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Some Aerobic Bacterial Degradation and Decolorization of Different Azo Dyes

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Abstract
In this present study an attempt was made to examine the potential of different bacterial strains for biodecolorization of Azo dye under aerobic condition. The bacterial strains used in the study were *Escherichia coli, Pseudomonas aerogenosia, Bacillus* sp. and *Klebsiella* sp. the selected bacterium shows higher decolorization *E. coli* as compared to another bacteria. The maximum removal was observed at 0.1 mg/L of direct orange, dispearse Brown and reactive green with Pseudomonas aerogenosa (94, 88, 72%) after 7 days incubation. The minimum removal was observed at 0.5 mg/L of D.O, D.B and R.G. with Klebsiella sp. (80, 45, 29) % after (6, 7, 1) days from incubation.
Keywords : Aerobic , bacteria, degradation , degradation , azo dyes

Introduction

Some microorganisms, including bacteria, fungi and algae, can degrade or absorb a wide range of dyes. The biological mode of treatment of dye bath effluents offers distinct advantages over the conventional modes of treatment. This method is more economical and leads to less accumulation of relatively harmless sludge. More importantly, biological treatment of bath effluents is ecofriendly. It causes mineralization of dyes to simpler inorganic compounds which are not lethal to life form (Tripathi & Srivastava, 2011).

When wastewater was discharged into the natural water body, it contaminants the entire system with it's color and the organic load it possess reduces the dissolved oxygen level in the water system making it toxic for the natural inhabitants. The azo dyes are biorecalcitrant under aerobic condition but could be cleaved under oxygen limiting conditions leading to the formation of toxic aromatic amines (Rajendran *et al*., 2011).

Several reduction products of azo dyes are aerobically biodegradable. Depending on the structure of azo compounds, the biodegradability is reduced in the presence of substituents such as sulfonate groups. *Shingomonas* sp. is known to aerobically degrade various substituted naphthalene sulfonic acids (Andreas *et al*., 1997).

Research on bacterial strains that are able to decolourize azo dyes under aerobic (xenphylus azovorans, *Bacillus* strain, *Kerstersia* sp. and *Staphylococcus* sp.) (Franciscon *et al*., 2009).

Reactive dyes usually have a synthetic origin and complex aromatic molecular structure, which make them stable and difficult to biodegrade and differ from all other dye classes in that they bind to textile fibers, such as cellulose and cotton through covalent bonds at present, a number of studies have focused on microorganisms, which are able to decolourize and biodegrade these dyes (Usman *et al*., 2011).

Bacillus subitils was gram-positive used to decolourize acid blue 1B. the bacterial culture exhibited 90% decolorization ability 50 h. The optimum dye decolorizing activity of the culture was observed at pH 7 and incubation temperature of 37 ºC (Gurulakshmi *et al*., 2008).

Escherichia coli is a common bacteria found in the environment has been used for treating textile effluent. These bacteria grown very fast and double after every 20 minutes under favorable conditions of pH in the range of (6 to 8) and optimal temperature at 35 ºC (Babu *et al*., 2000).

Pseudomonas aerugenosa is a gram-negative, motile, rod shaped bacterium. The bacterium is capable of both aerobic and an aerobic growth. It is abundant in various types of moist enviroment and can adapt to numerous others. The bacterium can be found soil, water fruits and respiratory therapy. *Klebsiella species* is a gram-negative, anaerobic facultatives (Rosli, 2006).

Increasing industrialization and urabanization leads to environmental pollution. The discharge of toxic effluents from various industries adversely affect water resources, soil fertlity, aquatic organisms and ecosystem integrity.

Azo dyes, which are aromatic compounds with one or more -N=N- groupds. Dyes that are absorbing light with wavelength in visible range (350-700) nm are colored. Dyes contain chromophores electron systems with conjugated double bonds and auxochromes usual chromophores are -C=C-, -C=N-, -C=O-, -N=N-, NO_2 and quinoid rings, and usual auxochromes are $–NH_3$, -COOH, $-SO_3H$ and –OH (Wong &Yuen, 1996 ; Isik,2003).

Dyes are classified inaccordance with either the chemical constitute or classification of dyes according to usage, the dyes are anionic (direct, acid, and reactive dyes), cationic (basic dyes), and nonionic dispersed. Anionic and nonionic dyes mostly contain azo or anthroquinone type of chromophores (Chung & Cerniglia,

1992).

