

## An assessment of the bio-remedial potential of *Rhodospirillum rubrum* under different conditions

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**Abstract:** *Rhodospirillum rubrum* is a purple nonsulfur bacterium that can grow aerobically or anaerobically. It has diverse metabolism as it has the ability to live through cellular respiration, fermentation, photosynthesis, or photoautotrophic growth. Therefore, it can be used as a potent bioremedial agent. An attempt was therefore made to evaluate its effectiveness in bioremediation of waste water, particularly contaminated with sewage waste. Certain physico-chemical parameters for the assessment of quality of sewage contaminated water were considered, including pH, BOD, nitrates, sulphates, chlorides and ammonia. Parameters were tested before, during and after the bioremedial treatment with *Rhodospirillum rubrum*. A comparison amongst three stages of testing was made, which has been of great help in assessing the bioremedial impact of *Rhodospirillum rubrum*. The outcome of the study indicates that *Rhodospirillum rubrum* is a potent bioremedial agent in improving the quality of water, contaminated with sewage waste. It can oxidise the organic load present in the sewage waste, even in the absence of oxygen.

**Keywords:** Bioremediation, Bioreactor, BOD, waste water, *Rhodospirillum rubrum*.

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### I. INTRODUCTION

Sewage waste disposal is a big problem in urban and semi urban areas. The un-decomposed sewage at several places is being mixed directly in to the water bodies, making them severely contaminated. The sewage water contains a wide variety of dissolved and suspended impurities, such as organic materials and nutrients that tend to rot. The sewage when enters a lake or stream, causes eutrophication, leading to excessive growth of algae and bacteria. Some of the organisms that do overpopulate from this can be disease-causing microorganisms.

Bioremediation is an effective method to degrade and detoxify various pollutants in the sewage and domestic waste. This approach uses simple micro-organisms that consume and degrade various organic pollutants. Bioremediation is a cost effective and efficient approach to reduce environmental pollution (B B Nepple, 2000).

In present investigation, *Rhodospirillum rubrum* was used as bioremedial agent. It is a rod-shaped, gram-negative, purple non-sulfur photo-heterotrophic bacterium. It is a great metabolically diverse organism that is capable of various modes of growth including aerobic respiration, anaerobic anoxygenic photosynthesis and fermentation (Furuhata et al, 2013).

### II. MATERIALS AND METHODS

The water samples were collected from various sites of the River Yamuna (At Mathura, India), which receive drains that contain domestic and sewage waste. Samples were collected in clean plastic bottles of 1 litre capacity. One part of the samples was analysed for physico chemical parameters in the laboratory using APHA guidelines. The parameters tested, include pH, BOD, nitrates, sulphates, chlorides and ammonia. The other part of the sample was used for bioremedial treatment. It was filtered and divided into four parts A, B, C and D.

Part A was inoculated with *Rhodospirillum rubrum* and kept in anaerobic light conditions. Part B was also inoculated with *Rhodospirillum rubrum* and kept in aerobic light conditions. Part C was also inoculated with *Rhodospirillum rubrum* and kept in anaerobic dark conditions. Part D was also inoculated with *Rhodospirillum rubrum* and kept in aerobic dark conditions.

The strains of the *Rhodospirillum rubrum* were obtained from ATCC (Global Bioresource centre), pure cultures (inoculum) were developed to increase the number of bacteria using Siström's minimal medium. Stirred tank Bioreactor (Batch type) was used for the bioremediation programme. The bioreactor has a two litre capacity glass column (tank). The glass tank bioreactor was selected to provide necessary lightening conditions for the growth and action of bacteria.

A tungsten lamp was placed 50 cm away from the glass column (200W/m<sup>2</sup> intensity). 500 ml of sterilized sample plus 500 ml Sistrof's minimal medium was taken into the reactor and 10 ml of inoculum was added to it. For optimal mixing the agitator system was set at 20 rpm. The bacteria were able to grow in the changed medium as log phase achieved well in time in both cases.

Two readings were taken - a. After 24 hours of mixing, b. After 48 hours of mixing.

### III. OBSERVATION AND DISCUSSION

The observations which were recorded have been summarized in following tables.

