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Biodecolorization and Biodegradation of Azo dyes by Some Bacterial isolates

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ABSTRACT: This study aims to isolate and optimize of bacterial strains having the ability to degrade and decolorize azo dyes produced in the final effluent of textile dying industries. In this regard, ten bacterial isolates were isolated from wastewater treatment plant(s) most of them subjected to the colored effluents resulted from dying houses. The ability of this bacterial isolates to use wide range of azo dyes as sole carbon source was determined. According to these screening tests, two bacterial isolates were selected as the most potent decolorizer for azo dyes and they were identified as *Comamonas acidovorans*-TM1and *Burkholderia cepacia*-TM5. Optimization process started with the addition of 1g/l yeast extract where the decolorization ability of the two strains increased sharply and according to this experiment the two azo dyes Acid orange 7 and Direct blue 75 were selected to complete the study. The effect of different conditional and chemical factors on the decolorization process of Acid orange 7 and Direct blue 75 by *Comamonas acidovorans*-TM1 and *Burkholderia cepacia*-TM5 was studied. These factors were in different pH, temperature, incubation period, inoculum sizes, carbon sources, nitrogen sources and different concentrations of yeast extract. This study recommended the application of the two most potent bacterial strains in the decolorization of the azo dyes generally and acid orange 7 and direct blue 75 specifically in the industrial effluents under all nutritional and environmental conditions in Egypt.

Keywords: Decolorization, Biodegradation, Azo dyes, *Comamonas acidovorans* and *Burkholderia cepacia*.

INTRODUCTION

Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and dwindling supply has made the treatment and reuse of industrial effluents an attractive option. One of the most important environmental pollution problems is the color in water courses, although some of this color is normally present and of "natural" origins (e.g. the color originates from the activity of some microorganisms in ponds), a considerable proportion, especially in the lower reaches of rivers draining large industrial conurbations, originates from industrial effluents. Some colored effluents are associated with the production and use of dyestuff.

Azo dyes, the largest chemical class of dyes with the greatest variety of colors, have been used extensively for textile, dyeing and paper painting (Carliell *et al*., 1995). These dyes cannot be easily degraded, while some are toxic to higher animals. Over 7×10^5 metric tons of synthetic dyes are produced worldwide every year for dyeing and printing and out of this about 5–10% are discharged with wastewater. The amount of dye lost depends on the class of dye applied; it varies from 2% loss while using basic dyes to about 50% loss in certain reactive sulfonated dyes. (Dafale *et al*., 2008).

Presence of the dyes in aqueous ecosystem diminishes the photosynthesis by impeding the light penetration into deeper layers thereby deteriorating water quality and lowering the gas solubility. Furthermore the dyes and /or their degradation products may be toxic to flora and fauna (Talarposhti *et al*., 2001). Azo dyes consist

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of a diazotized amine coupled to an amine or phenol, and contain one or more azo linkage. At least 300 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries. The effects of pH, temperature, type and concentration of respiration substrates and oxygen tension on the rate of biological reduction of a variety of azo dyes were investigated by (Wuhrmann *et al*, 1980; Deng *et al*., 2008).

Several combinations of treatment methods have been developed so far for effectively process cotton –textile wastewater; decolorization being among the main targets to achieve. Chemical coagulation flocculation techniques, usually combined with activated sludge treatment, have been among the most common processing methods mainly due to their application simplicity. However, the above methods require high amount of raw materials (coagulants) and also produce large amount of waste solids leading to the elevation of the total treatment cost. Advanced oxidation processes (Ozonation, UV/H_2O_2) are based on the generation of hydroxyl radicals, which are highly reactive oxidants. They are environmental friendly techniques since they produce no solid wastes. However, they don't cost effective high consumption of both energy and raw material (e.g. hydrogen peroxide). Activated carbon adsorption and nanofilteration techniques are able to remove dyes from wastewater. The main disadvantage of these methods though is the production of secondary waste stream (or waste solid) that requires further treatment or disposal.