Bioremediation is a pollution-control technology that uses natural biological species to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. Azo compounds constitute the largest and the most diverse group of synthetic dyes and are widely used in a number of industries such as textile, food cometics and paper printing (Majid *et al*., 2011).

The microorganisms being highly versatile are expected to develop enzyme systems for the decolorization and mineralization of azo dyes under certain environmental conditions.The biodegradation of azo dyes starts with the cleavage of dye structure releasing aromatic amine. Dye stuff toxicity (i. e. mortality, genotoxicity, mutagenicity and carcinogenicity) was studied on both aquatic organisms (fish, algae, bacteria and mammals) (Umbuzerio *et al*., 2005 ; Chang *et al*.,2004).

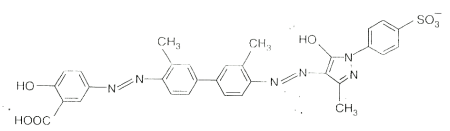
Several combinal anerobic and aerobic microbial treatments have been suggested to enhance the degradation of azo dyes. Some specialized strains of aerobic bacteia have developed the ability to use azo dyes as sole source of carbon and nitrogen, other only reduce the azo group by special oxygen-tolerant azo reductases (O'Neill *et al*., 2000; Balanca *et al*., 2007).

The gene encoded a protein with a molecular weight of 20, 557 Da, having conserved a putative NAD (p) H-binding site in the amino-terminal region. A part from these specific azoreductases, nonspecific enzymes catalyzing azo aye reduction also have been isolated from aerobically grown cultures of *Shigella dysenteria, E. coli* and *Bacillus* sp. (Zee, 2002; Ong, 2005) .

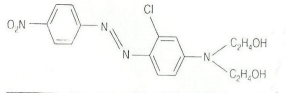
Materials and Methods

Azo dye used

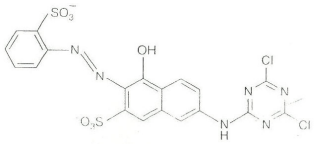
Azo dyes which are used in the research, direct orange dispearse Brown and reactive Green in concentration of (0.1, 0.3 and 0.5 mg/L) were kindly provided by the textile industries company, Hilla, Iraq the structures of the dyes are shown in following (Tacob *et al*., 2010).



Direct orange



Dispearse brown



Reactive green

Inocula

Growth of single culture, *Escherichia coli, Pseudomonas aerogenosa*, *Bacillus* sp., and *Klebsiella* sp. (10% inoculum) were grown in Erlemeyer flask (500 ml) containing 90% of azo dyes aqueous solution. All flasks were incubated at 37 ˚C for 7 days and take 10 ml from each flasks were centrifuged 2000 rpm for 10 min. for measured optical density. The changes in the dyes absorption spectra were recorded by using uv-spectrophotometer cintra-5 (Rosli, 2006).

Azo removal was determined from absorbance calibration curve of standard solution. The efficiency of dye removal was expressed as the percentage ratio of decolorized dye concentration to that of intial one.

$$\%\,\text{Decolorization} = \frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

Bacteria

The bacteria used in this study, *Escherichia coli, Pseudomonas aerogenosa*, *Bacillus* sp., and *Klebsiella* sp. were provided by Biology department, Babylon university.

Bacterial growth on plates

Nutrient agar plate was prepared by dissolving 20 g of powder in distilled water (1 L) and sterilozed by autoclaving at 121 ˚C, 15 bar for 15 minutes. The agar was then cooled down to about 5 ˚C before being poured in sterile petridish and incubated for 24 hours in a incubator.

Bacterial liquid cultures

Nutrient broth was prepared by dissolving 8 g of nutrient broth in distilled water (1L) and sterilized by autoclaving. The sterilized culture medium was then kept at 5 ˚C.

Results and Discussion

The present study include decoloruization of azo dyes by bacterial spp. (*Pseudomonas aerugenosa, E. coli, Bacillus sp.* and *Klebsella* sp.) were found bacteria spp. effect on azo dyes this agree with (Khalid *et al*., 2008; Hong *et al*., 2007; Yu *et al*., 2007; Tapana *et al*., 2008). Bacterial degradation of azo dyes is generally considered a specific reaction by azo reductase under aerobic condition. The efficient removal of these dyes is necessary and signficant for environmental protection.

Azo dye decolorization by bacterial species if often initiated by enzymatic reduction of azo bonds, the presence of oxygen normally inhibits the azo bond reduction activity since aerobic respiration may dominate ulitization of NADH; thus impeding the electron transfer from NADH to azo bonds.

