## 5.1 INTRODUCTION:

Azo dyes are permanent coloring agents that derive their name from the presence of azo group (-N=N-) [Chang *et al*. (2000)]. Due to availability of wide color combinations and its ability to bind with natural and synthetic fibers, these azo dyes find wide application in day to day life products [Spadaro *et al*. (1994)]. Presently, more than 3000 different kind of azo dyes are being used in textile and many other industries [Spadaro *et al*. (1992); Spadaro *et al*. (1994)]. Most of the azo dyes, except naturally occurring 4-4'-dihydroxyazobenzene, are produced synthetically [Gill *et al.* (1984)]. Synthetic azo dyes are the most versatile class of dyes widely used in various industries such as food, textile, carpet, pharmaceuticals, printings, cosmetics and many more. Synthetic dyes are widely used as compared to natural dye due to its ease of production, fastness and availability of various color combination thereby producing large quantities of dye based wastewater [Aksu *et al*. (2010); Li *et al.* (2010)]. The azo dyes are xenobiotics and owing to their chemical structure are recalcitrant to biodegradation posing short or long-term threats to living organisms [Qu *et al*. (2010); Sharma and Singh, (2011)]. Moreover, the byproducts of dye degradation i.e. aromatic amines are carcinogenic and mutagenic in nature that needs further degradation [Joshi *et al.* (2008)]. Moreover, dye based wastewater are discharged directly in the river system is a growing concern for river ecosystem. Apart from this, incorporation of this dye in food chain leads to various health hazards. The above mention problem clearly states the need for treatment of these dye based wastewater prior to its discharge with development of effective eco-friendly treatments methods for its removal from aquatic system.

Various treatment methods like coagulation, flocculation, sedimentation, photocatalytic degradation and others suffer limitations of low efficiency and hazardous byproducts. In recent years scientists have focused on biological methods as an alternative solution for remediation of dye based wastewater [Kaushik *et al.* (2009)]. The bioremediation technology widely utilized for dye degradation using indigenous microorganisms is considered as an eco-friendly and economical measure for treatment of wastewater. In this context, bacteria with its fast growth, multiple mechanisms for degradation of organic and inorganic materials and efficiency in dye degradation come up as an effective solution for the limitations of above mention methods. Studies suggest that pure line cultures of bacteria are effective for degradation of single pollutant [Singh *et al.* (2015); Mishra *et al.* (2012b)]. However for treatment of wastewater discharged to river system containing multiple pollutants diverse types of microbial strains in the form of consortia are required. Consortium with a richer metabolic network and each member specialized for uptake of a particular contaminant can results in successful removal of several contaminants from a mixed wastewater stream [Mishra *et al.* (2014a)]. Studies suggest that mixed consortia achieves a higher degree of biodegradation and mineralization of dye wastewater due to synergistic metabolic activities of its microbial community [Chang *et al.* (2004); Forgacs *et al.* (2004); Jadhav *et al.* (2008); Saratale *et al.* (2009)] however, the investigations in this direction are very scanty. The dye degradation by consortium is efficient and fast compared to dye degradation by isolated pure bacterial strain as the individual strains of the microbial consortium may attack the dye molecule at different positions or may utilize its toxic byproducts as metabolites for the growth of its different co-existing strains [Tony *et al.* (2009)]. The isolated mixed

consortia therefore have proven advantage for higher dye removal capacity and more stability against environmental fluctuations compared to single bacterial consortia in biological treatment [Tan *et al*. (2005).

The biological treatment of dye based wastewater involves anaerobic, anoxic or aerobic methods. Anaerobic method of degradation of azo dye is time consuming process reflected by its requirement of plentiful microorganism, long reduction rate and release of aromatic amines as byproduct due to reductive cleavage of azo bond. Degradation of dye in the anaerobic condition leads to formation of toxic, mutagenic and carcinogenic aromatic amines which further requires an aerobic pathway for their complete degradation. In contrast, aerobic treatment leads to complete degradation of the byproducts and is therefore preferred over anaerobic methods for biodegradation of azo dyes. However, rare studies have been conducted on the use of consortium for dye degradation under aerobic condition.

Carpet belt of Uttar Pradesh is perhaps considered as the most famous carpet-producing region in India. This belt is located near Varanasi in the south eastern corner of Utter Pradesh. Production and trade activities of these industries are concentrated primarily in the Mirzapur-Bhadohi belt and extend outwards to surrounding districts of Utter Pradesh. The wastewater produced by these industries is released directly into the nearby environment causing adverse impact on the aquatic ecosystem and its soil properties. The effluent having intense color results in reduction of light penetration potential with a reduction of the dissolved oxygen content of the aquatic system. Utilization of these effluents for irrigation purposes affects the physico-chemical

properties of soil as well as bioaccumulation of toxicants in food chains. Environmental hazards caused by this dye based wastewater highlights the need of its treatment before direct discharge to the surroundings. In purview of the above the study in this chapter is focused to (a) isolate bacterial consortia from dye contaminated soil for its application in the aerobic treatment of carpet effluent collected from Bhadohi area (b) optimization of various parameters affecting dye degradation by bacterial consortium BC1 (c) determining the kinetics of degradation of Scarlet 4BS dye and (d) estimating the pathway and phytotoxicity of dye degraded byproducts obtained using bacterial consortium BC1.

## 5.2. Results and Discussion

### *5.2.1 Identification of Bacterial Consortia (BC1)*

Efficiency of dye degradation under aerobic condition and their distinct morphology were considered as key criteria for selection of the bacterial culture**.** These isolates were identified using morphological, biochemical and 16S rRNA gene sequence analysis methods. Five indigenous consortia with potential dye degrading capacity were thus obtained from agar plate cultures in LB broth media**.** The five bacterial species constituent of the selected consortia namely (*Micrococcus* sp. BHUMC X14 (KM199787), *Arthrobacter* sp. BHUAS X16 (KM199789), *Plannococcus* sp. BHUP X11(KM199788), *Exiguobacterium* sp. IITES X12 (KM199786), *Pseudomonas putida* strain BHUPP X10 (KJ40223), as identified by 16S rRNA gene sequence analysis. Phylogenetic tree of the bacterial consortium thus formed (*Figure 5.1*) also includes sequences available with NCBI having similar action with their respective NCBI Gene Bank and Accession number (http://blast.ncbi.ntm.nih.gov).

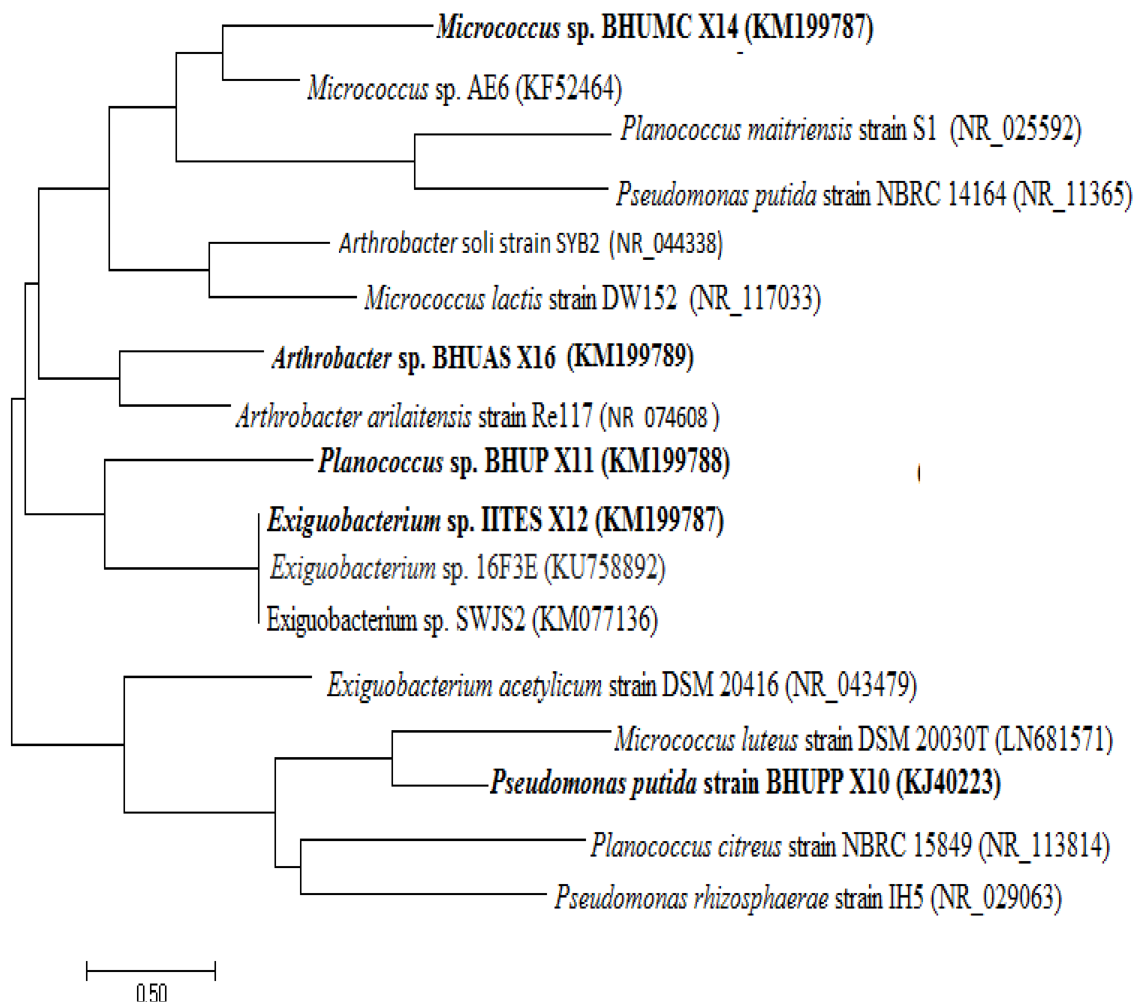



**Figure 5.1:** Phylogenetic tree of Bacterial Consortia (BC1) based on 16S rRNA gene sequences wherein the bar indicates the Jukes-Cantor evolutionary distance. Numbers at nodes indicate percentage bootstrap value.

### 5.2.2 Parameters optimization

Parameters like agitation, static condition, pH, temperature and initial dye concentration were assessed for two dyes namely Acid Red G (C.I. Acid Red 1) and Scarlet 4BS (C.I. Direct Red 23) and results for various parameters optimization are detailed below:

*5.2.2.1 Effect of Shaking and Static Condition*

*Figure 5.2 (a-b)* shows the effect of shaking and static condition on dye degradation investigated at 50 mg/l of initial concentration of Acid Red G (*Figure 5.2 (a))* and Scarlet 4BS (*Figure 5.2 (b)*). No significant difference was observed in color removal efficiency of the two respective dyes. However, 120 rpm was identified as the optimal rotation speed for the required inoculation size to be used for further experiments.