The anaerobic reduction of azo dyes to simpler compounds has been well researched (Brown and Laboureur 1983a&b; Razo-Flores *et al*. 1997; Chinwetkitvanich *et al*., 2000). These, and other studies have all demonstrated the ability of anaerobic microbes and sludge to effectively reduce azo dyes to their intermediate structures, thus destroying the apparent color. Many of these intermediates are aromatic amines with constituent side groups. By reducing the dye compounds to their intermediates, the problem of aesthetic pollution is solved, but a larger and more deleterious problem may be created. Most azo dyes are non-toxic, but a higher percentage of their intermediates have been identified as carcinogens. Because of the toxic potential of many aromatic amines, further degradation of the dye compound is necessary if toxicity is to be eliminated or reduced (Levine, 1991 and HeFang *et al*., 2004).

In the light of presumptive evidence, this investigation was designated to fulfill the following objectives(1) Investigate the potential of bacterial isolates from textile industries wastewater and drains (textile effluent adapted bacteria) in some azo dye biodegradation.(2) Bioremediation of secondary metabolites of azo dye degradation (secondary aromatic amines) to environmental friendly non toxic form.

MATERIALS AND METHODS

1. Isolation and cultivation of the most efficient decolorizing bacteria.

Six activated sludge samples were obtained from three wastewater treatment plants in (Eldawakhliah in Almahalla Alkopra, Bassiun and Mehalet marhome) Egypt. All samples were transported to the laboratory and screened to obtain the dye decolorizing organisms.

All bacterial isolations were cultivated on Mineral salts Basal medium with the following composition (g/l): Na_2HPO_4, 2.13; KH_2PO_4, 1.3; NH_4Cl, 0.5; $MgSO_4$, 0.2; tab water up to 1 liter and 1ml of trace element solution per liter. The trace element solution had the following composition (g/l):MgSO4.7H2O, 7.12; $ZnSO_4.7H_2O$, 0.044; $MnSO_4.4H_2O$, 0.081; $CuSO_4.5H_2O$, 0.0782; $Na_2MoO_4.2H_2O$, 0.025; $FeSO_4.7H_2O$, 0.498; Boric acid 0.1+0.27ml of H_2SO_4. The final pH was adjusted at 7.0. The mineral salts medium was supplemented with 1(g/l) yeast extract. The activated sludge samples (2.5 ml) were inoculated into 250 ml flasks containing 50 ml mineral salts medium and dye (prepared using a mixture of 9 types of dyes. All dyes were mixed together to get stock solution of mixture of dyes 0.9 (g/l) (0.1g/l of each dye). The stock solution was supplemented to basal salt medium to get final concentration of 0.1(g/l). Each flask contained 50 ml of sterile mineral salts liquid medium mixed with azo dyes mixture dispensed with one gram of yeast extract. Each flask was inoculated with 2.5 ml of activated sludge, all were incubated on rotary incubator at 150 rpm for 24 h. at 30^{o}C. Similar flasks were prepared and inoculated with 1 ml of the content of the flasks of

the first group, these were incubated for 24 h. This step was repeated for three times throughout 72 h. The last (third) group of flasks was incubated for five days. These flasks were used to isolate the aimed microorganisms by streaking mineral salts agar medium containing the same ingredients of the previous broth medium plus agar and 100 ppm of azo dyes mixture. Separate colonies of the predominant types of microorganisms were purified by re-streaking on the same medium. The purified isolates were examined microscopically to check their purity. Obtained pure cultures were maintained on nutrient agar at 4°C (in refrigerator) (Hayase *et al*., 2000; Kumar *et al*., 2005 and Chen 2002).

Ten morphologically different isolates were obtained from the previous step and studied for colony morphology, cell morphology, gram reaction, KOH reaction and catalase reaction. In order to select the effective strains, the ability to utilize different groups of dyes as the sole carbon source was screened.