The dye decolorization of azo dye (direct orange, dispearse brown and reactive green) was studied under static aerobic condition with dye concentration of (0.1, 0.3 and 0.5 mg/L) using different bacterial cultures of *E. coli*, *Pseudomonas aerugenosa, Bacillus sp.* and *Klebsella* sp. out of these cultures, *Pseudomonas aerugenosa* was the best decolorizer compared with other bacteria.

Absorption of pigment direct orange was decreased through the period of treatment from (3.175, 3.763 and 3.524 nm) before treatment to (0.201, 0.305 and 0.37 nm) after treatment. The percentage of removal pigment at first day was 69%, 78% and 66% respectively and continous to reach 94, 92 and 89 at seven day.

While dispearse brown pigment absorption was decreased continuously through period of treatment from (2.773, 3.010 and 2.312 nm) to (0.345, 0.605 and 0.741 nm). The percentage of removal was recorded at first day (76%, 58% and 26%) and continuous to reach 88% at seven day, 80% at five day and 68% at seven day respectively.

It also reactive green pigment absorption was decreased through period of incubation from (1.486, 2.306 and 2.817 nm) to (0.421, 0.753 and 1.999 nm). The percentage of removal was appeared at first day (25 %, 26% and 29 %) respectively and continous to reach 72%, 67% at seven day while stable treatment untile seven day 29% at 0.5 mg/L.

These treatments were occurred on ther types of bacteria (*E. coli, Bacillus* sp. and *Klebsella* sp.) found in concentration the same effect of treatment with Pseudomonas aerugenosa but in low percents. Where appeared direct orange result (89%, 83% and 86%), (85%, 81% and 81%) and (85%, 84% and 80%) in respectively.

While recorded treatment percents with dispearse brown (87%, 78% and 56%), (79%, 78% and 75%) and (77%, 80% and 45%) respectively.

Finally treatment percents with reacive green (35, 38 and 29%), (35, 35 and 29%) and (37, 32, 29%) respectively.

Table (1,2,3,4): Pigment concentration and absorption value during treatment period

Dye	Con.	Lmax (nm)	Absorption before treatment	Pseudomonas aerogenosa Day						
				1	2	3	4	5	6	7
Direct orange	0.1	667	3.175	0.713	0.435	0.428	0.355	0.349	0.183	0.132
	0.3	448	3.763	1.129	0.925	0.824	0.751	0.654	0.185	0.143
	0.5	435	3.524	0.983	0.744	0.489	0.402	0.369	0.189	0.142
Dispearse brown	0.1	464	2.773	0.964	0.867	0.723	0.656	0.442	0.406	0.362
	0.3	467	3.010	1.797	1.357	0.988	0.918	0.884	0.761	0.655
	0.5	463	2.312	1.999	1.999	1.999	1.999	1.486	1.361	1.265
Reactive green	0.1	664	1.486	1.097	1.097	1.077	0.993	0.984	0.978	0.962
	0.3	660	2.306	1.989	1.733	1.421	1.421	1.421	1.421	1.421
	0.5	656	2.817	1.999	1.999	1.999	1.999	1.999	1.999	1.999

LSD(0.05) = 0.231

Dye	Con.	Lmax (nm)	Absorption before treatment	Day Escherichia coli						
				1	2	3	4	5	6	7
Direct orange	0.1	667	3.175	1.377	1.151	0.713	0.435	0.428	0.349	0.349
	0.3	448	3.763	1.129	0.925	0.824	0.751	0.654	0.654	0.654
	0.5	435	3.524	1.345	0.983	0.983	0.744	0.744	0.489	0.489
Dispearse brown	0.1	464	2.773	0.964	0.867	0.723	0.656	0.442	0.406	0.362
	0.3	467	3.010	1.797	1.357	0.988	0.918	0.884	0.761	0.655
	0.5	463	2.312	1.999	1.881	1.536	1.350	1.092	1.085	1.007
Reactive green	0.1	664	1.486	1.097	1.097	1.077	0.993	0.984	0.978	0.962
	0.3	660	2.306	1.989	1.733	1.421	1.421	1.421	1.421	1.421
	0.5	656	2.817	1.999	1.999	1.999	1.999	1.999	1.999	1.999

