An Update on Bioremediation Potentials of Purple Non Sulphur Phototrophic Bacteria

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ABSTRACT

Photosynthetic bacteria can consume various type of organic matter with a relatively high growth rate and consume phosphorus and nitrogenous compounds. Photosynthetic bacteria have been used for water purification of fish breeding ponds in Japan. The species widely used for remediation of waste water are *Rb. sphaeroides*, *Rb. capsulatus*, *Rc. gelatinosus* and *Rps. palustris*. The practical application of an oxygenic phototrophic bacteria based in ecological studies is described with reference to their use in biological treatment and bioremediation of organic waste in the present communication.

KEY WORDS: Bioremediation, *Rhodo pseudomonas, Rhodo bacter,* detoxification.

1. INTRODUCTION

Bioremediation may be defined as the use of living organisms to reduce or eliminate environmental hazards caused due to accumulation of toxic chemicals and other wastes (Gibbon and Saylor, 1992). This process is also known as biotreatment or biorestoration. This technology is based on the use of naturally occurring or genetically engineered microorganisms to restore contaminated sites and protect the environment (Miller, 1994). Bioremediation techniques are economical attractive, ecofriendly and have shown promising results in the treatment of soil and water contaminated with organic compounds (Rhykerd, 1999). Bioremediation can be achieved by single or two groups of organisms based on the nature of the microorganisms. Biological processes are most suitable for waste treatment and microbial based treatments of industrial effluents offer an economical alternative to existing treatment methods. Significant efforts have been devoted to the studies of the biodegradation of the organic pollutants. However, different aspects need to be studied in more detail to obtain an overview of biodegradation processes in the environment and to optimize and predict the performance of degrading microorganisms. Several of environmental factors affect the metabolism and the rate of biochemical evaluation. Of particular importance are pH, concentration of suspended solids, BOD, COD, dissolved oxygen, number and species of microorganism present, nutrient contamination and bioavailability of the contaminants (Van Hamme, 2003). Approaches to analyze and assess biodegradation processes have been shifting towards the application of culture independent methodologies to characterize natural and engineered pollutant degrading microbial associations. Culture independent techniques for analysis of the genetic and metabolic potential of natural and model microbial communities that degrade organic pollutants revealed new metabolic pathways and enzymes for aerobic and anaerobic degradation. Genome sequencing of several biodegrading microorganisms has provided the genome insights into the bioremediation potentials of bacteria.

Photosynthetic Purple Non Sulphur Bacterial Remediation: The ubiquitous presence of microbes in the air, water, soil significantly affects day to day life as well as industrial activities. Bacterial species belonging to genera *Bacillus, Rhodopseudomonas*, *Rhodospirillum* are known to help in mineralization of organic wastes (Thomas, 1992). The concept of using bacterial products needs further research to determine the factors contributing to their effectiveness in augmenting production. Lots of new microbes are being identified as having bioremediation potential in different ecosystems. A species of facultative photo-organotrophic, purple, non-sulfur bacterium was isolated from mixed-species microbial mats, Characterized and examined for metal tolerance and bioremediation potential. Constructed microbial mats were used for bioremediation of heavy metals and organic chemical pollutants. Purple non-sulfur bacteria are characteristically found in lower strata of intact mats, but their contributing function in mats survival and Function by mediating the chemical environment has not been explored. The presence of photosynthetic bacteria along with the heterotrophic bacteria have been reported in various aquatic environments like Indian tropical waters (Srinivas Munjam, 2000; Ramchander, 2007) salt marshes (Bergstein, 1993) industrial effluents (Ramasamy, 1990; Munjam, 2002; Ramchander Merugu, 2008) sea water (Kobayashi, 1982), sewage (Kobayashi, 1995), waste water (Sunitha and Mitra, 1993; Vasavi, 2004), hot water springs (Demchick, 1990), earthworm casts (Vasavi, 2001), paddy fields (Archana, 2004; Vasavi, 2007). Ocean waters and aquaculture (Andreas Kappler and Claudia Pasquero, 2006) brackish lagoon (Anthony, 2006), ponds (Srinivas, 2007) and black sea (Overmann and Manske, 2006; Blankenship, 1995) studied taxonomy of anoxygenic photosynthetic bacteria. The photosynthetic bacteria are reported to be quite active in the degradative activity in view of their ability to grow under high osmotic concentrations and withstand anaerobic conditions. However, studies on their distribution in this region are limited (Srinivas Munjam, 2001). Purple non sulphur bacteria are the most diverse and versatile of all photosynthetic bacteria with vast potentials for applications. These bacteria preferably grow by a photoheterotrophic metabolism with organic substances as electron donors during their photosynthetic activity. Most species are also capable of growing photoautotrophically with molecular hydrogen as donor. These organisms have potential applications which are précised in table.1.

