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BACTERIAL DECOLOURIZATION OF TEXTILE DYE-REACTIVE RED -M5B

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Abstract: **The dye decolorizing isolates *Bacillus sp* and *Pseudomonas sp* were isolated from textile effluent sample collected from Erode, Tamilnadu. Different parameters such as pH, temperature, different concentration of dye, carbon source, Nitrogen source and different incubation periods were optimized for decolorization of reactive red M5B by using bacterial isolates. The optimal condition for decolorization of reactive red M5B for both *Bacillus sp* and *Pseudomonas sp* was found to be 1% glucose, 1% peptone, pH 7.0, 37^{0}c, 1% dye concentration. *Bacillus sp* and *Pseudomonas* showed highest decolourization of 76% and 79% at the end of 96hrs under optimum condition. The present study concluded that bacterial isolates like *Bacillus sp* and *Pseudomonas sp* can be used as good microbial source for treatment.**

Keywords: **Decolourization, *Bacillus sp, Pseudomonas sp*, Reactive red M5B.**

I. INTRODUCTION

Textile dyes are chemicals of complex aromatic structures designed to resist the impact of detergents, sunshine and temperatures (Nigam *et al*., 1996). They are chemically and photochemically stable and are extremely persistent in natural atmospheres. In 1856, the world's first commercially successful synthetic dye, mauveine, was discovered for practical uses. Over 10,000 different dyes with an annual production of over 7×10^5 metric tons worldwide are commercially available (Zollinger1987; Gupta *et al* . ,2011]. The fixation rates of several textile dyes are not 100 % and around 30-70 % of the amount of dyestuff used to get eliminated into effluent during the wet processing operations (Khaled *et al*., 2009; Bumpus, 1995). The estimated dyes concentration in the textile effluent has been reported to be in the range of 10-200 mg L^{-1} (Kadam *et al*., 2011). Textile dyes are chemically diverse in nature and are broadly divided into azo, reactive, triphenylmethane, heterocyclic, polymeric structures, etc (Cheunbarn *et al*., 2008). Among these various types, the azo dyes constitute about 70 % and are used widely for dyeing purposes such as textile dyeing, paper, food, leather, cosmetics, and pharmaceutical industries (Chang *et al*., 2009). This makes them the largest and most important group of synthetic colorants released into the environment (Ambrósio and Campos-Takaki, 2004).

In order to satisfy the standards necessary for industrial applications like stability and long lasting of colorants, the azo dyes are manufactured with various colours, molecular structures and resistance to attenuation upon exposure to sunlight, water and several chemicals (Correia *et al*., 1994) .The amount of dye lost depends upon the class of dye application, varying from 2% loss while using basic dyes to 50% loss in certain reactive sulfonated dyes, leading to severe contamination of surface and ground waters in the vicinity of dyeing industries [Neill *et al* .,1999].

In India, an average mill discharges about 1.5 million litres of contaminated effluent per day, which leads to chronic and acute toxicity (Sandhya *et al*.,2005) . Improper textile dye effluent disposal in aqueous ecosystems leads to the reduction in sunlight penetration which in turn decreases photosynthetic activity, dissolved oxygen concentration, and water quality and depicts acute toxic effects on aquatic flora and fauna, causing severe environmental problems worldwide (Vandevivere *et al*.,1998). They can also cause human health disorders such as nausea, haemorrhage, ulceration of the skin and mucous membranes, and severe damage to kidneys, the reproductive system, liver, brain and central nervous system (Verma and Madamwar, 2003).In addition, azo dyes also have an adverse impact in terms of total organic carbon (TOC), biological oxygen demand (BOD), and chemical oxygen demand (COD) (Saratale *et al* .,2009) . Many synthetic azo dyes and their metabolites are toxic, carcinogenic, and mutagenic (Myslak and Bolt 1988). Therefore, the treatment