LSD(0.05) = 0.023

Dye	Con.	Lmax (nm)	Absorption before treatment	Day Bacillus sp						
				1	2	3	4	5	6	7
Direct orange	0.1	667	3.175	1.417	1.273	1.273	0.918	0.733	0.476	0.476
	0.3	448	3.763	1.623	1.623	1.237	1.113	1.113	0.697	0.697
	0.5	435	3.524	1.834	1.051	0.819	0.819	0.677	0.677	0.677
Dispearse brown	0.1	464	2.773	1.711	1.711	1.232	0.972	0.972	0.578	0.578
	0.3	467	3.010	1.094	0.955	0.873	0.830	0.650	0.650	0.650
	0.5	463	2.312	1.273	1.273	1.118	1.040	0.906	0.584	0.584
Reactive green	0.1	664	1.486	1.198	1.107	1.107	1.104	1.040	1.021	0.972
	0.3	660	2.306	1.507	1.507	1.507	1.507	1.507	1.507	1.507
	0.5	656	2.817	1.507	1.507	1.507	1.507	1.507	1.507	1.507

LSD(0.05) =0.007

Dye	Con.	Lmax (nm)	Absorption before treatment	Day *Klebsella sp*						
				1	2	3	4	5	6	7
Direct orange	0.1	667	3.175	0.935	0.791	0.677	0.564	0.564	0.475	0.475
	0.3	448	3.763	1.237	0.998	0.799	0.799	0.612	0.612	0.612
	0.5	435	3.524	1.876	1.455	1.346	1.098	0.978	0.695	0.695
Dispearse brown	0.1	464	2.773	1.072	0.943	0.783	0.632	0.632	0.632	0.632
	0.3	467	3.010	1.479	1.248	1.099	0.725	0.663	0.614	0.602
	0.5	463	2.312	1.999	1.999	1.999	1.999	1.486	1.361	1.265
Reactive green	0.1	664	1.486	1.311	1.170	1.118	1.072	1.043	0.971	0.932
	0.3	660	2.306	1.571	1.571	1.571	1.571	1.571	1.571	1.571
	0.5	**656**	2.817	1.999	1.999	1.999	1.999	1.999	1.999	1.999