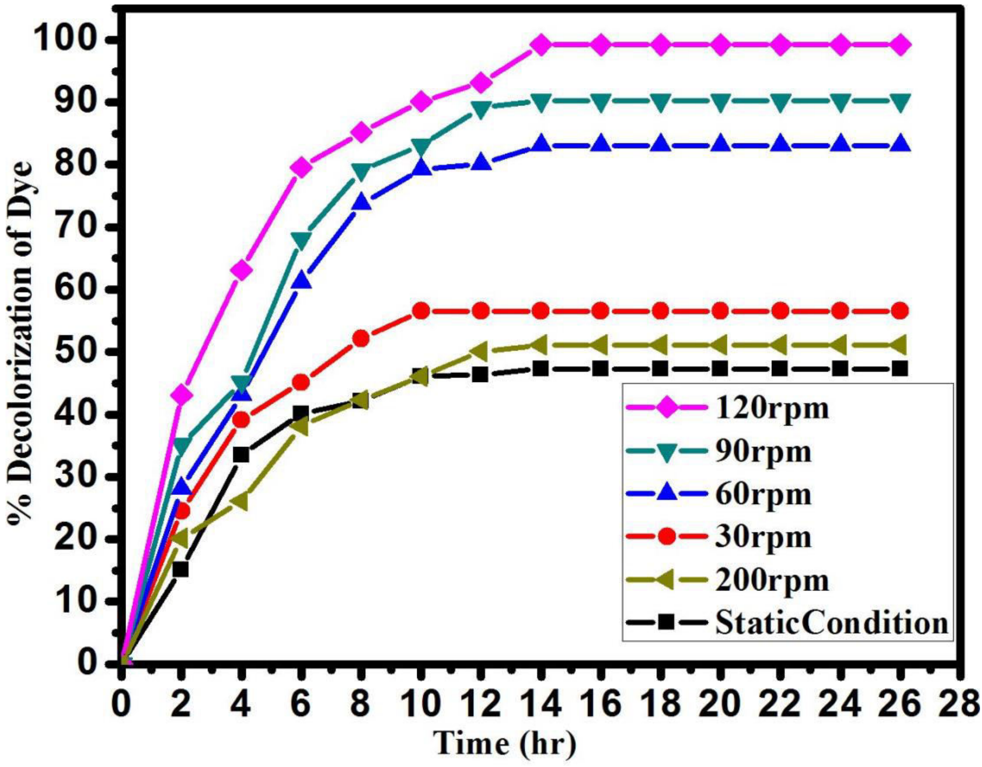



**Figure 5.2:** **(a)** Effect of different Shaking and Static condition on efficiency of degradation of ACID RED G dye by mixed Consortia BC1 (dye concentration 50 mg/l and bacterium=$1.5\times10^{6}$cells/ml)

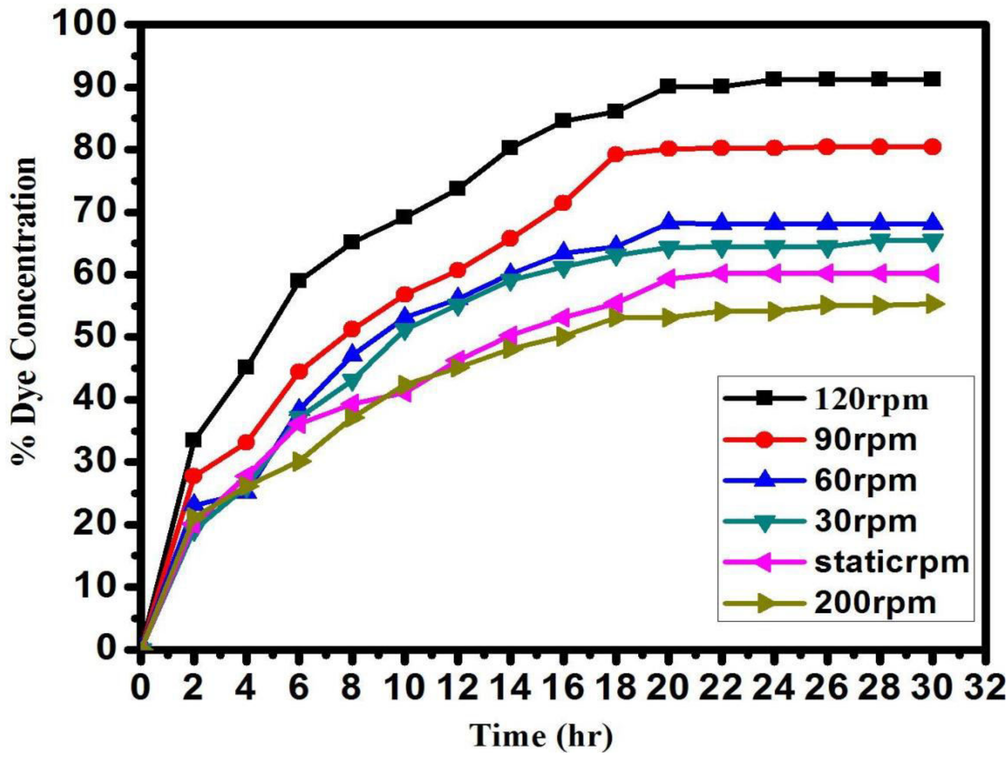



**Figure 5.2:** **(b)** Effect of different Shaking and Static condition on efficiency of degradation of Scarlet 4BS dye by mixed Consortia BC1 (dye concentration 50 mg/l and bacterium=$1.5\times10^{6}$cells/ml).

### *5.2.2.2 Effect of pH*

Effect of various pH conditions on the efficiency of dye degradation by bacterial consortium BC1 is illustrated in (*Figure 5.3 (a-b)*). The optimal pH for dye degradation ranges from 5-11 with most suitable value at pH 8 for 50 mg/l initial concentration of Acid Red G (*Figure 5.3 (a))* and Scarlet 4BS *(Figure 5.3*(*b*)) using BC1 consortium in MSM. The growth of bacterial consortia BC1 and thereby dye degradation was higher in alkaline condition as compared to acidic condition. Reduction in the efficiency under acidic condition may be attributed to accumulation of its intermediated product in the culture medium resulting in feedback inhibition of dye degradation.

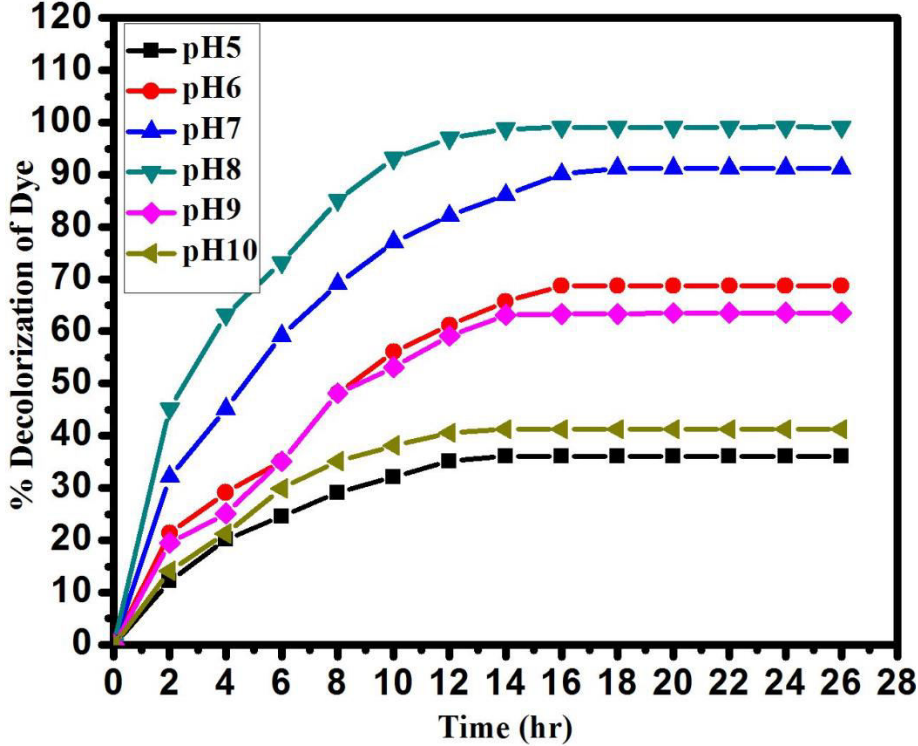



**Figure 5.3: (a)** Effect of different pH condition on efficiency of degradation of RED G dye by mixed Consortia BC1 (dye concentration 50 mg/l, bacterium=$1.5\times10^6$cells/ml and temperature 35 °C under Shaking Condition 120rpm)

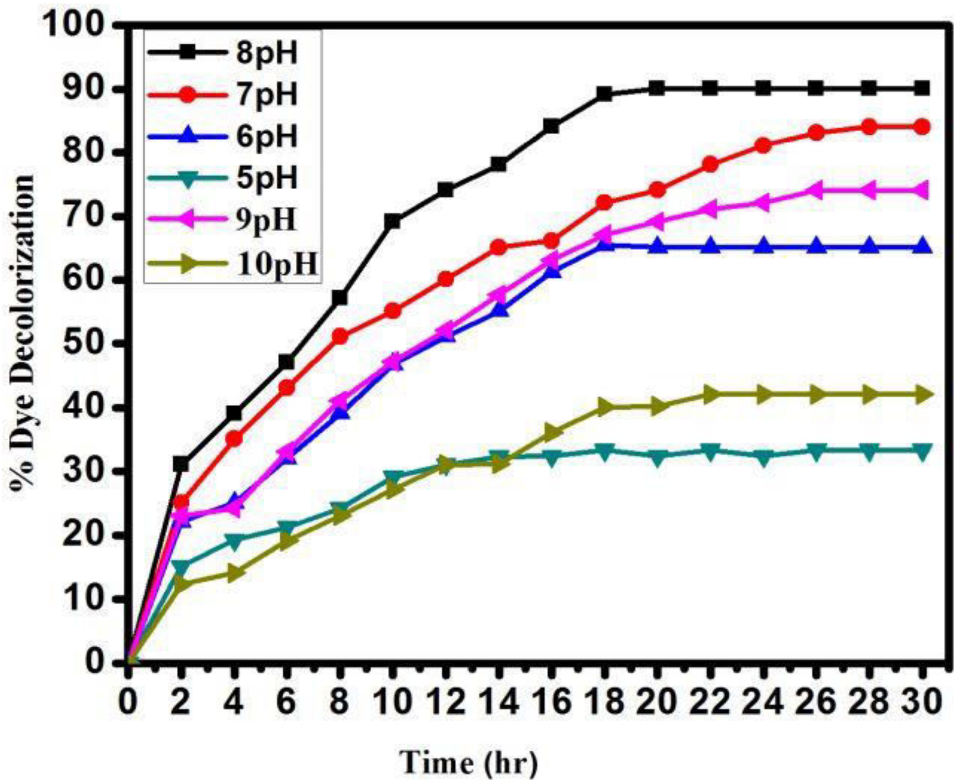



**Figure 5.3: (b)** Effect of different pH condition on efficiency of degradation of Scarlet 4BS by mixed Consortia BC1 (dye concentration 50 mg/l and bacterium=$1.5\times10^6$cells/ml, temperature 35 °C under Shaking Condition 120rpm)

### *5.2.2.3 Effect of temperature*

*Figure 5.4* shows the effect of different temperature condition on dye degradation investigated at 50 mg/l of initial concentration of Acid Red G (*Figure 5.4(a)*) and Scarlet 4BS (*Figure 5.4(*b)). Results indicate that the optimal temperature ranges from 20 to 45 °C with most effective temperature being at 35 °C. However the dye degradation rate decreases below and above 35 °C. Acid Red G dye degradation initiated at 20°C reaching its 91% and 99% potential at 30 °C and 35 °C temperature respectively (*Figure 5.4(a)*) whereas in case of scarlet 4BS dye at these mentioned temperatures the potential reached to 85% and 90% respectively (*Figure 5.4(*b)). At higher temperature, the rate of dye

degradation decreases which can be either due to loss of cell viability or deactivation of the enzyme responsible for degradation [Cetin *et al*. (2006); Luangdilok *et al*. (2000)]. The consortium degrades dye at a relatively broad range of temperatures which might prove advantageous for bioremediation of dye in natural environments exhibiting large temperature differences.