2. Screening program (testes) to select the most potent organisms:

First, screening to test the ability of the purified isolates to utilize different groups of dyes as the sole carbon source. Screening to test the ability of isolated organisms to utilize direct blue75, direct blue71, reactive blue 194 and direct red 89 as the sole carbon source was carried out in Mineral salts Basal medium used in isolation. Yeast extract was replaced by 0.1 g/l individual dye. Organisms were selected on the basis of their ability to grow and reduce color under these conditions. Colonies of an overnight growth were suspended in normal saline to obtain optical density of 0.6 at wavelength 610 nm. One milliliter of cell suspension was used to inoculate hundred ml bottles capacity containing 25 ml Mineral salts Basal medium supplemented with 0.1g/l individual dye, bottles were incubated for seven days at 30°C. Second, screening to ensure the ability of selected isolates to utilize different groups of dyes as the sole carbon source.

Six isolates were obtained from the previous step. In order to ensure the ability of the selected six isolates to utilize azo dyes as the sole carbon source, therefore the previous step was rebated.

3. Identification of the most potent of azo dyes-decolorizing bacterial isolates:-

The two most potent bacterial isolates TM1 & TM5 having highest decolorization potentiality were selected to complete the study. They were identified on the basis of cell shape, cell arrangement, relation to oxygen, nutritional characteristics, physiological and biochemical characteristics as *Comamonas acidovorans* and *Burkholderia cepacia.* The two strains *Comamonas acidovorans* and *Burkholderia cepacia* were re-examined with another group of azo dye (reactive red, mordant brown, acid orange and acid black).

4. Analytical methods:

A. Decolorization assay:-

All samples (2 ml) were centrifuged at 11,000 rpm for 10 min. The supernatant was read at absorbance with maximum (λ max) values using thermo spectronic- Genesys 20 spectrophotometer. The uninoculated dye free medium was used as blank. All dyes were prepared in duplicate and compared with inoculated controls. To ensure that the pH shifts in the dyes solutions did not influence decolorization potentiality, the visible absorption spectra were recorded between pH 3 and 9 and pH did not affect spectrum. The efficiency of color removal was expressed as the percentage of the decolorized dye concentration to that of the initial one, i.e. the difference between the initial dye concentration, Dye (i), and the residual dye concentration, Dye(r), of the sample:

$$\text{Color removal (\%)} = \frac{\text{Dye (i)} - \text{Dye(r)}}{\text{Dye (i)}}$$

B. Determination of total protein

The total protein determination was made according to method of Lowry *et al*., (1951) using serum albumin as a standard protein.

C. Determination of biochemical oxygen demand (BOD).

BOD determination was made according to method of standard methods for examination of water and waste water 20Th edition.

D. Determination of chemical oxygen demand (COD).

COD determination was made according to closed reflux, colorimetric method standard methods for examination of water and waste water 20Th edition.

E. UV- visible, infra red and HPLC analysis.

Metabolites produced during biodegradation of acid orange 7 , direct blue 75 and their mixture were extracted with equal volumes of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness in rotary evaporator then dissolved in ethanol and used for all analysis Kalyani, *et al*., (2008).

The decolorization was monitored using UV–vis spectroscopy analysis (Hitachi U 2800). The active groups of degradation metabolites were monitored by using FT-IR spectroscopy Analysis using (Jasco, FT-IR 460 PLUS- Japan).

HPLC analysis was carried out on a Cecil model Adept CE 4900 chromatograph equipped with a Cecil model CE 4200 UV detector, an oven column model CE 4601, and a lichrosorb C18 column with a 4.6 mm inside diameter and 25 cm height. A mobile phase composed of 50% methanol, 0.3% H_3PO_4, and 49.7% water was used at a flow rate of 0.5 ml min^{-1}. The elutes were monitored by the UV absorption at 300 nm **(Asad, *et al*., 2007)**. In all cases parent azo dyes acid orange 7 and direct blue 75 were used as a control.