LSD(0.05) =0.001

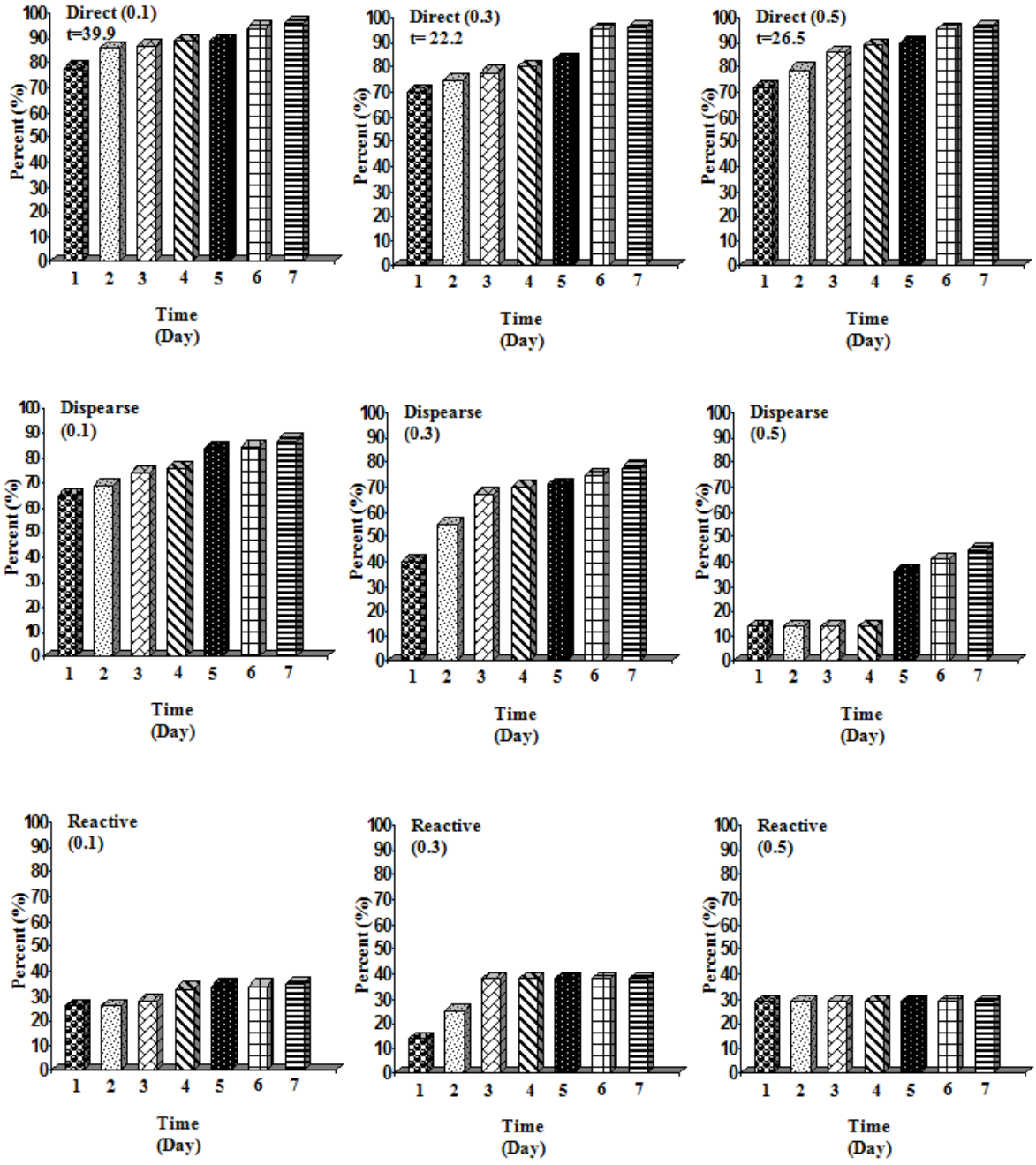


Figure (1): Color value of pigment during treatment by *Pseudomonas aerogenosa.*