of industrial effluents containing azo dyes and their metabolites is necessary prior to their final discharge to the environment. Various physicochemical methods like adsorption, chemical precipitation and flocculation, photolysis, chemical oxidation and reduction, electrochemical treatment, and ion pair extraction have been used for the removal of dyes from wastewater (Wang *et al* ., 2009). The major drawbacks of these methods have been largely due to the high cost, low efficiency, limited versatility, interference by other wastewater constituents, and the handling of the waste generated (Kaushik and Malik,2009). Conversely, biological processes provide an alternative to existing technologies because they are more cost-effective, environmental friendly and do not produce large quantities of sludge. In contrast, remediation of dyeing industry effluent by using microorganisms has proved to be the best solution (Salar *et al*.,2012). Since numerous bacterial species including *Bacillus, Pseudomonas, Enterobacter, Halobacter,* and *Aeromona*s have been reported to exhibit tremendous capability to decolourize and detoxify a wide range of azo dyes composed of phenylamine, benzene diazonium chloride or phenol(Telke *et al*,.2008 ; Mendes *et al* ., 2011 ; Feng *et al.*, 2008) .

In most cases, bacteria disintegrate azo bonds of the dyes, which result in the formation of colourless amines and subsequently simpler compounds(Stolz 2011). In recent days, the use of technologies based on bioremediation has got much attention for the treatment of textile dye effluent because of simple structural set-up, low cost, easy to operate, less sludge volume, environmental benignity, and wider application **(**Walker, 1970 ; Zimmermann *et al.*,1984 **:**Pasti-Grigsby *et al.*, 1992).

Apparently, the development of novel biological decolourization system consisting one or more acclimatized microorganisms in habitat concentration is urgently needed for the effective clean up of the excess dyes in effluent. In the present study, an attempt was made to evaluate the potential of bacterial strains for decolorization effluent containing a dye, Reactive red M5B with respect to various pH, temperature, carbon (glucose) source, nitrogen (peptone) source and different dye concentration were optimized.

2. MATERIALS AND METHODS

Chemicals and media

Textile dye Reactive Red M5B and dye effluents were collected from a dying industry located at Erode, Tamil Nadu. All microbiological media and medium ingredients were procured from Hi Media Laboratories, Mumbai (India).

Isolation, screening and identification of dye degrading bacteria

Microbial isolations were carried out by serially diluting textile effluent in sterile distilled water subsequently plated onto nutrient agar medium added with 100mg/l Reactive red M5B and incubated at 37° C for 12 hrs. After incubation the presence of dye degrading bacterial colonies were checked by observing the zone of clearance/decolorization on the respective plates (Khalid *et al* .,2008). Those colonies that exhibited dye degradation were isolated and maintained in nutrient broth medium. Further the identification of dye degrading bacterial strains were carried out on the basis of morphological and biochemical analysis.

Decolourization Assay

Decolourization activity was expressed in terms of percentage decolourization and was determined by monitoring the decrease in absorbance at absorption maxima (λ max) of respective dyes (i.e. 423nm for reactive red M5B). The uninoculated nutrient broth supplemented with respective dye was used as reference. The culture suspension was centrifuged at 8,000 rpm for 10 min for removal of the biomass. The degree of decolorization of the tested dye was measured at its respective maximum absorbance wavelength using supernatant by UV-visible spectrophotometer. Based on decolorization potential, strain was selected for further studies. The decolorization assay was calculated according to the following formula (Ali *et al* ., 2009).

Percentage of Decolorization = [A-B /A] × 100

Where A= Initial absorbance

B= Observed absorbance

Effect of pH and temperature on dye decolourizing activity

The effect of pH on the dye decolourization was determined by measuring the relative activity using different buffers at 37°C. Sodium acetate (pH 4.0 to 5.5), Tris (pH 6.0 to 8.5) sodium carbonate (pH 9.0) buffers were used for the estimation of relative activity at different pH conditions. The maximum activity was considered as 100%, and used as reference in

determining relative activities at different pH values. The effect of temperature on the reaction rate was determined by performing the standard reaction at different temperatures in the range of 28-42°C. The relative activity was expressed considering maximum activity as 100%.