5. Optimization of decolorization ability for the selected isolates

a. Addition of yeast extract:

One g/l yeast extract was supplemented to the mineral salt medium used in screening experiments in a trial to support growth and increase the degradation ability of the selected bacterial isolates. The experiment proceeded in triplicates at pH 7 and incubation temperature 30 °C in bottle contain 25 ml medium.

6. Decolorization under different culture conditions:-

The effect of various culture conditions such as pH, temperature, inoculum size and incubation period on decolorization of acid orange 7 and direct blue 75 by *Com. Acidovorans*-TM1 and *Bur. Cepacia*-TM5 was examined.

a. Effect of pH on the decolorization of azo dyes acid orange7 and direct blue 75 by *Com. acidovorans* -TM1 and *Bur. cepacia*-TM5.

Colonies of an overnight growth were suspended in normal saline solution to obtain optical density of 0.6 at 610 nm wavelength. One milliliter of cell suspension was used to inoculate hundred ml bottles capacity containing 25 ml Mineral Salts Basal Medium supplemented with 0.1g/l individual dye, and 1 g/l yeast extract. The medium was adjusted to pH 4, 6, 7, 8 and 9 with (1N) hydrochloric acid and (1N) sodium hydroxide. Bottles were incubated for seven days at 30 °C.

b.Effect of different incubation temperatures on the decolorization of azo dyes acid orange 7 and direct blue75 by *Com. acidovorans* TM1 and *Bur. cepacia* TM5.

The experiment was carried out in hundred ml bottles capacity containing 25 ml Mineral Salts Basal Medium supplemented with 0.1g/l individual dye, and 1 g/l yeast extract. The medium was adjusted at pH 8 and each bottle was inoculated with predetermined equal cell density for the two strains. Bottles were divided to be incubated at different temperatures 10, 25, 30, 35 and 40°C.

c. Effect of different incubation periods on the decolorization of azo dyes acid orange 7 and direct blue75 by *Com. acidovorans* -TM1 and *Bur. cepacia*- TM5. This experiment was carried out in order to investigate the effect of different incubation periods on the decolorization process of the two azo dyes by *Com. acidovorans*-TM1 and *Bur. cepacia* -TM5. The two strains were allowed to grow on the two azo dyes under the optimum conditions resulted from the previous experiments and incubated for 6, 12, 24, 48, 72, 120 and 168 hours respectively. At the end of each incubation period azo dyes decolorization (%) and protein content were assayed.

d. Effect of different inoculum sizes on the decolorization of azo dyes acid orange

7 and direct blue75 by *Com. acidovorans* -TM1 and *Bur. cepacia* -TM5.

Different inocula sizes of heavy cell suspension of the two bacterial isolates *Com. acidovorans*- TM1 and *Bur. cepacia*- TM5 (prepared by washing each slant by 20 ml of sterile saline solution under aseptic conditions and optical density was adjusted to obtain optical density of 0.6 at 610 nm wavelength) were used. The following inocula sizes were applied via 0.2, 0.5, 1, 2 and 3ml per flask. All other optimal culture conditions were taken into consideration. At the end of incubation period azo dyes biodegradation was determined for each flask as previously mentioned.

e. Effect of different carbon sources on the decolorization of azo dyes acid orange 7 and

direct blue75 by *Com. acidovorans* -TM1 and *Bur. cepacia* -TM5.

Different carbon sources were introduced into the two azo dyes mineral salts media at an equimolecular level located at 0.5 g /l. parallel experiment was made without sugar as a control. The carbon sources were represented by sucrose, glucose, maltose, starch, dextrin, fructose, mannitol and lactose. In all cases, other previously mentioned optimal conditions were taken into consideration.

f. Effect of different nitrogen sources on the decolorization of azo dyes acid orange 7 and direct blue75 by *Com. acidovorans* -TM1 and *Bur. cepacia* -TM5.