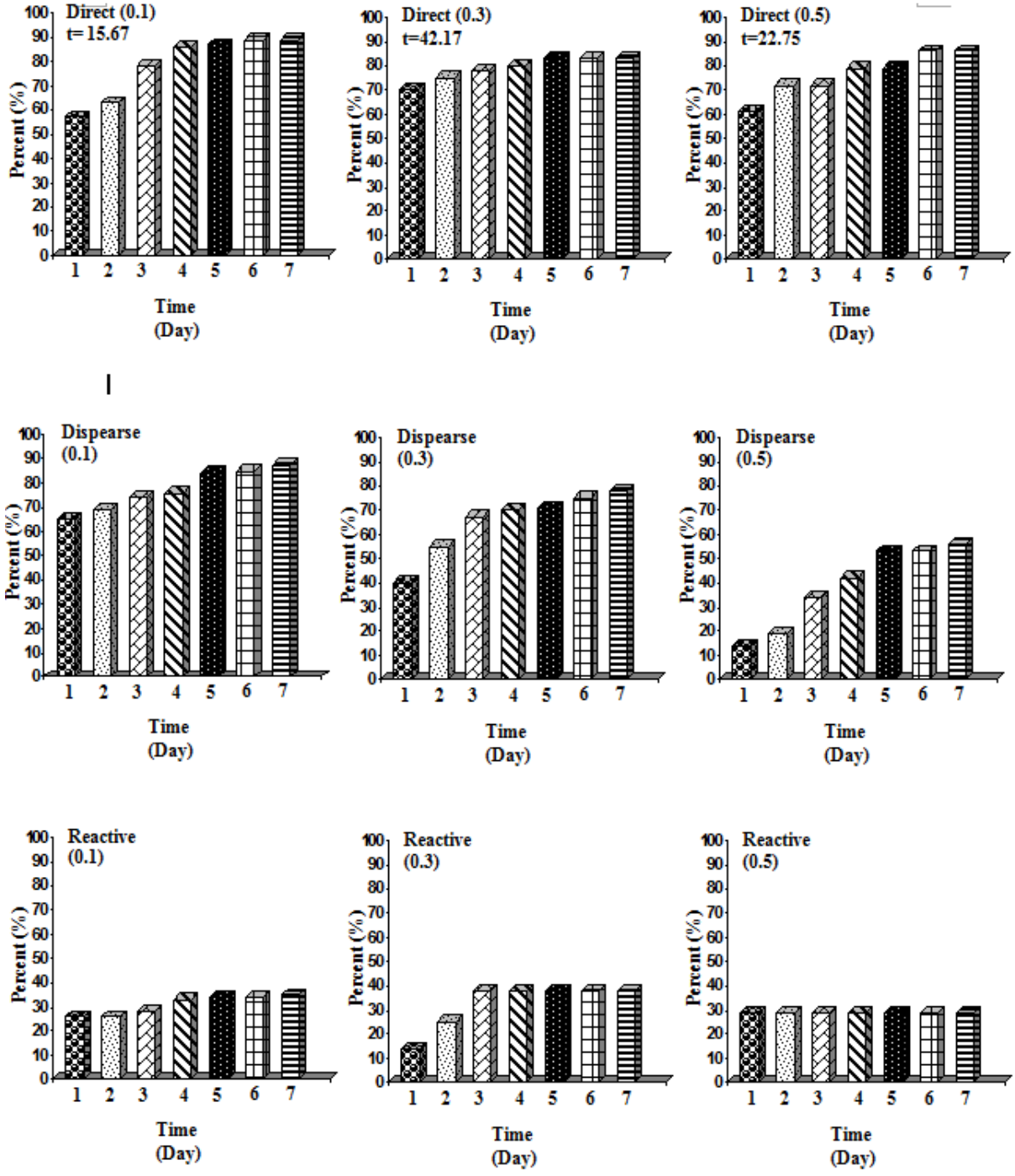


Figure (2): Color value of pigment during treatment by *Escherichia. coli.*

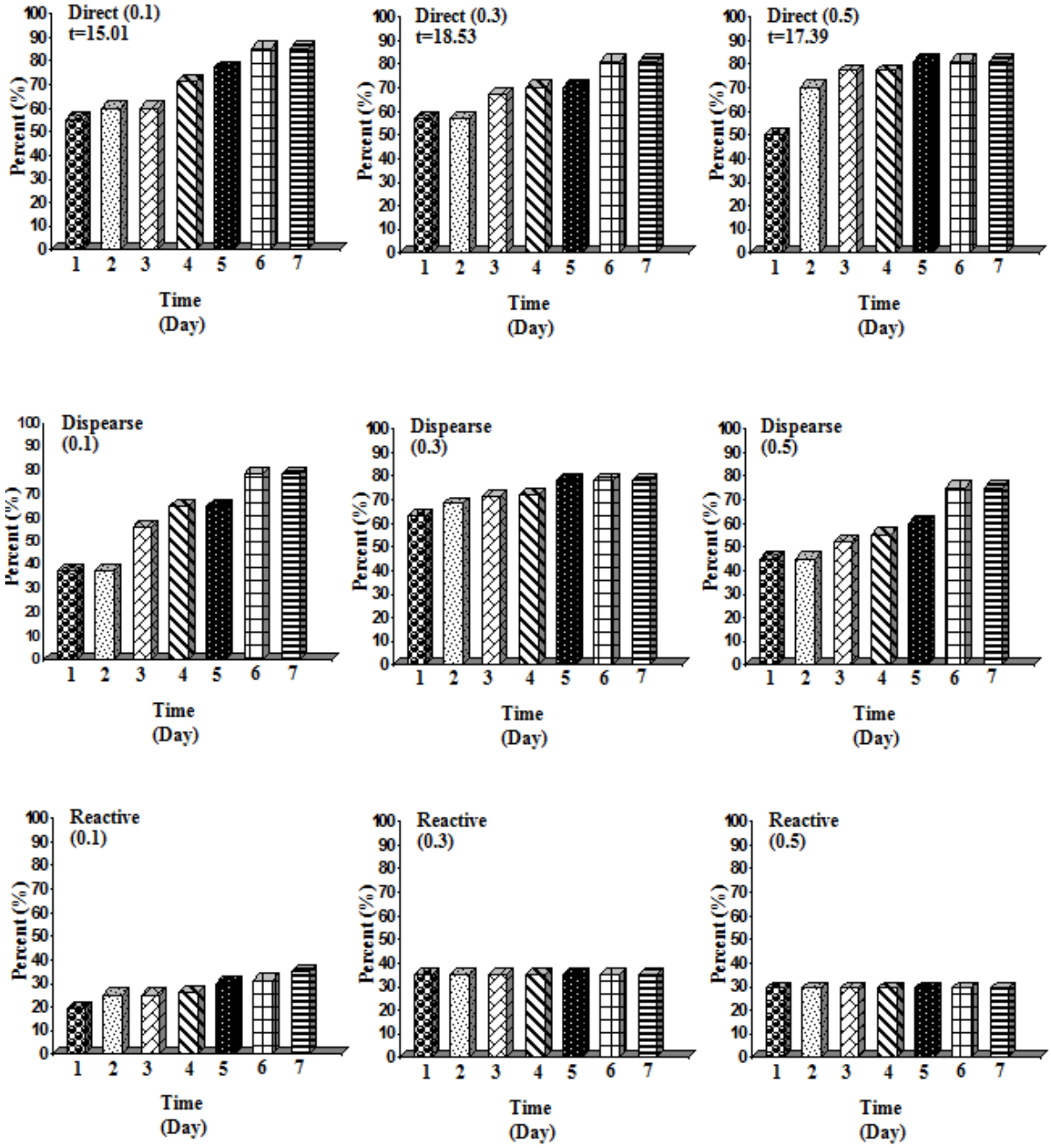