Effect of different concentration of dye on dye decolourizing activity

The effect of different concentration of dye was determined by measuring the relative activity using different concentration (1%,2%,3%,4% and 5%) at 37° C.

Effect of different concentration of carbon and nitrogen source on dye decolourizing activity

The effect of different concentration of carbon (glucose-0.25%,0.5%,0.75% and 1.00%) and nitrogen source (peptone-0.25%,0.5%,0.75% and 1.00%) individually on decolourizing activity were monitored at specific wavelength at 37°C.

Effect of different incubation periods on dye decolourizing activity

To test the effect of different incubation period on the decolourization of textile dye effluents by bacterial isolation, 100 ml of nutrient broth in 250 ml Erlenmeyer flasks were prepared and supplemented with Reactive red M5B of 100 ml L^{-1} concentration. The media were sterilized and inoculated with 1 ml of the standard inoculums of different bacterial treatment. The flask was incubated at 30^0c for rotary shaker running at 180 rpm at different incubation periods *viz.*, 12 hrs, 24 hrs, 36 hrs, 48 hrs, 60 hrs, 72 hrs, 84 hrs and 96 hrs.

3. RESULTS AND DISCUSSION

Isolation, screening and identification of dye degrading bacteria

Textile and dyeing industry are one of the industry which contribute to the soil and water pollution. The Industrial units are now looking forward to cost effective solutions for reduction of pollution. Numerous bacterial cultures capable of dye decolourization have been reported , an array of bacteria as degraders of dyes such as *Bacillus subtilis*, *Escherichia coli*, *Enterococcus* sp., *Pseudomonas* sp., *Rhabdobacter* sp., *Lactobacillus* sp., *Staphylococcus* sp., *Clostridium* sp., *Micrococcus dermacoccus*, *Acinetobacter* sp., *Geobacillus* , *Rhizobium*, *Proteus* sp., *Morganella* sp., *Aeromonas* sp., *Alcaligenes* sp. and *Klebsiella (*Sudha *et al* ., 2014). In the present study the textile dye effluent sample was collected from textile industries located at Erode and the isolated morphologically distinct colonies showed decolourization zone on Nutrient Agar plate containing. Reactive red M5B dye. By microscopic and biochemical characters the bacterial cultures were identified as *Bacillus sp* and *Pseudomonas sp*. This results were in accordance with Saran raj *et al* ., 2014 who isolated five different bacterial isolates from the textile dye effluent and identified as *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas fluorescence*, and *Staphylococcus aureus*.

Effect of pH and temperature on dye decolorization

The effect of pH for both *Bacillus sp* and *Pseudomonas sp* on dye decolourization activity was determined in different buffers (pH 3.0 to 9.0). The decolourization rate was increased when pH increased from pH 3.0 (43%) to pH 7.0 (60%) for *Bacillus sp* and from pH 3.0 (49%) to pH 7.0 (65%) for *Pseudomonas sp*. The highest percentage decolourization of rate was observed at pH 7.0 *i.e* 60% and 65% activity were observed for *Bacillus* sp and *Pseudomonas* sp, respectively **(Fig.1)**.

The effect of temperature for both *Bacillus sp* and *Pseudomonas sp* on dye decolourization activity was determined in various temperatures (28°C, 37°C and 42°C). The highest percentage decolourization of rate was observed at temperature 37°C. At this temperature *Bacillus sp* and *Pseudomonas sp* showed 57% and 67% activity, respectively (**Fig.2)**. Increase in temperature resulted in marginal reduction in decolourization activity of the bacterial culture.