Natural waters are reported to be contaminated with several heavy metals arising from variety of industrial effluents (Grousset, 1999). Unlike organic contaminants which can be degraded to harmless chemical species, heavy metals cannot be degraded. The uses of microbial cells as biosorbents of heavy metal offer a potentially inexpensive alternative (Knorr, 1991; Khoo and Ting, 2001). The unique character of the phototrophic bacteria is their diverse metabolic activity and the ability to grow in different cultural conditions, the gram-negative rod-shaped bacterium, produced a dark red culture under phototrophic conditions, reproduced by budding and formed a lamellar intra cytoplasm membrane (ICM) system parallel to cytoplasm membrane, which contained bacterial chlorophyll a and carotenoids both in synthetic and naturally occurring substrates. The potential of purple non-sulphur bacteria for bioremediation was assessed by investigating the ability of *Rhodobacter spheroid's* strain to grow photo synthetically in heavy metal contaminated environments. *Rhodopseudomonas palustris* belong to the same lineage, and are distinct from all other *Rhodopseudomonas* in bioremediation properties. Within the mat significantly the mat changed from dark green to reddish purple in color metal tolerances indicates that this microbe maybe important in the bioremediation of other metals as well. Many members of the Rhodospirillaceae show a high resistance towards toxic heavy metal oxides and oxyanions (Moore and Kaplan 1992), *Rhodobacter sphaeroides* and *Rhodovulvum sp*. are capable of cadmium removal in a batch culture system (Watanabe, 2003). Removal of phosphorus from oyster farm mud sediment using *Rhodobacter sphaeroides* Il 106 was reported (Utsunomiya, 2003). *Rsp. rubrum* is reported to reduce and detoxify elemental selenium (Takeno, 1999). Biosorption characteristics of cadmium and lead ions of *Rhodobacter sphaeroides* and *Alcaligenes eutrophus* H16 were discussed by (Kessi, 1999). Anoxygenic phototrophic bacteria are the major groups of microorganisms existing in paddy soils and contribute significantly to soil fertility (Seki, 1998). Hable & Alexander (1980), have reported the adverse effect of pesticide on diazotrophic growth and nitrogenase activity of some anoxygenic phototrophic bacteria isolated from paddy soils. On the other hand carbendazim was shown to be photo assimilated by *Rps. palustris* strain as sole carbon and nitrogen source (Chalam, 1997). *Rhodoferax* species was reported to degrade phenoxyherbicides (Rajkumar & Lalitha Kumari, 1992). Berne (2005), have reported tributyl phosphate (TBP) degradation by *Rps. palustris* and other photosynthetic bacteria.