Figure (3): Color value of pigment during treatment by *Bacillus sp.*

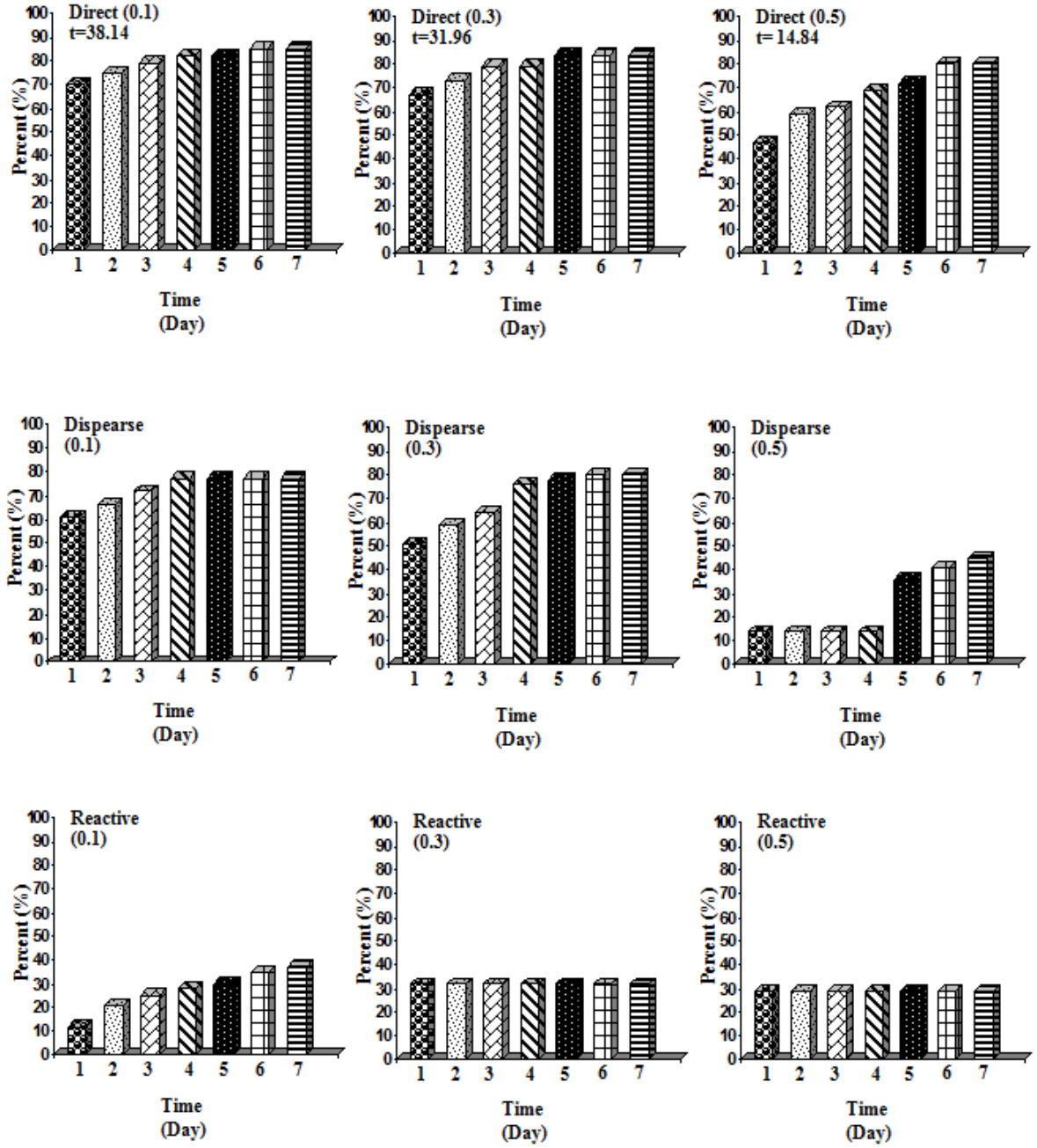


Figure (4): Color value of pigment during treatment by *Klebsella sp.*