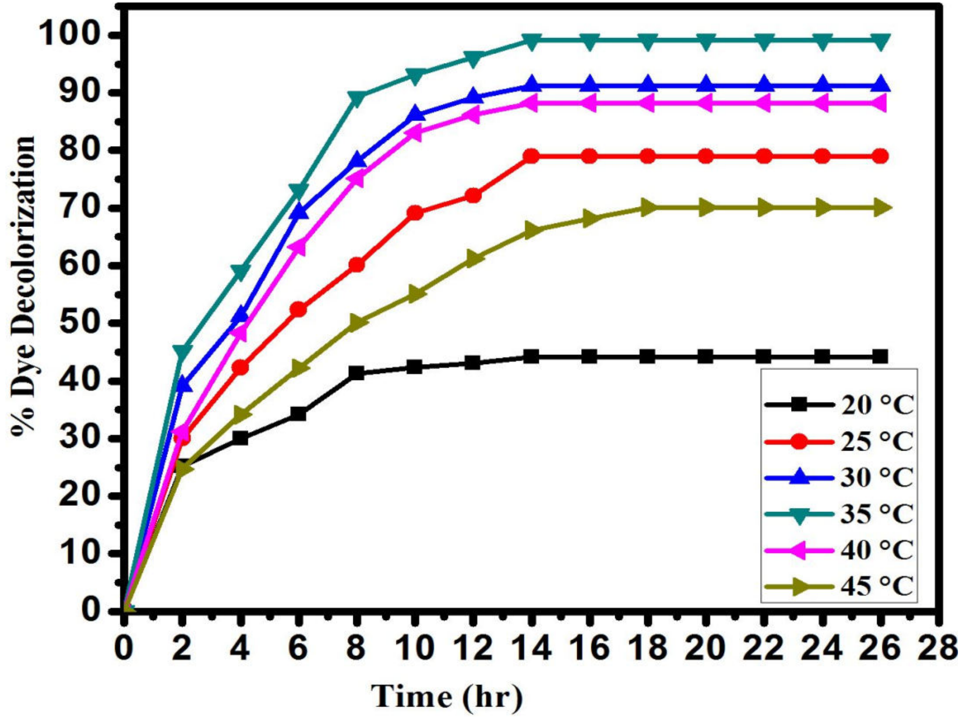



**Figure 5.4: (a)** Effect of different temperature condition on efficiency of dye degradation of Red G by Mixed Consortia BC1 (dye concentration 50 mg/l and bacterium=$1.6\times10^{6}$cells/ml under Shaking Condition 120rpm)

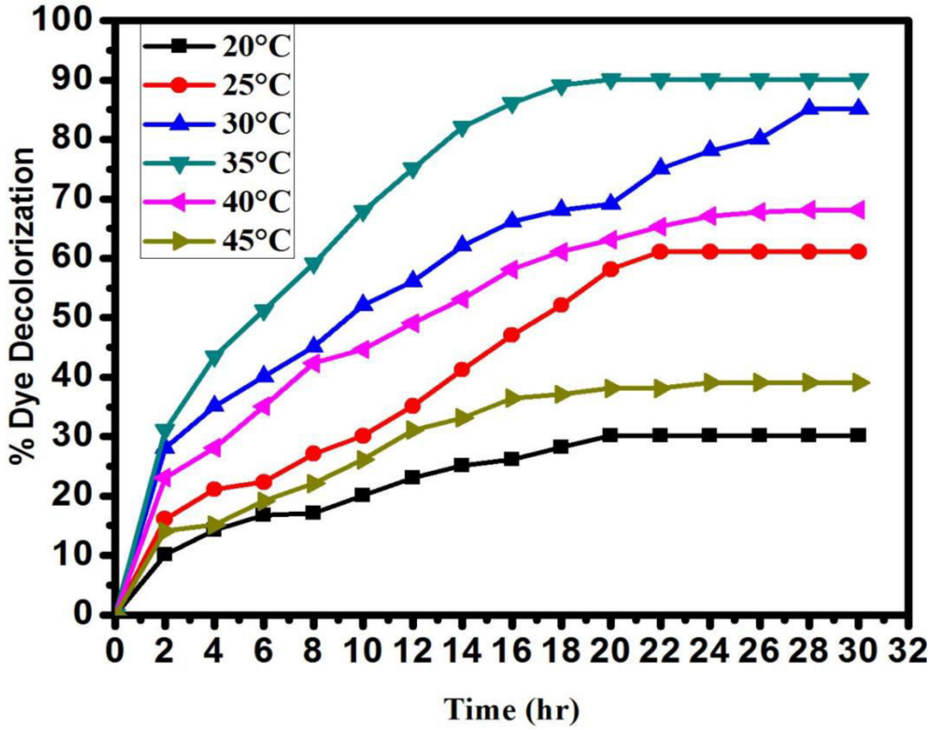



**Figure 5.4:** **(b)** Effect of different temperature condition on efficiency of dye degradation of Scarlet 4BS by Mixed Consortia BC1 (dye concentration 50 mg/l and bacterium=$1.6\times10^{6}$cells/ml under Shaking Condition 120rpm)

***5.2.2.4 Effects of initial dye concentration***

*Figure 5.5* illustrates the degradation processes of Acid Red G (*Figure 5.5(a)*) and Scarlet 4BS (*Figure 5.5(b)*) at different dye concentration by the mixed consortia. More than 90% removal was observed in 24h with 50 mg/l of initial dye concentration for both the dyes. The dye degradation decreases with increase in concentration of the dye which can be attributed to the toxicity of intermediate products of dye degradation on microorganism [Pearce *et al.* (2003)]. Moreover, about 35% degradation of dye was observed at 500 mg/l concentration using isolated mixed consortia BC1. Further increase in concentration inhibited the growth of the bacteria of mixed consortia.

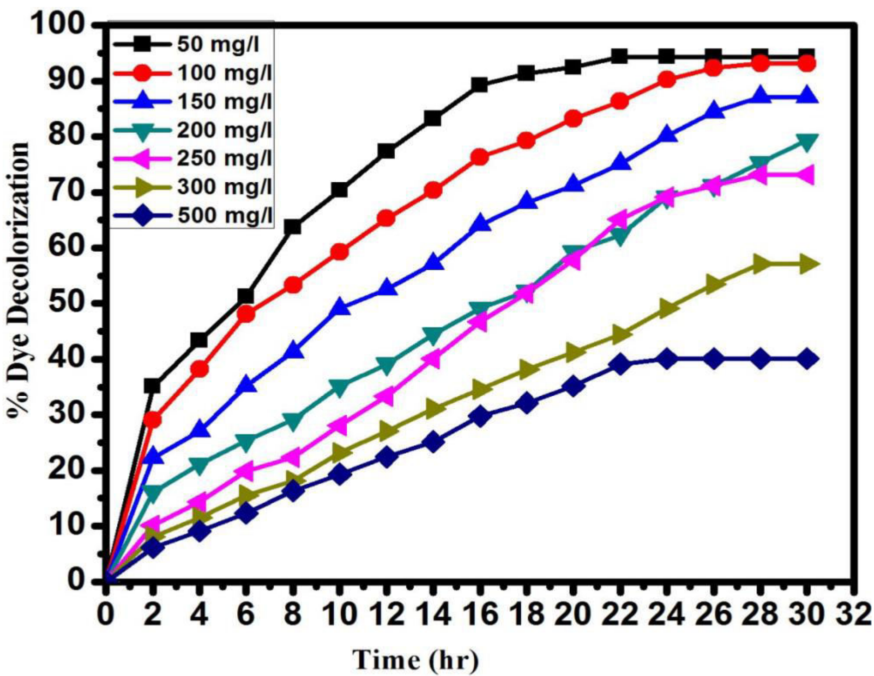



**Figure 5.5: (a)** Effect of different initial dye concentration on rate of degradation of Red G by Mixed Consortia BC1 (bacterium=$2.6\times10^{6}$cells/ml and temperature 35 °C under Shaking Condition 120rpm).

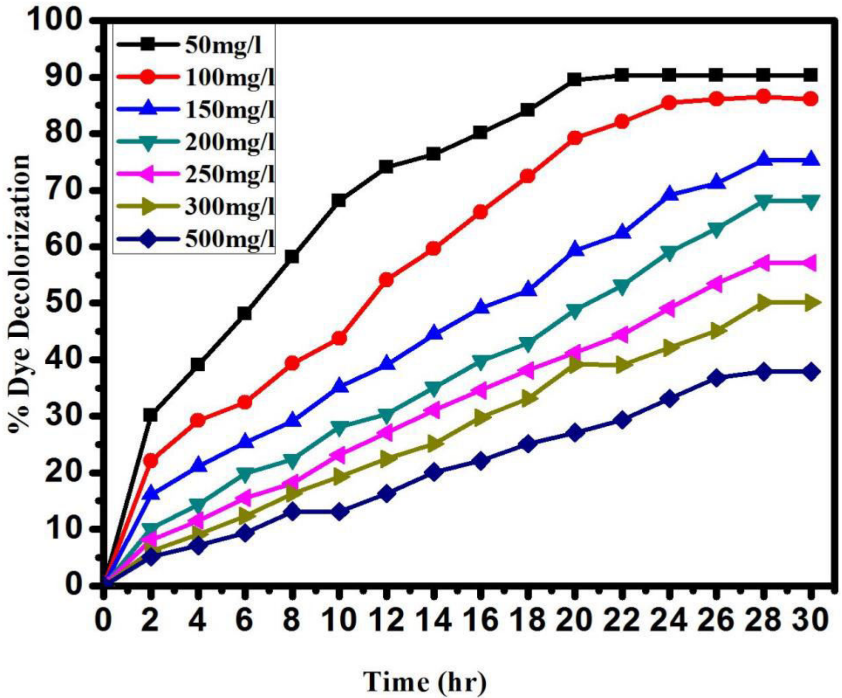



**Figure 5.5: (b)** Effect of different initial dye concentration on rate of degradation of Scarlet 4BS by isolated Mixed Consortia BC1 (Bacterium=$1.6\times10^{6}$cells/ml and temperature 35 °C under Shaking Condition 120rpm).