Similar result were reported on *Bacillus subtilis* showed (76%) Methyl red decolourization at 37^0c (Ezhilarasu 2016). The decreased in decolourization at higher temperature may be due to the loss of cell viability (or) deactivation of enzyme responsible for decolorization at 40^0c(Cetin and Donmenz 2006) .The decolourization of the dye Acid orange 10 by. *Pseudomonas Putida* MTCC 102 showed optimum pH and temperature of 7.0 and 37^oc respectively (Tripathi and Srivastava .2011).

Effect of different concentration on dye decolourization

Decolourization activity of both *Bacillus sp* and *Pseudomonas sp* was studied at different concentrations of dye varying from 1%2%,3%,4% and5%. The optimum dye concentration for decolourization by *Bacillus sp* and *Pseudomonas sp* was

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found to be 1% **(Fig.3)**.Similar results were also observed by Neil *et al* ., (2014) showed that further increase in dye concentration resulted in reduction in decolourization rate. Lower decolourization efficiency is due to higher inhibition at high dyestuff concentration .

Bhoomi Joshi *et al*., (2013) observed that the *Bacillus sp* showed maximum dye degradation at 1% concentration. Chen *et al* ., (2003) also observed that decrease in Decolourization ability at high substrate concentration might be dye to the toxicity of dye. The dyes generally contain one or more sulphonic acid groups on aromatic rings which might act detergents to inhibit the growth of microorganism.

Effect of glucose as carbon source on dye decolourization

Different concentrations of glucose (0.25%,0.50%,0.75% and1.00%) as carbon source were evaluated for dye decolourization. Among various concentrations, the highest concentration i.e 1.0% of glucose showed highest activity for decolourization of dye for both *Bacillus sp* and *Pseudomonas sp* as shown in (**Fig.4)**. *Pseudomonas aeruginosa* GSM3 showed complete decolourization in the presence of glucose when compared to lactose and sucrose (Mallikarjun *et al* ., 2014).

Effect of peptone as nitrogen source on dye decolourization

In present study, different concentrations of peptone (0.25%,0.50%,0.75% and1.00%) as nitrogen source were evaluated for dye decolourization. Among them 1.0% of peptone concentration was found to be optimum for both *Bacillus sp* and *Pseudomonas sp* to make the dye decolourization process economical **(Fig.5)**. There is a report that peptone is the ideal nitrogen source for decolourization of Red 3BN dye by *Bacillus sp* (Mohan *et al* ., 2013).

Effect of different incubation periods on dye decolourization

In the present study different Incubation periods on the dye decolourization was studied. Among the eight different Incubation periods The highest mean percentage of decolourization was recorded at 96 hours by both *Bacillus sp* and *Pseudomonas sp* These results are in agreement with results of Raja ganesh and Ameer basha (2014) found that bacterial isolate namely *Bacillus subtilis* showed maximum decolourization ability followed by *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* at 96hrs of incubation (**Fig.6)**.

4. CONCLUSION

The results of the optimization study recorded that the highest percentage of dye decolourization was recorded at a PH 7.0 , temperature of 37^0C .In the presence of 1% glucose and 1% peptone are the essential conditions for attaining maximum degradation efficiency. From this study, it has been concluded that *Pseudomonas species* had degraded effectively, when compared to *Bacillus sp* Nevertheless, both the species of bacteria can be inferred as good agents for the degradation of Reactive red M5B.