Biodegradation mineral salts media were supplemented with different nitrogen sources with equivalent amount of nitrogen that present in 0.5 g/l NH_4Cl. The applied nitrogen sources were KNO_3, $NaNO_3$, NH_4Cl, NH_4NO_3, $(NH4)_2NO_3$, Amm.SO_4, Amm.oxalat, Amm. molybdenat and peptone. All other optimal factors were carried out as previously mentioned. The azo dyes biodegradation was assayed for each separate nitrogen source as previously mentioned.

g. Effect of different Yeast extract concentrations on the decolorization of azo dyes acid orange 7 and direct blue75 by *Com. acidovorans*- TM1 and *Bur. cepacia* -TM5.

This test was performed to investigate the effect of different concentrations of yeast extract on the azo dyes biodegradation by *Com. acidovorans*-TM1 and *Bur. cepacia* -TM5. This experiment was carried out by applying different concentrations of yeast extract (0.1, 0.25, 0.5, 1 and 2 g/l). All concentrations were proceeded as previously mentioned optimal conditions.

h. Effect of different incubation types (oxygen concentrations) on the decolorization of azo dyes acid orange 7 and direct blue75 by *Com. acidovorans*- TM1 and *Bur. cepacia*- TM5.

This experiment was carried out in order to investigate the effect of incubation conditions on the biodegradation of azo dyes acid orange 7 and direct blue 75 by *Com. acidovorans*- TM1 and *Bur. cepacia*-TM5. This was carried out by incubating the flasks containing the mineral salts media with each azo dye in addition to the optimal sources of carbon and nitrogen resulted from the previous optimization in four ways: static incubation, shaking incubation at 150 rpm, anaerobic and aerobic-anaerobic incubation. At the end of incubation period azo dyes decolorization were assayed as previously mentioned.

i. Effect of consortium as *Com. acidovorans*-TM1+*Bur. cepacia*.-TM5 on the decolorization of azo dyes acid orange 7 and direct blue75:-

This experiment was carried out in order to investigate the effect of consortium of *Com. acidovorans*-TM1 +*Bur. cepacia*-TM5 on enhancing biodegradation of azo dyes acid orange 7 and direct blue 75. This was carried out by inoculating the two strains *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5 into the biodegradation media and incubation under all the optimal nutritional and environmental conditions. At the end of incubation period, the two azo dyes biodegradation was assayed as previously mentioned.

RASULTS

I. Isolation, purification and screening test for azo dye decolorizing bacteria.

Ten bacterial isolates were isolated from aeration tank and input effluent of three wastewater treatment plants (Eldwakhlia-Elmahalla Alkopra, Mehalet Marhome and Bassiun) Egypt. Table (1). Experiments on growth and dye removal ability of the pure isolates growing on mineral salts agar medium containing 100 ppm dye revealed that isolates TM1&TM2 grew rapidly with more visible colonies than other isolates. Ten bacterial isolates were confirmatory tested for the ability to utilize direct blue75, direct blue71, reactive blue 194 and direct red 89 as the sole carbon source in mineral salts basal medium used for isolation.

Table (1): Code numbers, isolates locality, cell morphology and gram, catalase reaction of the ten bacterial isolates used for azo dye biodegradation process.

No	Code	Isolation locality	Cell morphology	Gram reaction	KOH reaction	Catalase reaction
1	TM1	Aeration tank of Eldwakhliah WWTP	Rods	-	+	+
2	MKD3	Industrial input effluent of Eldwakhliah WWTP	Cocci	-	+	+
3	MKD5	Industrial input effluent of Eldwakhliah WWTP	Rods	-	+	±
4	MKD7	Industrial input effluent of Eldwakhliah WWTP	Rods	-	+	+
5	TM5	Aeration tank of Eldwakhliah WWTP	Rods	-	+	+
6	MM5	Aeration tank of Mahalet marhome WWTP	Rods	-	+	+
7	MM7	Aeration tank of Mahalet marhome WWTP	Rods	-	+	+
8	BO 3	Aeration tank of BassionWWTP	Rods	-	+	+
9	TM7	Aeration tank of Eldwakhliah WWTP	Rods	-	+	+
10	BO 4	Aeration tank of Bassiun WWTP	Rods	-	+	+

Data presented graphically in fig. (1) Showed different capabilities of the ten bacterial isolates concerning this respect, this can be clearly detected by reviewing protein content values and their relation to decolorization percentage concerning different dyes.