### *5.2.3 Kinetics of the dye decolorization of Scarlet 4BS*

General kinetic model of dye degradation is given on the basis of dye and biomass concentration. Kinetic study for degradation process of Scarlet 4BS dye using BC1 bacterial consortium is given by Michaelis-Menten rate model equation. The temperature effect on the enzymatic rate constant is analyzed assuming Arrhenius equation as this model neglects the effect of temperature on Km from the two model parameter i.e. Vmax and Kmax. The general kinetic equation relating Vmax and T is given (Eq 5.1) as:

$$\frac{ds}{dt} = K_1 S^n M^m \tag{5.1}$$

Where, S is concentration of dye (mg/l), M is cell mass concentration (mg/l) & n, m are reaction order with respect to dye and cell mass concentration respectively at time period (h), $K_1$ is decolonization rate constant. M value is taken as constant since growth and death was not observed so equation 1 is simplified to Eq. 5.2 and 5.3:

$$\frac{ds}{dt} = K_0 S^n \tag{5.2}$$

$$K_0 = K_1 M^m \tag{5.3}$$

Integrating equation 1 and 2

$$\ln\left(\frac{S}{S_0}\right) = K_0 t \tag{5.4}$$

A plot between the values of ln ($S_0$/S) with time gives the rate of the reaction kinetics involved in the experiment process. A double reciprocal plot of 1/$V_o$ versus 1/S of Scarlet 4BS with Eq.5 was used to determine the kinetics of enzyme activity.

### *5.2.3.1 Determination OF $K_{MAX}$ AND $V_{MAX}$*

$$V_o = -\frac{dE}{dt} = \frac{\text{V max E}}{\text{E+ Km}} \tag{5.5}$$

Where, E is substrate concentration, $K_m$ is Michaelis constant, $v_{max}$ is maximum degradation rate and $K_m$ value is dependent on temperature, pH, specific dye etc. The plot between the values of ln ($S_0$/S) with time for Scarlet 4BS was used to derive the rate constant ($K_0$) at each temperature. These constant value thus obtained is used to generate a plot between ln ($K_0$M) versus 1000/T, the slope of which is multiplied by universal gas constant (R= 8314JK$^{-1}$M$^{-1}$) to get activation energy value.

Activation energy for reaction was given by Arrhenius equation (Eq. 5.6)

$$K_0 = -K' \exp\left(\frac{-E_a}{RT}\right) \tag{5.6}$$

Where, K' is frequency factor, Ea activation energy (cal K-$M^{-1}$), T is temperature (K), $K_0$ are rate constant.

Equation 5.6 can be rewritten as Equation (5.7) to give overall relationship between concentrations, temperature and cell mass concentration

$$\ln\left(K_0\right) = \ln\left(K'\right) - \frac{Ea}{RT} \tag{5.7}$$

The rate of degradation for the study of kinetics was measured using a test solution containing 50 mg/l of dye which was incubated under aerobic condition at 35 °C for 34h at pH 7. This kinetic of dye degradation using bacterial consortium is first of its kind wherein kinetics constants of the enzymatic mixed for dye degradation are determined from the entire time course data. Kinetic study for this degradation process is based on the parameters such as agitation, pH, temperature, dye concentration and glucose concentration. Study on the effect of these parameters towards rate of degradation was carried out to understand key factors affecting efficacy of dye degradation.

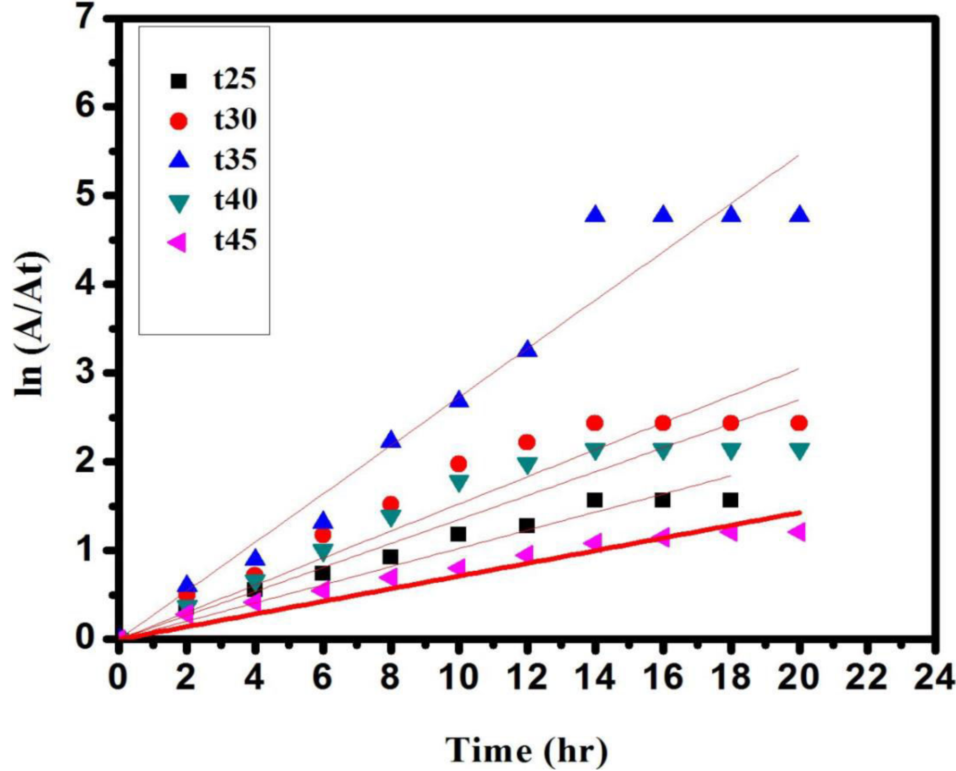



**Figure 5.6: (a)** Graph of $\ln(S/S_0)$ versus time for the degradation of Scarlet 4BS dye using BC1.

From *Figure 5.6(a)* is used to find pseudo first order rate $K_0$ constant at each temperature with high degree of linearity was observed in plot. These constant value at different temperature obtained with $R^2 > 0.9$ can be used to obtain a plot between ln ($K_0M$) versus 1000/T to get activation energy value as shown in *Figure 5.6(a)*. The slope of this plot was multiplied by universal gas constant ($R = 8314 JK^{-1}M^{-1}$). Activation energy for reaction was calculated by Arrhenius equation 5.6.

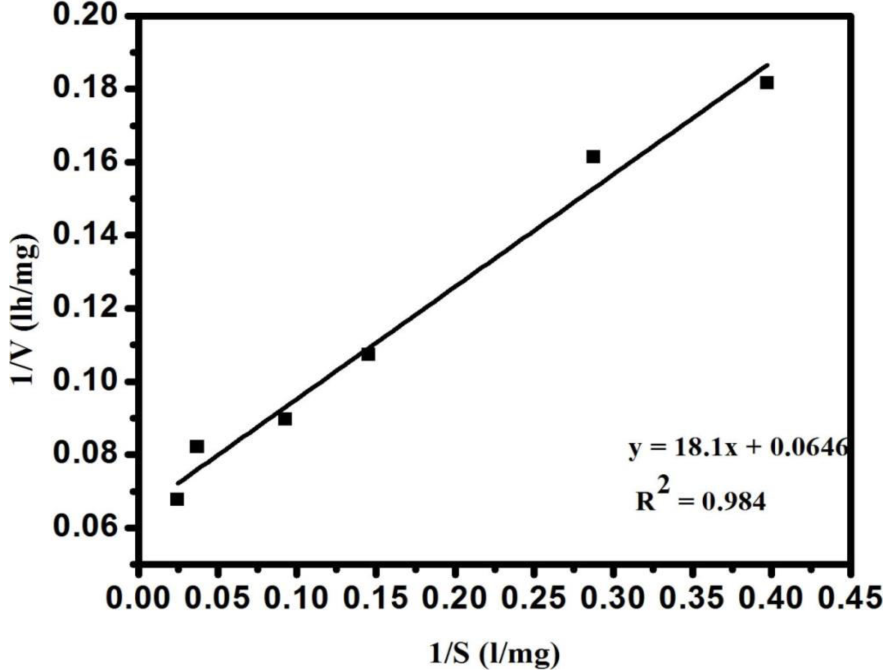



**Figure 5.6: (b)** Estimation of activation energy of scarlet dye from graph ln ($K_0M$) versus 1000/T

*Figure 5.6* shows the kinetics of Scarlet 4BS degradation by BC1. Plot of ln($S_0$/S) with time using experimental values for the degradation of Scarlet 4BS (*Figure 5.6a*) gives a straight line with high degree of linearity as depicted by the value of $R^2$ at different temperature except at T= 20 °C indicating it to be a first order kinetics. The kinetic data was found to be highly significant ($R^2$ > 0.9) at all temperatures except at 20°C. In contrast, kinetic study for Acid Red G reveals it to be of pseudo first order kinetics.

A double reciprocal plot of initial rate of dye degradation (1/V) and scarlet dye concentration (1/S) (*Figure 5.6(b)*) gives a straight line with value of intercept i.e. 1/$V_{max,}$ and slope i.e. $K_m/V_{max}$ is found to be 16.6 $h^{-1}$ and 301.546 mg/l respectively. Maximum value of $V_{max}$ was achieved when bacteria was saturated with dye from all its sides.