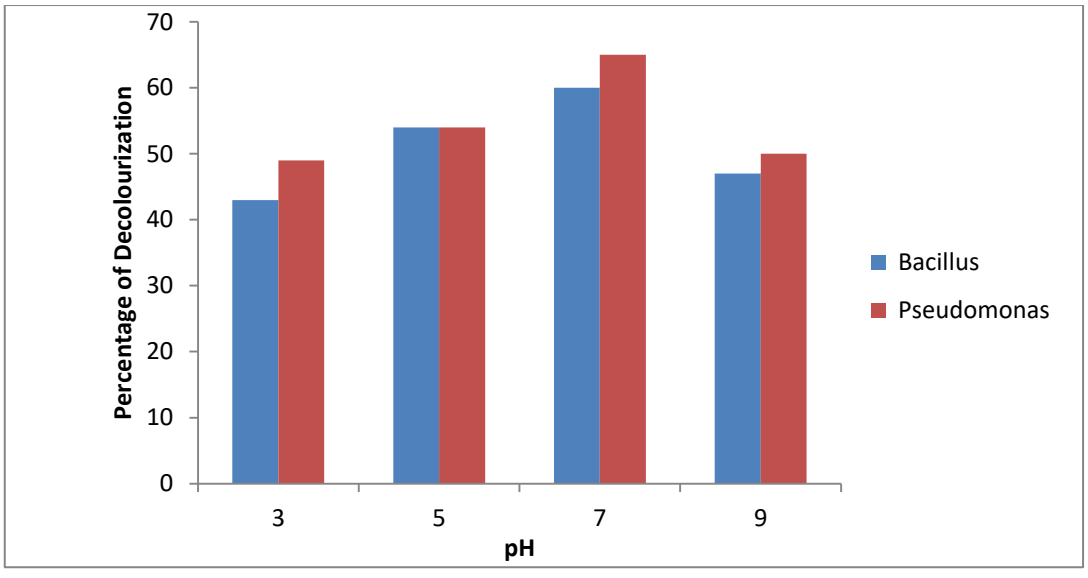

Fig 1: Effect of different pH on Dye Decolourization

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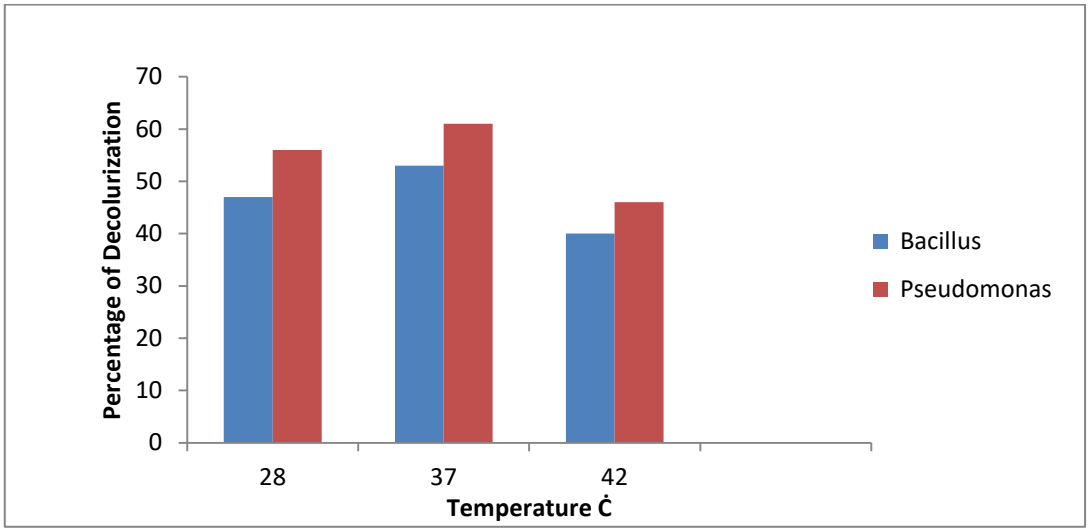