The composition of water bodies changes with environment. Vasavi (2007) and Merugu (2008), have reported significant changes in physicochemical characteristics of water under the influence of phototrophic bacteria. A strain of *Rps. palustris* capable of growth with acrylamide under photoheterotrophic conditions was isolated and being successfully used. *Rb. sphaeroides* is also being widely used for bioremediation of heavy metal contaminated environments. Light dependent transformation of aniline to indole esters was mediated by the phototrophic bacterium *Rb. sphaeroide*s. Leather industry effluents remediation by *Rb. capsulatus* revealed decrease in dissolved oxygen (12%), COD (55%) and BOD (12%). Similarly, other components such as bicarbonates, chlorides, sulphates and organic matter decreased significantly due to the activity of *Rb. capsulatus*. Changes in physicochemical parameters and photosynthetic microorganisms in a deep waste water self depuration lagoon have been reported by Soler (1991). Anaerobic treatment of domestic and industrial waste waters releases large amounts of phosphorus and nitrogen which are directly responsible for eutrophication of rivers, lakes and seas worldwide (Trepanier, 2002). Narula (2007), have investigated various factors like pH, organic acids and sugars on phosphate solubilization by phosphate solubilising microorganisms. Recent molecular studies techniques such as Fluorescence *in situ* hybridization (FISH) analysis showed that bacteria of the beta-2 sub class of proteobacteria and acinetobacteria have extremely good phosphorus removal capacity (Bond, 1995). Purple non sulphur bacterium *Rhodobacter capsulatus* immobilized on cellulose beads removed organic carbon, ammonium ions and phosphate ions from a diluted growth medium over a period of 19-22 days (Sawayama, 1998). *Rhodobacter sphaeroides, Rhodobacter sphaeroides* NR-3 and *Rhodopseudomonas palustris* immobilized in porous ceramic removed phosphates, nitrates and H_2S from synthetic waste water (Nagadomi, 2000). Julie (2002), investigated phosphorus removal by *Rhodocyclus* spp. Several workers studied the efficiency of anoxygenic phototrophic bacteria in the degradation of organic wastes mostly agricultural and food industries which include molasses wastes (Sasikala, 1992), refinery, lactic acid wastes (Sasikala and Ramana, 1995), high strength organic waste water (Ogbonna, 2000) and oil containing sewage water (Takeno, 2005). The advantages of using anoxygenic phototrophic bacteria for waste treatment over activated sludge processes are dilution of waste water is not required, requires minimum facilities and provides a rich biomass of proteins, vitamins and carotenoids and does not produce undesirable gases (Merugu, 2008). The denitrification and deammonification capabilities of anoxygenic phototrophic bacteria are of special interest because in industrial effluents the ammonia and nitrate concentration is often much higher, causing many problems because such waste waters are discharged into rivers and lakes (Weismann, 1994; de-Bashan Luz and Bashan Yoav, 2004). Hassan (1997), have reported efficient treatment of palm oil by *Rhodobacter sphaeroides*. Treatment of oil containing sewage waste water using immobilized photosynthetic bacteria was reported (Takeno, 2005). *Rps. palustris* could degrade tributyl phosphate which is widely used in nuclear fuel processing and other waste generating chemical industries (Berne, 2007). Rajoka (2004), have studied different factors influencing depollution of sago effluent by *Rps. palustris* strain B1.

Phototrophic bacteria are reported to degrade efficiently aromatic compounds such as benzoates, benzoate derivatives (Wright, 1991), amines (Cabello, 2004), thiols (Pieter and Visscher, 1993) and phenols (Blasco and Castillo, 1992). Mixed culture of *Rb.sphaeroides*, *Chlorella sorokiniana* and *Spirulina platensis* proved to be better for treating organic waste water (Ogbonna, 2000). Due to the widespread use, chromium is considered to be a serious environmental pollutant. Cr (VI) is highly toxic whereas Cr (III) is less toxic. Many bacteria which are reported to reduce Cr(VI) to Cr (III) are *Pseudomonas putida* (Ishibashi, 1990), *P. fluorescence* (Bopp and Ehlrich, 1988), *Bacillus* spp (Flavia, 2005), *Rhodobacter sphaeroides* (Nepple, 2000), *Desulfovibrio vulgaris* (Humphries, 2005) and *Microbacterium liquefaciens* (Pattanapipitsal, 2001). Cr(VI) reduction by immobilized cells of *Desulfovibrio vulgaris* NCIMB 13776 was analyzed (Humphries, 2005). Chromate reduction was detected in *Rhodobacter sphaeroides* and the enzyme reducing chromate was characterized (Nepple, 2000). Photosynthetic bacteria can consume various type of organic matter with a relatively high growth rate and consume phosphorus and nitrogenous compounds. Photosynthetic bacteria have been used for water purification of fish breeding ponds in Japan (Sasaki, 1998). Treatment of aquarium water by using immobilized phototrophic bacteria by *Rb. sphaeroides* was investigated by Nagadomi (2000). The practical application of anoxygenic phototrophic bacteria based in ecological studies is described with reference to their use in biological treatment and bioremediation of organic waste (Kobayashi, 1995). The species widely used for remediation of waste water are *Rb. sphaeroides*, *Rb. capsulatus*, *Rc. gelatinosus* and *Rps. palustris* (Sasikala and Ramana, 1995). David (2005), have isolated *Rps. palustris* capable of growth with acrylamide under photoheterotrophic conditions. The potential of *Rb. sphaeroides* for bioremediation of heavy metal contaminated environments was assessed (Livia, 2006). Light dependent transformation of aniline to indole esters was mediated by *Rb.sphaeroide*s (Vijay, 2006).