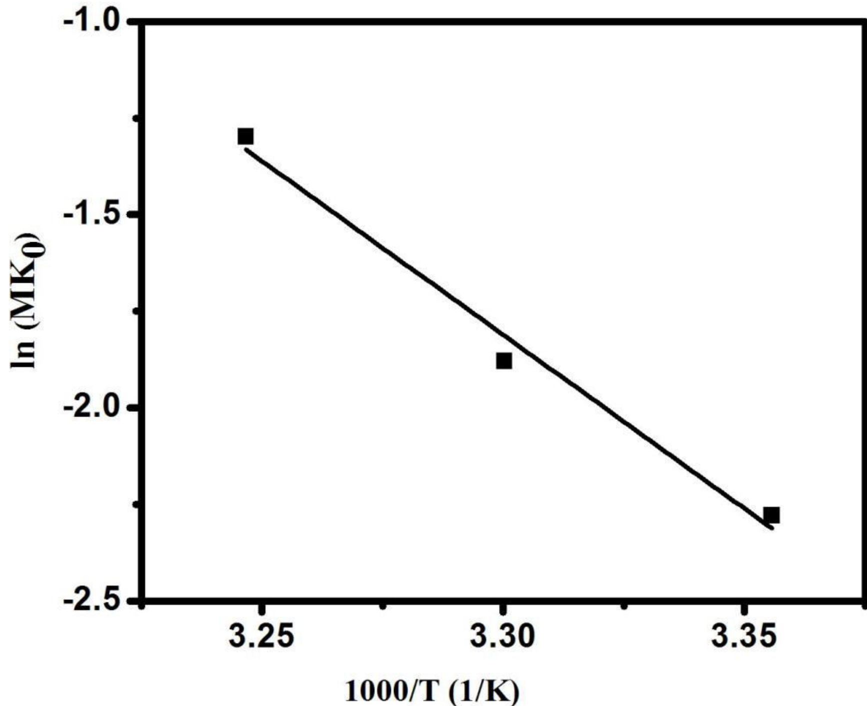



**Figure 5.6: (c)** A double reciprocal plot of initial rate of dye degradation (1/V) and scarlet dye concentration (1/S).

Plot between ln ($K_0M$) versus 1000/T to derive the activation energy of Scarlet 4BS is illustrated in *Figure 5.6(c)*. Using this plot and equation 5.6, the value of activation energy (Ea) and frequency factor was found to be 17.869 (Kcal $M^{-1}$) and $1.2\times10^{-12}$ respectively. Low value of activation energy depicts it be a faster and efficient method for dye degradation.

**Effect of various parameters on the kinetics of dye degradation using BC1 is explained below:**

### *5.2.3.2 Effect of pH*

A plot of effect of pH on the rate of dye degradation is illustrated in (*Figure 5.6(d)*. From the graph it is evident that rate of degradation increases with increasing pH at the range of 5-7, above which the rate of degradation decreases. Alkaline pH plays an

important role in binding of the dye to the fabric. Results indicate that neutral or weak basic pH as optimal condition for azo-dye degradation at 37°C using mixed consortia BC1 (*Figure 5.6 (a-d)*).

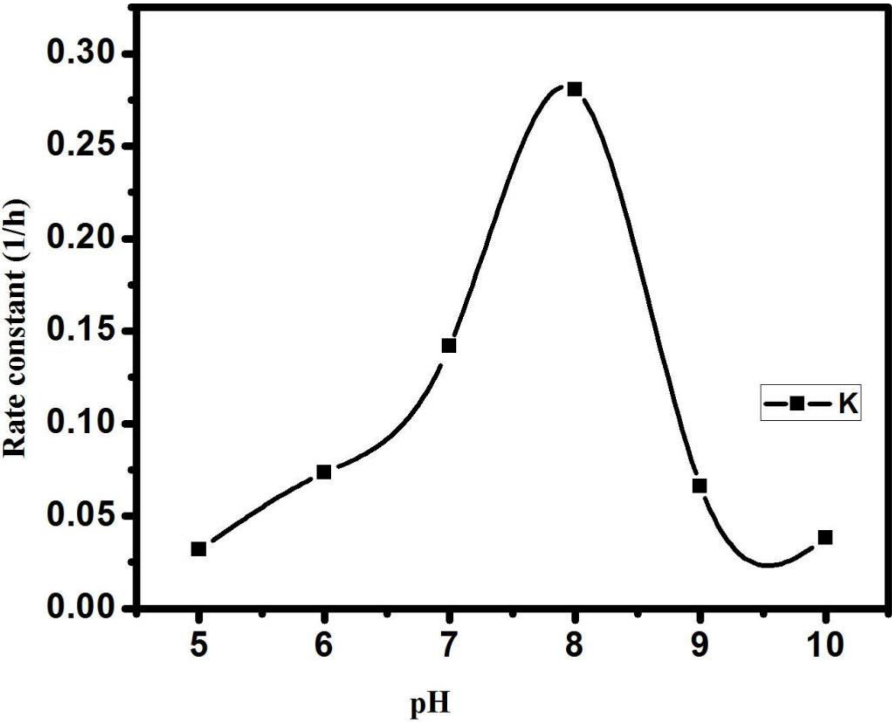



**Figure 5.6: (d)** Effect of pH on rate constant at 35 °C, 50 mg/l dye.

***5.2.3.3 Effect of dye concentration***

The efficiency of dye removal depends on two key factor i) toxicity of dye at higher concentration ii) ability of enzyme to recognize the dye at low concentration. *Figure 5.6 (e)* shows the effect of dye concentration on rate of its degradation. It is observed that at high concentration of dye i.e. from 50 to 500 mg/l the growth and thereby extent of degradation is suppressed.

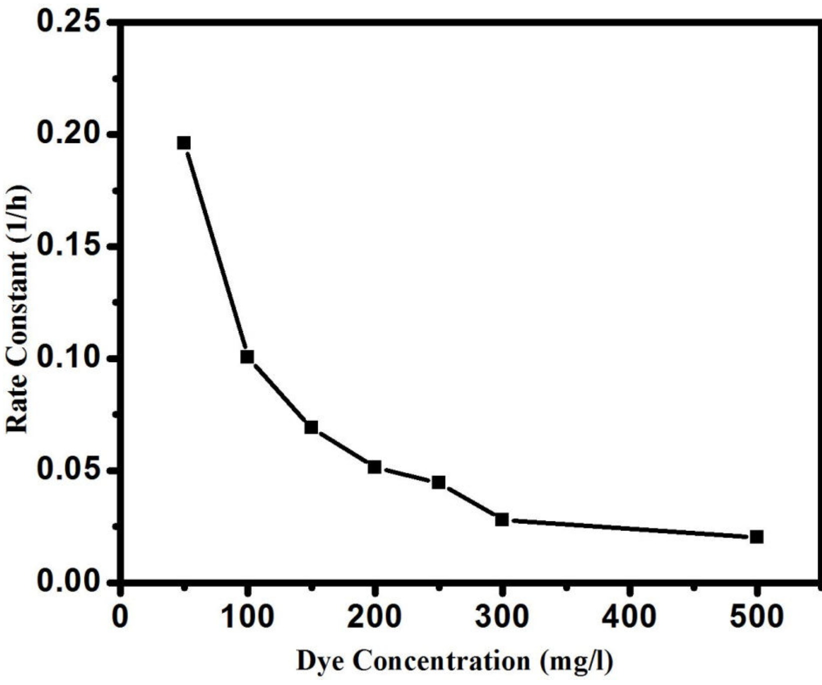



**Figure 5.6: (e)** Effect of dye concentration on rate constant at 35 °C and 50 mg/l dye

### *5.2.3.4 Effect of initial glucose concentration and glucose to microbe ratio*

Supplementary carbon sources provide energy to decolorizing bacteria thus enhances the performance of decolorizing system. Glucose is found to be one of the best supplementary carbon sources for the biodegradation of synthetic dye. *Figure 5.6 (f-g)* respectively shows the effect of glucose concentration and substrate to microbial weight on the rate of dye degradation. The correlation between different concentration of glucose and substrate to microbe weight ratio shows the reaction to follow a first order kinetics. A decrease in degradation constant is observed with increasing glucose concentration (*Figure 5.6 (f)*). It is due to limitation of growth rate of specific bacterial species at high glucose concentration. Negative effect at high glucose concentration may be attributed to the fact that higher concentration of glucose is being used by the bacteria for its growth rather than for dye degradation.

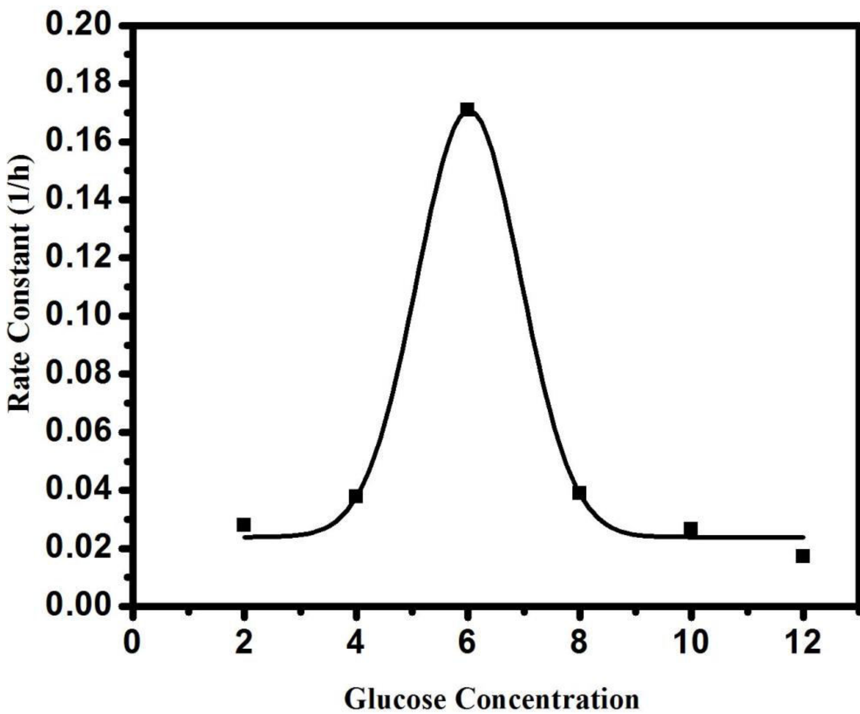



**Figure 5.6: (f)** Effect of glucose concentration on rate constant at 35 °C and 50 mg/l dye.

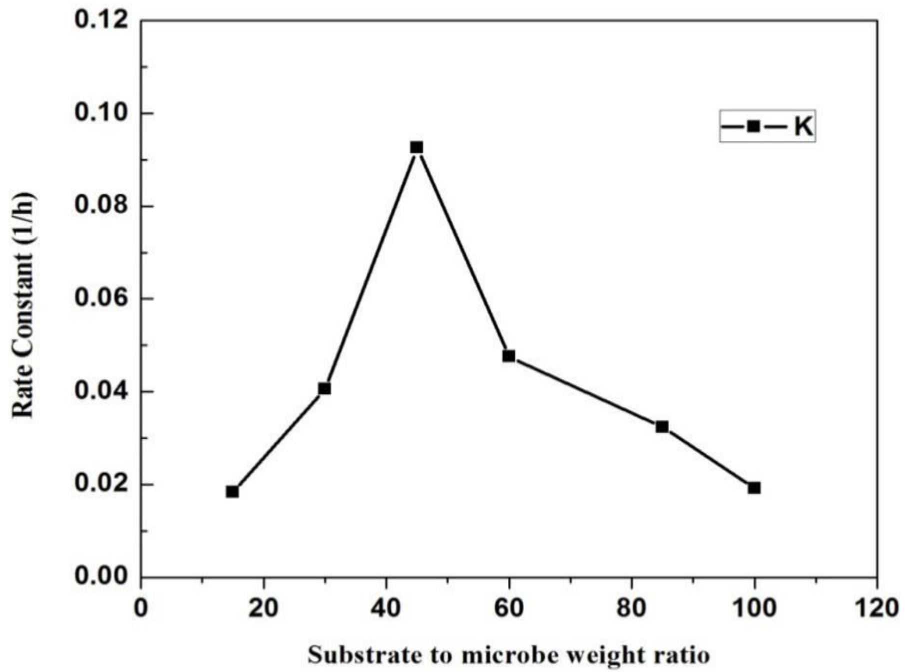



**Figure 5.6: (g)** Effect of substrate to microbe weight on rate constant 35 °C and 50 mg/l dye

**5.3 Effluent Degradation Bioassay**

Possible pathway of dye and its degraded metabolites were analyzed using UV-Vis spectrophotometer, HPLC and GC-MS spectra analysis. *(Figure 5.7 (a-b))* illustrates the byproducts of dye degradation analyzed using UV-Vis spectrophotometer at different time intervals. Maximum absorbance for Acid Red G were obtained at 200, 230, 325 and 500 nm (*Figure 5.7a*) and at 200, 230, 260, 400 and 500 nm for Scarlet 4BS dye (*Figure 5.7b*). The peaks thus obtained are due to the presence of phenyl and naphthyl rings in the structure the two tested dyes. There was a remarkably decrease in the peaks which virtually reached zero after 24h. Disappearance of peak at 500 nm (*Figure 5.7(a-b)*) indicates cleavage of azo bonds from corresponding intermediates products. A decrease