Fig 2: Effect of different temperature on Dye Decolourization

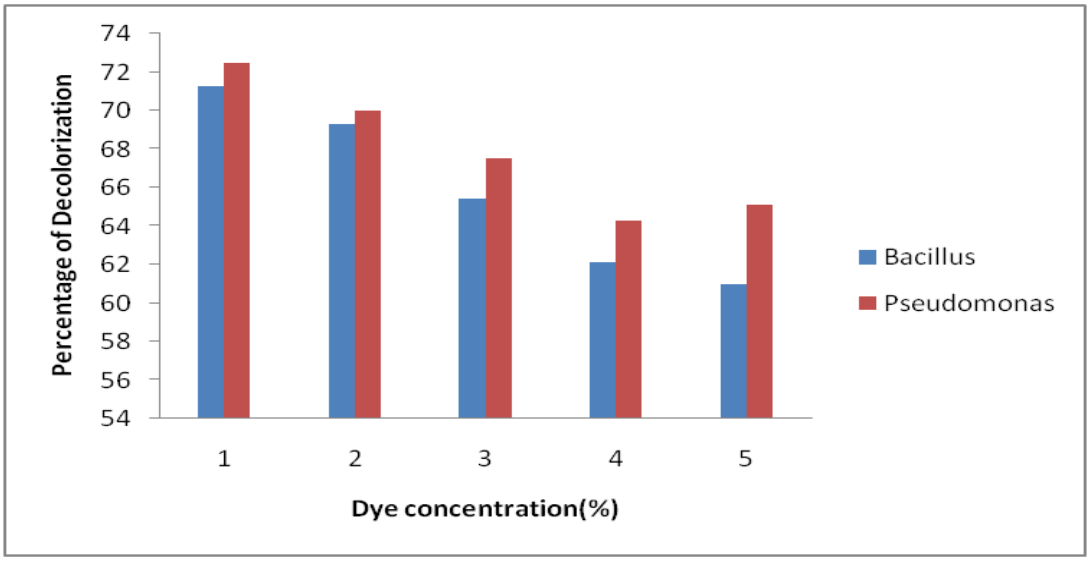

Fig 3: Effect of different concentration of dye on dye decolourization

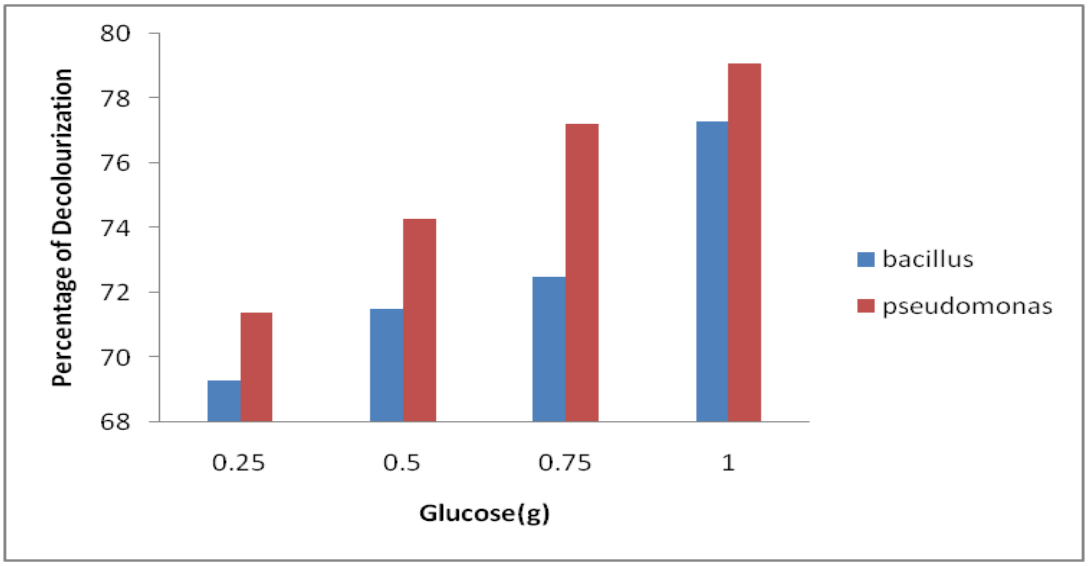

Fig 4: Effect of glucose as carbon source on dye decolourization