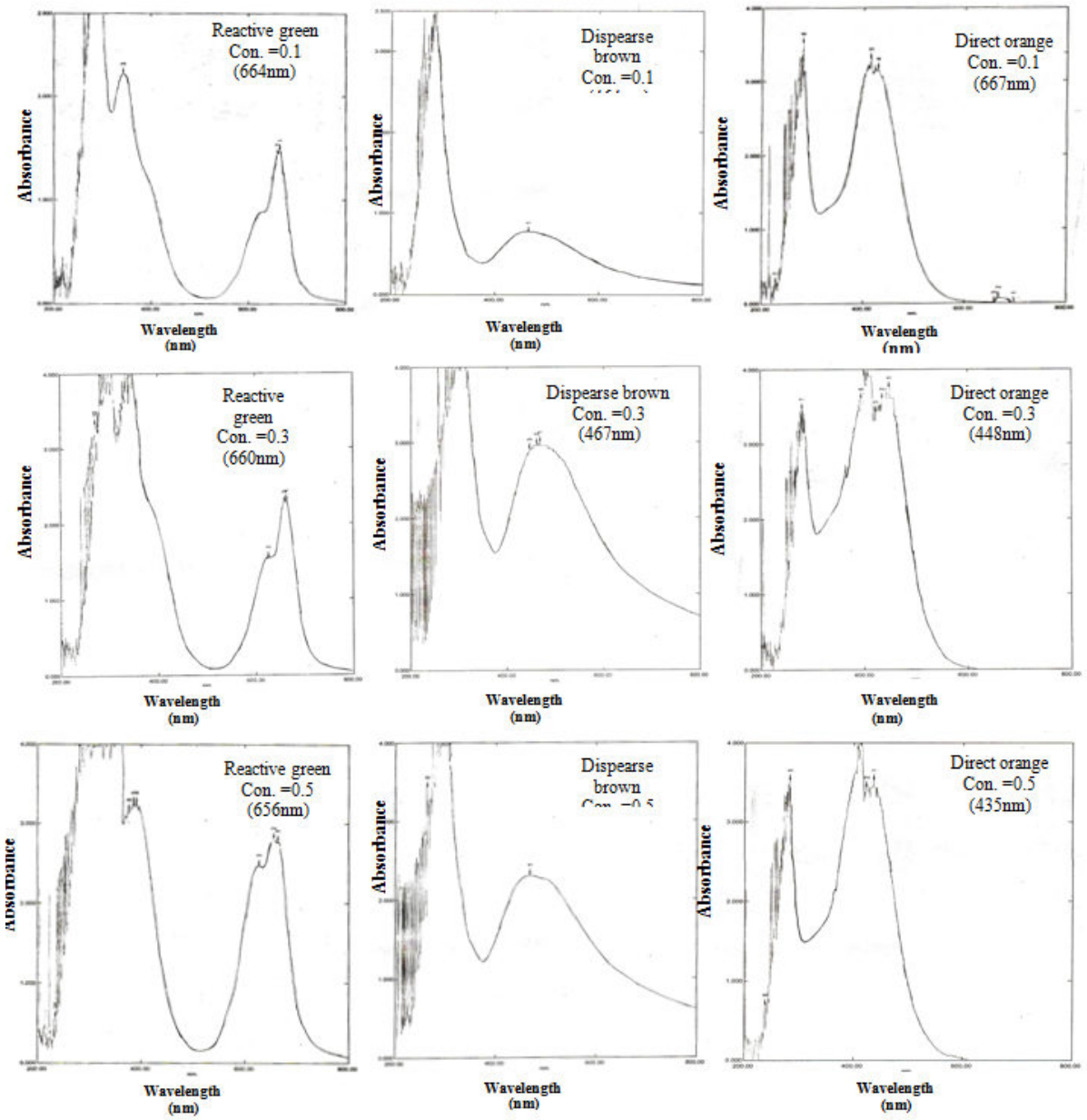


Figure (5): Spectrum peak pick report of pigment

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