**Table 1- Changes in Physico-chemical parameters by *Rhodospirillum rubrum* in light conditions.**

Parameters	unit	PART A of the sample (Anaerobic light conditions)			PART B of the sample (Aerobic light conditions)		
		Before Mixing	After 24 hours of mixing	48 hours of mixing	Before Mixing	After 24 hours of mixing	48 hours of mixing
pH		8.5	8.2	7.9	8.5	8.3	7.9
BOD	mg/l	16.8	8.56	3.28	16.8	8.21	5.25
Sulphates	mg/l	6.13	6.20	6.58	6.13	6.57	7.01
Sulphides	mg/l	6.25	5.18	3.74	6.25	3.27	2.98
Nitrates	mg/l	4.21	4.51	4.31	4.21	5.09	5.78
Ammonia	mg/l	18.22	12.41	8.14	18.22	10.17	6.43
Chlorides	mg/l	6.27	4.21	2.57	6.27	4.07	3.08

**Table 2- Changes in Physico-chemical parameters by *R. sphaeroides* in dark conditions**

Parameters	unit	PART C of the sample (Anaerobic Dark conditions)			PART D of the sample (Aerobic Dark conditions)		
		Before Mixing	After 24 hours of mixing	48 hours of mixing	Before Mixing	After 24 hours of mixing	48 hours of mixing
pH		8.5	8.3	8.1	8.5	8.2	7.9
BOD	mg/l	16.8	13.01	11.26	16.8	12.22	9.25
Sulphates	mg/l	6.13	6.01	5.98	6.13	5.12	4.74
Sulphides	mg/l	6.25	5.28	4.44	6.25	4.34	3.71
Nitrates	mg/l	4.21	4.77	4.11	4.21	5.98	5.74
Ammonia	mg/l	18.22	16.61	13.26	18.22	11.45	9.36
Chlorides	mg/l	6.27	5.12	4.22	6.27	5.28	4.65

pH is an important valuable indicator which shows the acidic or alkaline nature of water. The pH of the sample was found to be alkaline, mainly due to high ammonical contents (Agrawal et al, 2000). A reduction in the pH was noted during and after the treatment in all the cases because of decrease in ammonia. So, changes in pH were found to be in perfect correlation with the values of ammonia. Accordingly, decrease in alkalinity indicates towards the oxidizing capacity of *Rhodospirillum rubrum* (Ritchie, 2012).

Due to increasing concentration of oxygen, ammonia was oxidized to nitrates. So, nitrates exhibited a trend opposite to that of ammonia. Hence, a higher value of nitrate contents was observed, especially under aerobic conditions. In anaerobic light conditions, no external air was given to the sample but significant reductions in ammonia values were noted. In these cases, the oxidizing conditions were developed by microbial photosynthetic oxygen (Blankenship et al, 1995). This clearly shows that *Rhodospirillum rubrum* is a good oxidizing biological agent which in the presence of light, even under anaerobic conditions, can have a strong oxidizing impact. (Tanya Krutz, 1995).

The conclusion further gains strength from the fact that in case of part C (where the condition was dark and anaerobic), no significant decrease in the values of ammonia was noted after 24 hours of mixing. Also, there was no significant change in the pH value.

Similar to nitrates, the sulphates also exhibited a slight increase in the anaerobic light conditions and aerobic conditions. This was mainly because of the oxidation of sulphides to sulphates (S Kalpan, 2005). This idea further gains strength from the fact that during both light as well as dark conditions, a reduction in the value of sulphides was noted. This also proves that under anaerobic light condition, the oxidation of sulphides to sulphates was because of the photosynthetic oxygen, produced by the *Rhodospirillum rubrum*. Higher values of nitrates and sulphates show a great degree of oxidation by the *Rhodospirillum rubrum* (Focht, 1997).

Similarly, the improvement of chloride values was found maximum in anaerobic light conditions. This also suggests that anaerobic light conditions are the most suitable for the maximum output from this bacterium.

#### IV. CONCLUSION

Above discussion and analysis suggest that *Rhodospirillum rubrum* is a metabolically diverse species, being capable of growing in a wide variety of growth conditions. It can be used commercially on a large scale to treat both domestic and municipal waste water. The best growth conditions for the species were found to be anaerobic light conditions, where its oxidizing impact becomes intense due to its extreme photosynthetic capacity.

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