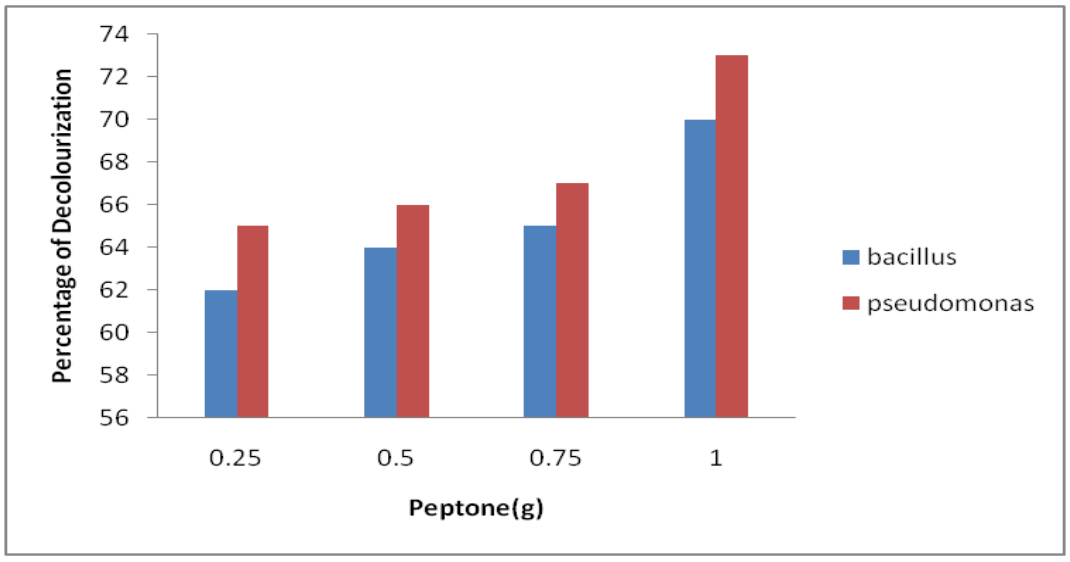

Fig 5: Effect of peptone as nitrogen source on dye decolourization

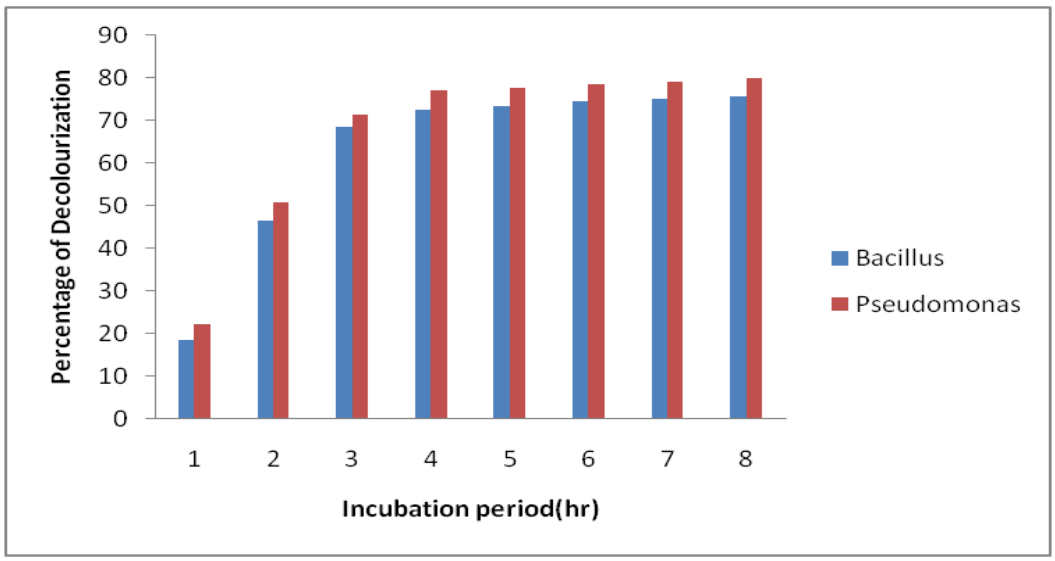

Fig 6: Effect of different incubation periods on dye decolourization

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