Yegani (2006), have shown growth enhancement of *Rb. capsulatus* in presence of iron. *Rb. capsulatus* was reported to grow in presence of cadmium (17µM) containing media (Sanaa, 2006) *Rhodospirillum rubrum* was reported to accumulate cadmium (Smiejan, 2003). Molybdenum was also reported to influence the growth of *Rb. capsulatus* (Tsygankov, 1996). Anoxygenic phototrophic bacteria are more resistant to pesticides than cyanobacteria which make them more suitable for use as a biofertiliser. Brown (1990), have reported that *Rps. palustris* and *Rc. gelatinosus* were inhibited, while *Rps. acidophila* could tolerate herbicide atrazine. Such a species variation in response to different pesticides was also reported (Chalam, 1997). Studies on *Rb. capsulatus* (Merugu, 2008) reveals that it is most suitable for improving soil fertility in pesticide contaminated soils. Studies on the effect of pesticides on growth of phototrophic bacteria have shown that *Rb. capsulatus* was more resistant than *Rps. acidophila.* Studies on solubilisation of di and tricalcium phosphate by two anoxygenic phototrophic bacteria revealed their potential of solubilising tricalcium and dicalcum phosphate (Merugu, 2008). *Rps. palustris* and *Rc. gelatinosus* were also reported to be efficient in phosphate solubilization (Munjam, 2002). Vasavi (2008), also reported that *Rps. rutila* and *Rsp. rubrum* solubulised phosphate. Berne (2007), reported that CYP201A2, a cytochrome p450 plays a key role in phosphate degradation in *Rps. palustris* which required 21 days for its degradation. Fernandez (2007), reported phosphate solubilization upto 60 days by *Bradyrhizobium* PER2H strain. *Pseudomonas fragi* CS11RH1 (Rajasekar, 2007). *Bacillus subtilis* and *Bacillus megaterium* (Sharan and Darmwal, 2008) and *Xanthomonas campestris* (Thangaraj, 1994) were reported to be efficient solubilisers of phosphorus. Biological wastewater treatment is one of the most important biotechnological applications of anoxygenic phototrophic bacteria. In any research aimed at applied goals, it is important to keep economic limitations of a large scale application. Hence the substrates used to drive a biological hydrogen production system must be readily available natural resources in large quantities as with waste waters. Though *Rb. capsulatus* and *Rps. acidophila* could remediate waste water, the degree of remediation, varied with the conditions. Similarly, *C. okenii* and *T. roseopercina* in a mixed culture with *Rps. acidophila* (Kobayashi, 1995). *Rps. palustris* (Bai, 2008), *Rps. rutila* and *Rsp. rubrum* are reported to be efficient in the remediation of effluents and waste water. Both the organisms not only decreased different constituents of waste waters but were also responsible for decrease in their BOD and COD. The mechanism and kinetics of cadmium removal by growing *Rhodobacter sphaeroides* were investigated (Ponsano, 2008). Furthermore, it was found that the removal mechanism of cadmium was predominantly governed by bioprecipitation as cadmium sulfide with biosorption contributing to a minor extent. Also, the results revealed that the activities of cysteine desulfhydrase in strains grown in the presence of 10 and 20 mg/l of cadmium were higher than in the control, while the activities in the presence of 30 and 40 mg/l of cadmium were lower than in the control. Content analysis of subcellular fractionation showed that cadmium was mostly removed and transformed by precipitation on the cell wall. *Rubrivivax gelatinosus* was grown in Pfennig's synthetic medium (PM) and in treated wastewater from poultry slaughterhouse (TW) to assess growth profiles for biomass production (Nepple, 2000). Ponsano (2008), also have demonstrated the ability of different phototrophic bacteria to reduce chromium. It is clear from the present investigations that anoxygenic phototrophic bacteria can be exploited for the purification of effluents and sewage. A COD decrease of 91% was observed in (Getha, 1998). *Rhodopseudomonas palustris* strain B1 from a sago-starch-processing decanter showed a 77% reduction of chemical oxygen demand (COD) of the effluent (Azad, 2004). *Rhodovulum sulfidophilum* was used for the treatment and utilization of sardine processing wastewater (Mehrabi, 2001). A species of facultative photo organotrophic, purple non-sulfur bacterium was isolated from mixed-species microbial mats was found to have multiple metal resistances and to be effective in the reductive removal of Cr (VI) and the degradation of 2,4,6-trichlorophenol (Song, 2003). *Rhodobacter sphaeroides* AS1.1737 decolorized more than 90% of several azo dyes in 24 hours. Bioremediation of waste waters was as reported in earlier studies (Merugu, 2015). In view of the above it can be concluded that PNSB can be used for bioremediation and in this process many useful products can be obtained.

