8	9	12	-	-	-	-	-	-	-
4	52	35	41	54	25	41	-	-	-	-	-	-	-
5	74	54	65	78	50	58	22	24	29	28	54	53	40
6	70	50	64	91	62	76	42	49	62	48	64	80	72
7	66	45	64	92	61	71	49	75	82	78	90	89	95
8	60	42	63	90	60	65	51	72	82	81	87	88	94
9	-	-	-	-	-	-	42	66	74	67	84	84	91

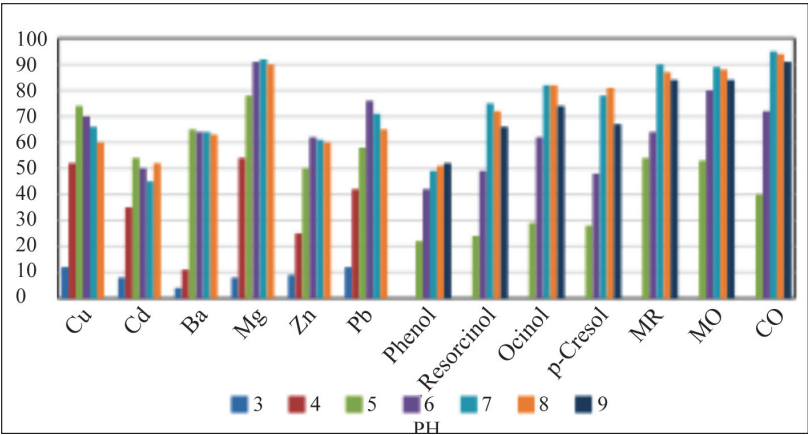


Figure 11: pH effect on biosorption and biodegradation by *Halobacterium cutirubrum*

from data that the sorption and degradation rates are very slow in highly acidic environments due to the interference of H⁺ ions (Fourest *et al.*, 1992). A significant increase is observed as the pH rises from 4 to 7 before plateauing. We propose that this is due to the compounds getting converted to their respective salts, which hinders the attack of microbial mass.

Effect of Contact Time (Incubation Period)

To understand the influence of incubation period on the capability of the biomass for heavy metal sorption, phenol, and azo compounds degradation under similar experimental conditions are depicted in Tables 8 and 9, and pictorially represented in Figures 12 and 13. The data reveals that beyond 20 % of metal

Table 8: Contact time (incubation period) effect on metal sorption (%)

Contact Time (minutes)	Cu	Cd	Ba	Mg	Zn	Pb
30	22	15	28	25	21	21
60	39	22	42	39	34	37
90	46	34	52	55	41	49
120	58	45	67	67	48	59
150	65	48	73	81	56	69
180	75	54	78	92	64	74
240	75	52	78	91	64	74

sorption are completed within 30 minutes and continually increase with increase in duration of incubation period before reaching saturation

by 180 minutes. No considerable changes were found thereafter. However, the biomass needed 10 days for effective degradation.

Table 9: Contact time (days) effect on degradation (%) of phenolic compounds

Contact Time (days)	Phenol	Resorcinol	Orcinol	p-Cresol
0	0	0	0	0
2	10	28	15	36
4	18	69	53	51
6	23	73	69	82
8	40	75	89	83
10	52	78	90	86

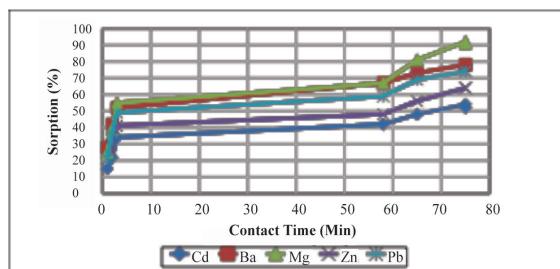


Figure 12: Contact time effect on biosorption of metals by *Halobacterium cutirubrum*

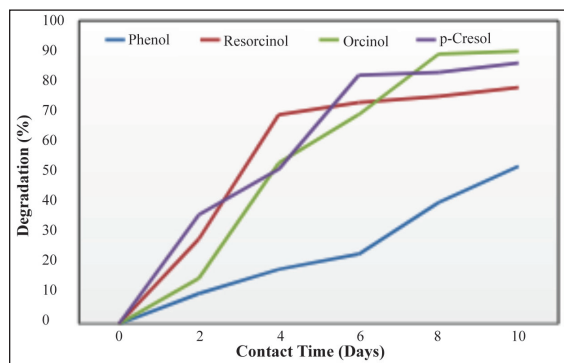


Figure 13: Contact time effect on biodegradation of phenols by *Halobacterium cutirubrum*

Effect of Temperature

The studies were carried out at different initial reaction temperatures ranging from 20°C to 50°C while maintaining the other factors. It was found that the *Halobacterium cutirubrum* showed its maximum effect of either sorption or degradation in the temperature range between 30°C to 35°C.

Conclusions

The aim of this investigation was to develop a green technology approach to two important sustainable goals, clean water, and climate change. The microbe *Halobacterium cutirubrum* was tested for its biosorption and biodegradation abilities on very important

industrial effluents and found to be successful. It was found that remediation was absolutely structurally dependent. Important parameters for optimisation viz., biomass concentration, pH, incubation period and temperature, were elucidated.

Acknowledgements

The authors acknowledge the authorities, Godavari Global University (GGU), Rajamahendravarm, Andhra Pradesh, India for their support in arranging the necessary facilities.

Author Contributions Statement

Suryanarayana Raju Sivangi planned, carried out the research, proposed the mechanisms and wrote, and revised the article. Srinivas Venkata Ramana Kandarpa developed mathematical tabulations and graphical developments.

Conflict of Interest Statement

The authors agree that this research was conducted in the absence of any self-benefits, commercial, or financial conflicts.

Ethical Conduct

Procedures adopted in this study were approved by the University Research Committee, Godavari Global University, Rajamahendravarm, Andhra Pradesh, India C through RD193, on 10 March 2025.

Declaration of AI-usage

During the preparation of this work, the authors did not use any AI tools.

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