in the intensity of various other peaks (*Figure 5.7(a-b)*) suggests opening of all the aromatic nuclei. The final products could possibly be simple hydrocarbons without any conjugated bonds or amines, alcohols and others or $CO_2$ and $H_2O$ due to complete mineralization of dye degradation byproducts.

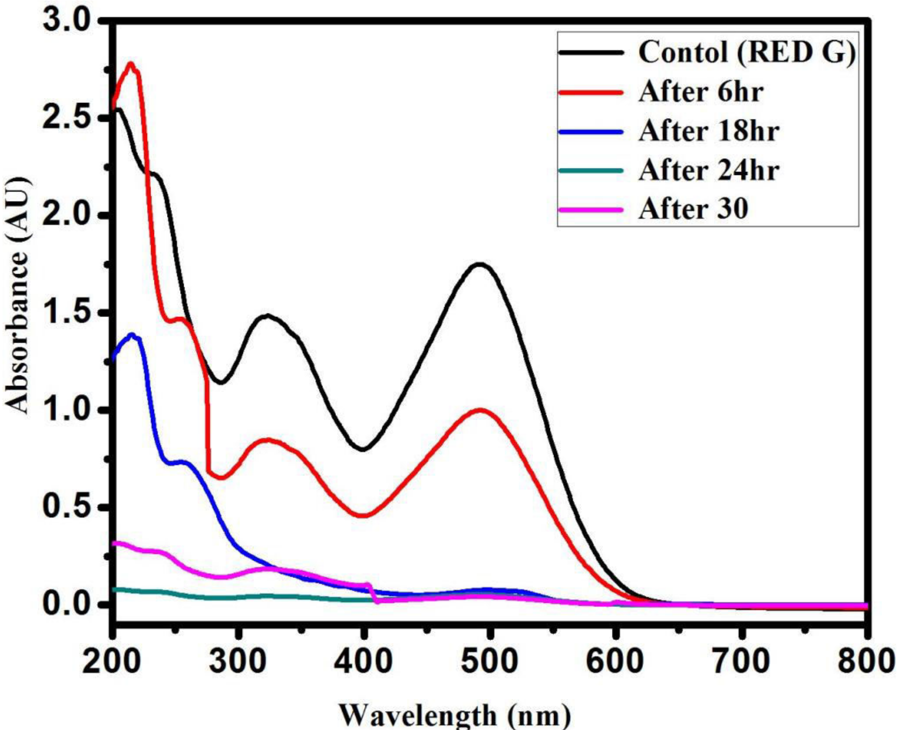



**Figure 5.7: (a)** UV-Vis spectrophotometer of RED G (50 mg/l) biodegraded by BC1 before and after optimized condition at T=35°C, pH=8.0 bacterium= $1.5\times10^6$ cells/ml at Shaking Condition (120 rpm).

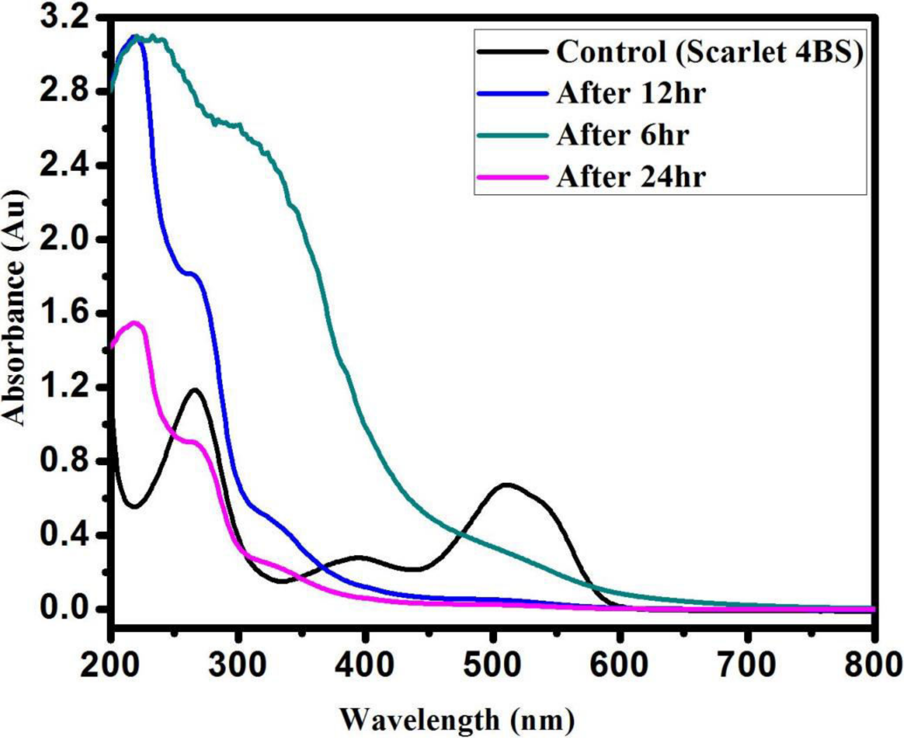
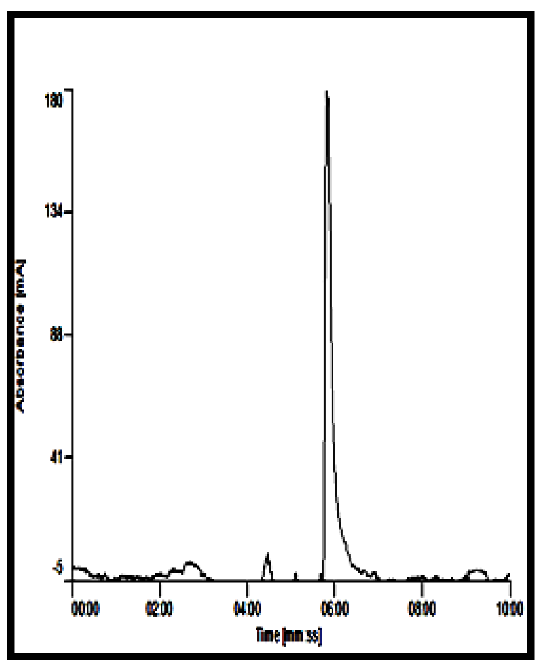



**Figure 5.7: (b)** UV-visible spectra of Scarlet 4BS by mixed consortia before and after degradation under optimized condition i.e. temperature = 35 °C, pH= 8.0, bacterium=$1.6\times10^6$cells/ml and shaking condition (120 rpm).



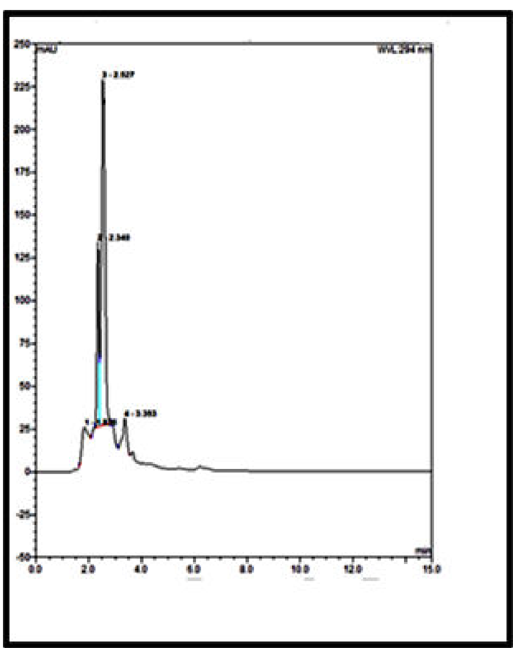
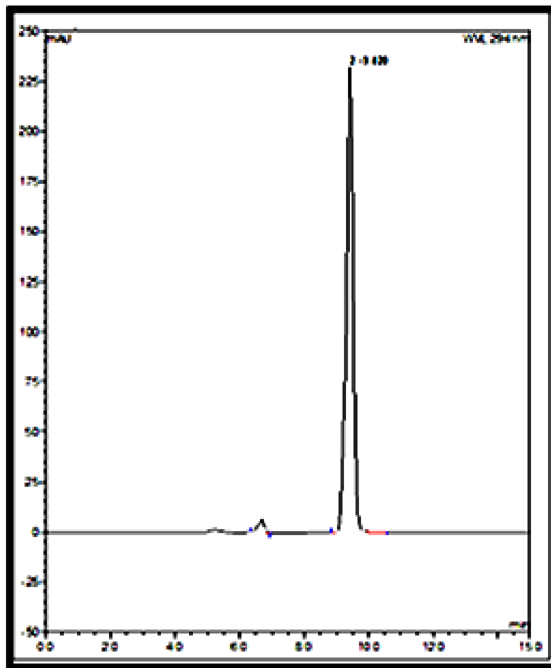