Table.1. Bioremediation Potential of Purple Non Sulphur Bacteria

Bioremediation	Organism	Reference
Biodegration of organic and inorganic waste & Waste water treatment	*Rhodobacter sphaeroides*, *Rhodobacter sphaeroides* NR-3 and *Rhodopseudomonas palustris*	Sanaa, 2006; Blasco and Castillo, 1992; James, 2000; Ramana, 2000; Nagadomi, 2000; Ramana and Sasikala, 2002; Takeno, 2005
Chromate reduction	*Rhodobacter sphaeroides*	Nepple, 2000
Phosphate solubilisation	*Rhodobacter sphaeroides* Il 106, *Rps. palustris,Rhodocyclus spp.*	Takeno, 1999; Berne, 2005; Julie, 2002
Cadmium removal	*Rhodobacter sphaeroides* and *Rhodovulvum sp.*	Watanabe, 2003
Selenium detoxification	*Rsp. rubrum*	Takeno, 1999
Lead ions biosorption	*Rhodobacter sphaeroides*	Kessi, 1999
Molasses wastes, citric acid fermentation wastes refinery	Photosynthetic bacteria	Sasikala, 1992; Yu, 1991; Sasikala, 1995
Carbendazim, phenoxy herbicides and tributyl phosphate (TBP) degradation	*Rps. Palustris*, *Rhodoferax*, *Rps. palustris*	Rajkumar and Lalitha Kumari, 1992; Ehrig, 1997; Berne, 2005
Removal of organic carbon, ammonium ions and phosphate ions	*Rhodocyclus* spp., *Rhodobacter capsulatus*	Sawayama, 1998
Removal of phosphates, nitrates and H_2S from synthetic waste water phosphorus removal	*Rhodobacter sphaeroides*, *Rhodobacter sphaeroides* NR-3, *Rhodopseudomonas palustris*	Nagadomi, 2000; Julie, 2002
Aromatic compounds, Benzoate, amines	Phototrophic bacteria	Wright and Madigan, 1991; Cabello, 2004
Thiols	Photosynthetic bacteria	Visscher and Taylor, 1993
Phenols	Photosynthetic bacteria	Blasco and Castillo, 1992
Organic waste water	Mixed culture of *Rb. sphaeroides*, *Chlorella sorokiniana* and *Spirulina platensis*	Ogbonna, 2000
Palm oil	*Rhodobacter sphaeroides*	Hassan, 1997
Oil containing sewage waste water	Oil containing sewage waste	Takeno, 2005
Tributyl phosphate	*Rps. palustris*	Berne, 2005
Sago effluent	*Rps. palustris* strain B1	Ibrahim, 2006
Purification of fish breeding ponds	Photosynthetic bacteria	Sasaki, 1998
Treatment of aquarium water	*Rb. sphaeroides*	Nagadomi, 2000
Bioremediation of organic waste	*Rps. palustris*	David and Ensign, 2005; Kobayashi, 1995
	Rps. acidophila	Thangaraj and Kulandaivelu, 1994

Transformation of aniline to indole esters	*Rb. sphaeroide*s	Vijay, 2006
Cadmium accumulation	*Rhodospirillum rubrum*	Smeijan, 2003
Phosphate solubilisation	*Rps. Acidophila*, *Rps. palustris* and *Rc. gelatinosus*	Srinivas, 2000
	Rps. rutila and *Rsp. rubrum*	Vasavi, 2008

2. CONCLUSION

In view of the above it can be concluded that PNSB can be used for bioremediation and in this process many useful by-products can be obtained such as hydrogen, polyhydroxy butyrate, phytohormones, aminolevulinic acid etc.

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