**Figure 5.8**: HPLC analysis for dye degradation (Acid Red G) a) Dye control Sample b) Degraded sample.



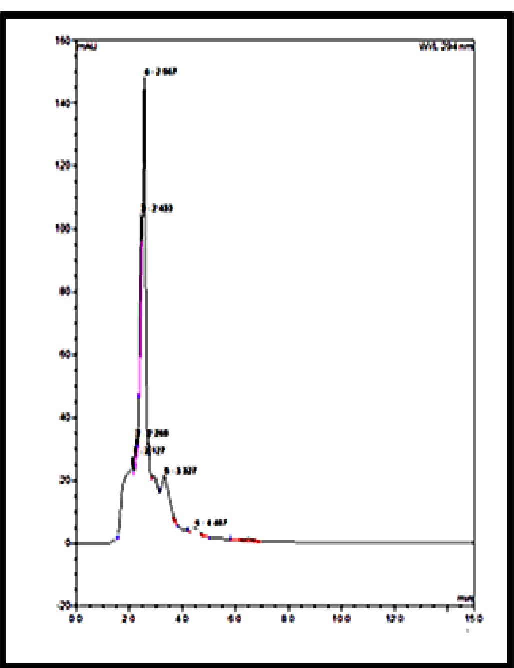
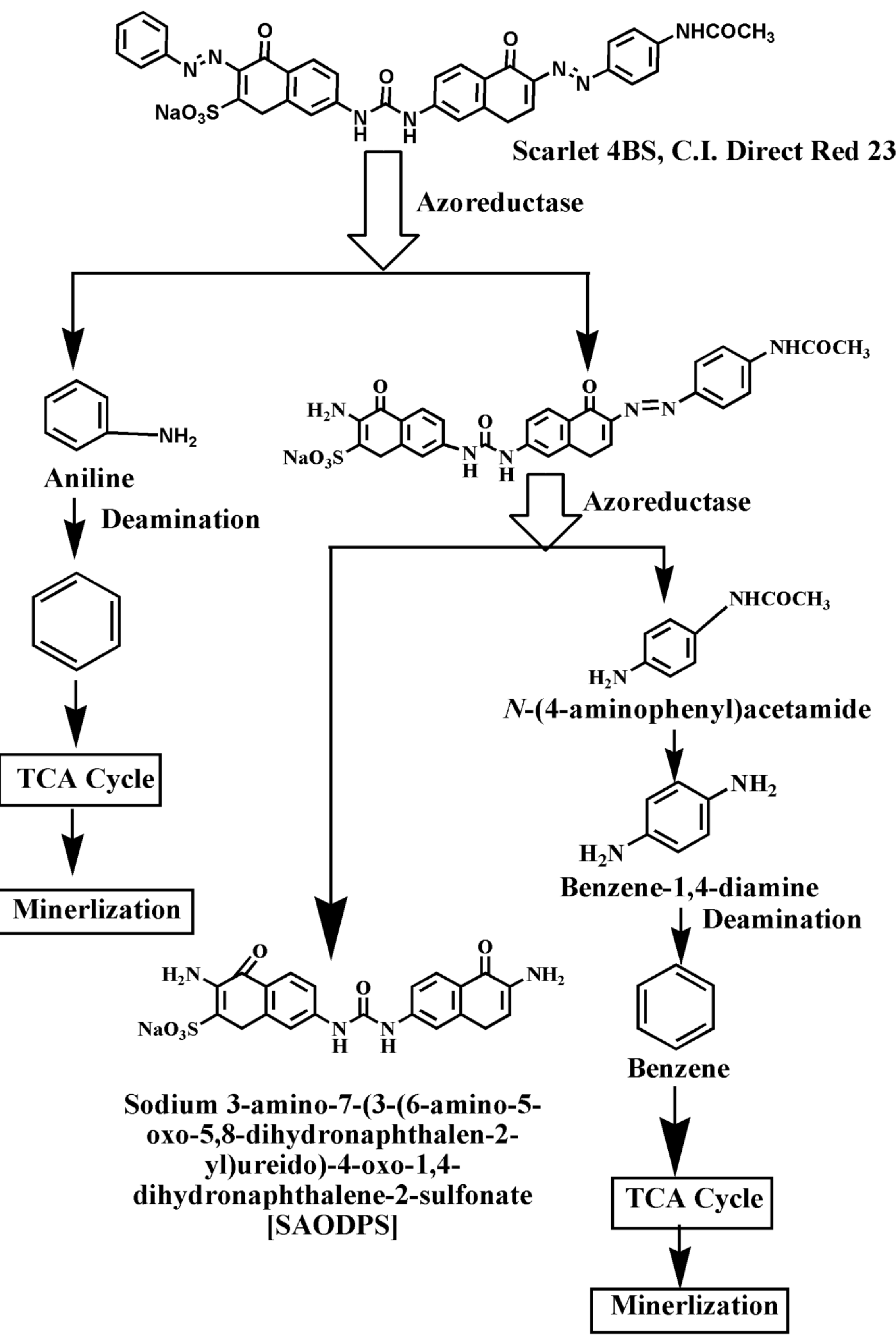




**Figure 5.9:** HPLC analysis for dye degradation (Scarlet 4BS) a) Dye control Sample b) Degraded sample

### *5.3.1 Possible pathway of Acid Red B: (Proposed Dye Degradation Pathway)*

GC-MS analysis was carried out to investigate byproducts of dye degradation by BC1 bacterial consortium. Both Acid Red G and Scarlet 4BS dye shows aerobic degradation pathway. The azoreductase enzyme of the bacterial consortium is responsible for degradation of the azo groups of both the dyes. Kumari *et al.* (2015) reported similar degradation of Acid Red G following an anaerobic pathway. Aniline a toxic agent is expected to be the first byproduct formed in the anaerobic pathway was found absent in the aerobic degradation pathway (not shown in *Figure*). Acid Red G being a monoazo dye in contrast to Scarlet4BS (diazo dye) exhibits differences in its degradation byproducts. *Figure 5.10* shows possible pathway and its byproducts for the degradation of Scarlet 4BS dye using BC1. Intermediates formed during degradation were taken up in the TCA cycle showing complete mineralization and utilization of the resulting products as nutrient for the bacterial consortium (*Figure 5.10*). GC-MS spectra of various intermediates of Scarlet 4BS degradation are illustrated in *Table 5.1*. Scarlet 4BS gave (N (4 aminophenyl acetamine), Benzene, 3-7, diamino-4 hydroxynapthalene-2sulfonic acid, Naphthalene, and Sodium 3,7-Diamini-4-oxo-3,4-dihydronaphthalene-2-sulfonate as byproducts (*Figure 5.10*) in contrast to 3-aminonaphthalen-2-ol, 3-hydroxyphthalic acid, pyrocatechol formed as byproduct of Acid RED G similar to its anaerobic pathway [Kumari *et al.* (2015)]. Moreover toxic aromatic amines are not formed in the aerobic degradation pathway in contrast to anaerobic pathway making it as an eco friendly technique for dye degradation.



NHCOCH3
NaO3S
Scarlet 4BS, C.I. Direct Red 23
Azoreductase
NH2
Aniline
Deamination
H2N
Azoreductase
NHCOCH3
N-(4-aminophenyl)acetamide
NH2
Benzene-1,4-diamine
Deamination
TCA Cycle
Minerlization
Benzene
Sodium 3-amino-7-(3-(6-amino-5-oxo-5,8-dihydronaphthalen-2-yl)ureido)-4-oxo-1,4-dihydronaphthalene-2-sulfonate [SAODPS]
TCA Cycle
Minerlization

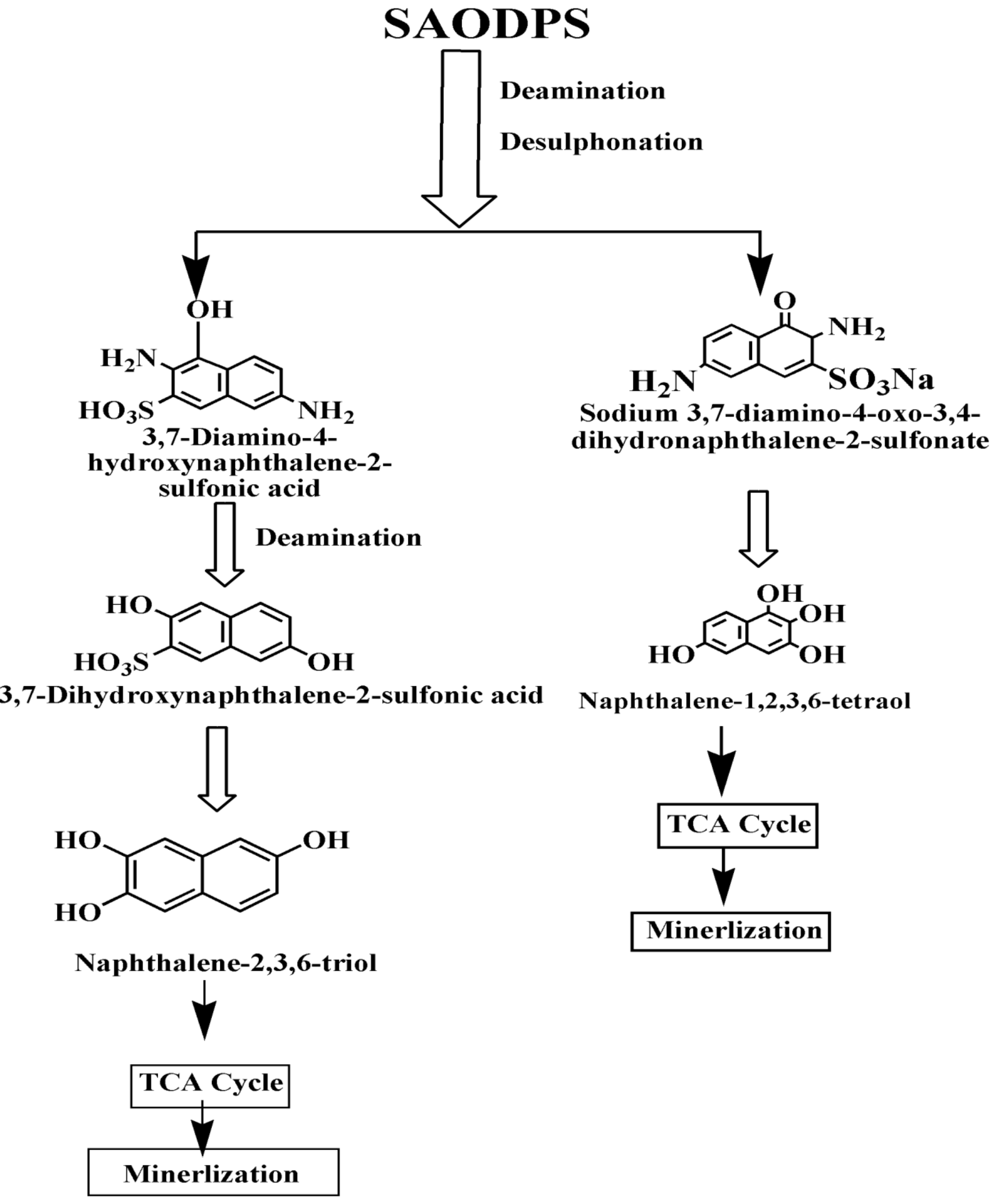
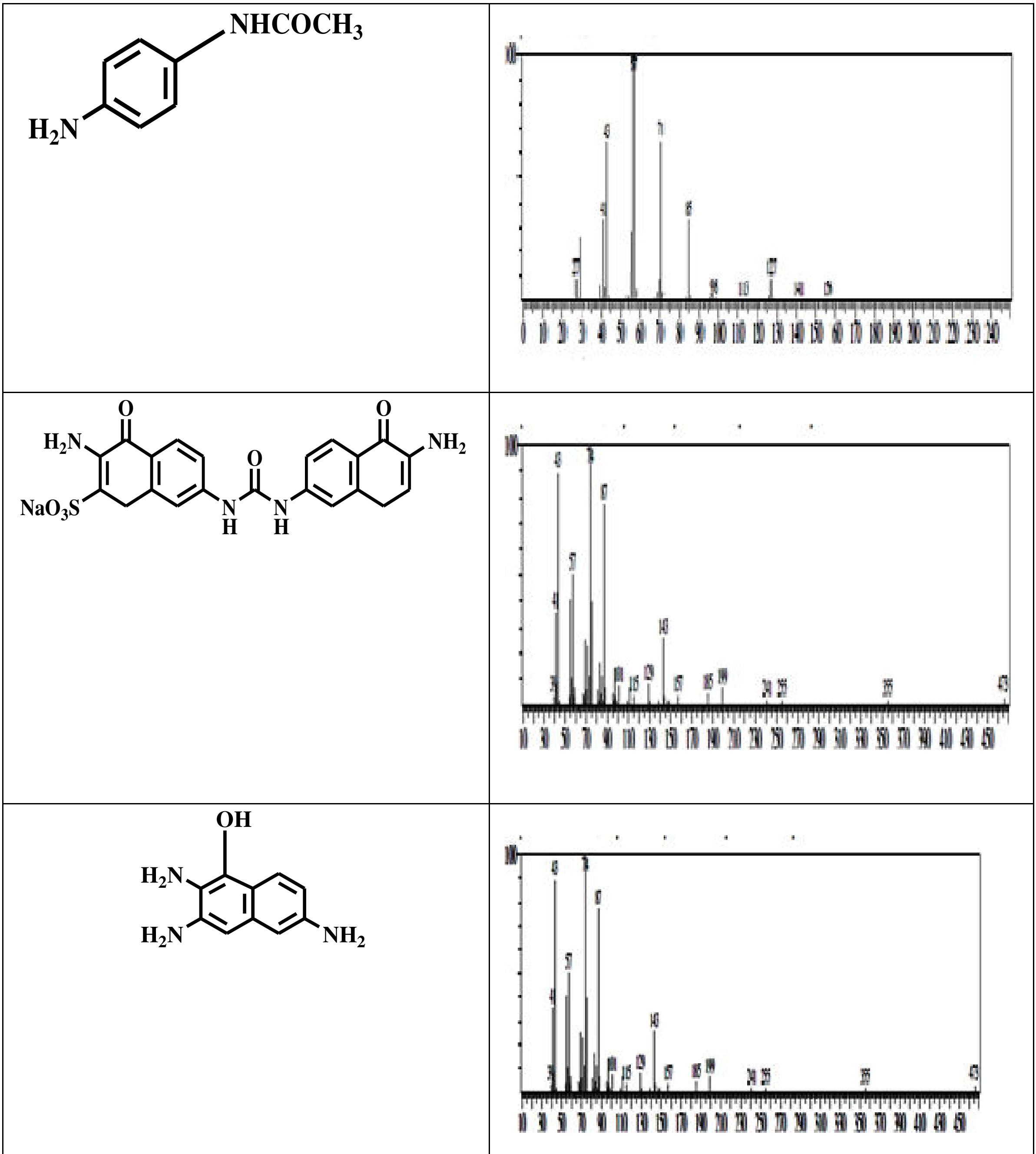
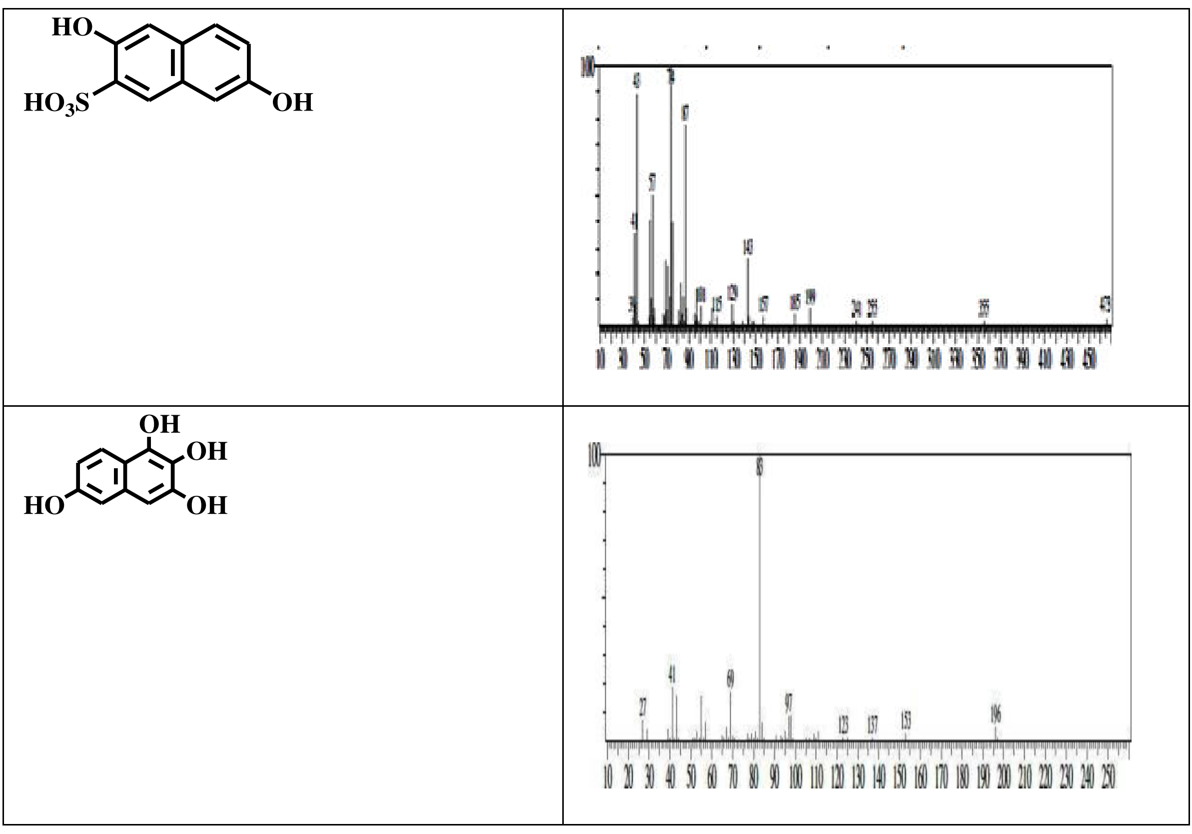



**Figure 5.10:** Proposed pathway for the degradation of Scarlet 4BS by isolated bacterial consortia (BC1) on the basis of identified metabolites, enzyme activity and utilization profile of different substrates.

**Table 5.1:** GC-MS spectra of intermediates metabolites of degradation of Scarlet 4BS using BC1.






## 5.4 Phytotoxicity studies

The effluent coming out from these industries which are either being discharged directly into water bodies or used in agricultural practices are toxic in nature and causes serious environmental hazards. In river ecosystem it causes great loss to aquatic biodiversity with alteration in the physicochemical properties of water [Pandey *et al.*, (2007)]. This wastewater causes toxic effect on the germination rates and biomass of plant species when used for agricultural practices. In the present study, carpet industries of Bhadohi have significant direct wastewater discharge causing harmful impacts on the nearby flora and fauna. Therefore it becomes important to assess the toxicity of the effluent before and after degradation. Plant populations with great commercial and agricultural significance can be used as biosensors of genetic toxicity for the environmental pollutants. *Table 5.2* shows phytoxicity study of degraded dye metabolites

and its control set (prepared using distilled water) on *Sorghum vulgare*. The germination of plant was observed after 7days of incubation on the basis of its length of radical and plumule. Results shows 100% germination in both dye degraded metabolites and control set as compared to only 20% germination in dye sample. There was a significant reduction in zone of inhibition of plumule and radical in the biotransformed products of Scarlet 4BS compared to untreated dye thereby showing significant increase in yield and biomass of the plants. Further, the metabolites generated after the degradation of dye are less toxic than the original dye. These observations suggest that the degraded byproduct of dye using BC1 bacterial consortium is much safer to the environment and can be utilized for agricultural uses or can be discharged directly in the aquatic system thereby showing high potential of this bacterial consortium for treatment of dye based wastewater.

**Table 5.2:** Phytotoxicity Study of Scarlet 4BS dye and its degradation metabolites (*Sorghum vulgare*).

| Plants | Observation | Treatments | | |
|---|---|---|---|---|
| | | Distilled water | Untreated wastewater | Aerobic treated effluent (32h) |
| ***Sorghum vulgare*** | **Germination (%)** | **100** | **20** | **100** |
| | **Plumule (cm)** | **14.8±2.2** | **2.5±3.4** | **8.8 ± 3.6** |
| | **Radical (cm)** | **7.6±1.4** | **0.9±1.3** | **7.5 ± 1.9** |

### 5.5 CONCLUSION:

Discharge of untreated effluent into nearby industries causes serious environment and health hazards. Isolated mixed consortia (BC1) used in this study which is actively growing in dye contaminated soil was found to have remarkable properties of dye degradation. Present study demonstrates that an isolated mixed consortium BC1 from dye contaminated soil was efficient at degrading the toxic dyes scarlet 4BS and Red G under aerobic condition. Various factor optimized for efficient dye degradation were pH at 8, 35°C temperature, 120 rpm shaking condition with 50 mg/l initial dye concentration. UV-Visible spectrophotometer, HPLC and GC-Ms analysis shows complete mineralization of toxic byproducts resulting in simple products like $CO_2$ and $H_2O$ by bacterial consortium. The phylotoxicity study on *Sorghum vulgare* test plant confirms the detoxification of dye as the rate of germination and length of plumule and radical was found to be same as control setup. The greater extent of degradation of dye by BC1 under natural environmental conditions indicates this bacterial strain as ecofriendly with high economic potential to be employed in the biological treatment of dyeing mill effluents. The ability of BC1 can be considered as potential bioremediation tool for the treatment of industrial wastewater which can be discharged directly in aquatic system or can be used for agricultural purpose. Our results indicates that isolated bacterial strain to be a better environmentally safe biotransformation option for dye degradation compared to previously reported bacterial species. The prospect plan of this study focused on pilot scale treatment of carpet effluent using mixed consortia in packed bed reactor. The ability of isolated bacterial consortia to tolerate, decolorize and degrade azo dye at high concentration gives it advantage over other dye wastewater treatment methods. However, potential of bacterial degradation of azo dyes needs further investigations using appropriate bioreactor system and toxicity study for its application in natural environments.