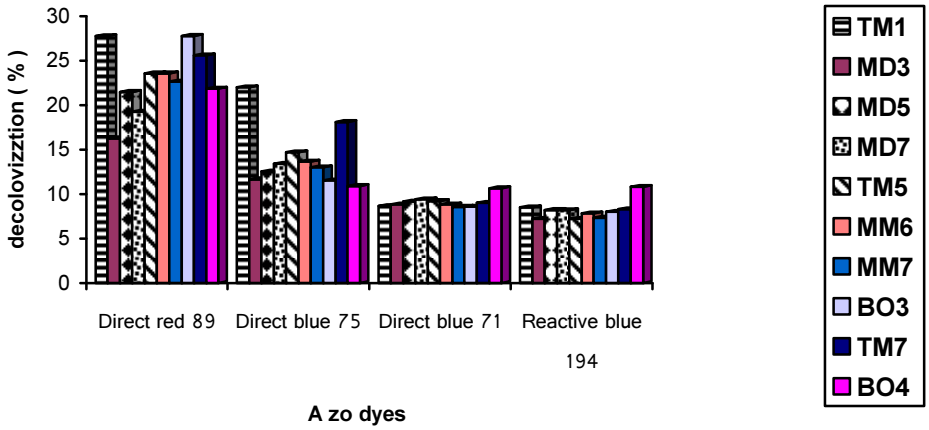

Fig. 1: First Survey to test the ability of bacterial isolates to utilize azo dyes as sole carbon source.

Concerning the second survey, data revealed that direct red 89 and direct blue 75 gave the most promising results among all the four azo dyes under investigation. It is worthy to mention that reactive blue 194 failed to fulfill carbon source requirement for all ten bacterial isolates. With regard to the ten purified bacterial isolates under investigation, six isolates were recorded to give highest decolorization percentage and protein content. Obtained six bacterial isolates (TM1, MKD7, TM5, BO3, TM7 and BO4) were tested to grow on the same medium containing higher concentrations (500ppm).

In the second screening, values of decolorization percentage and protein content were generally decreased, and the best results were attained in case of the isolates TM1& TM5 so this isolates were selected to complete the study (fig. 2).

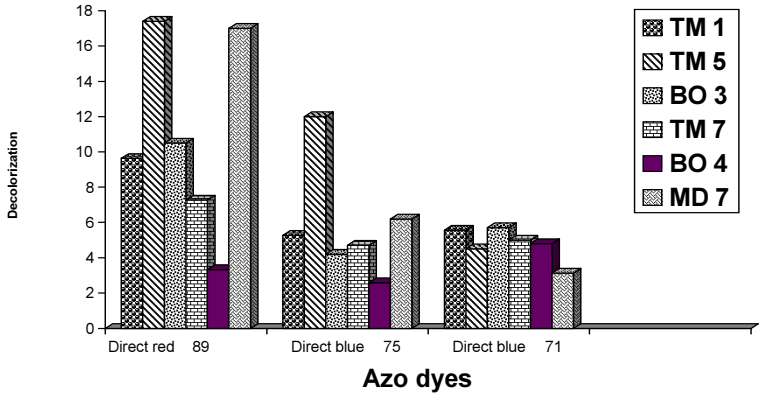

Fig. 2: Second confirmatory survey by the selected six isolates on the same azo dyes.

In order to test the ability of TM1& TM5 to deal with wide range of azo dyes and the ability of the two isolates to utilize another members of azo dyes that were tested. The new azo dyes were reactive red, mordant brown, acid yellow, acid orange 7 and acid black. Data presented graphically in (fig.3) showed that there was no great differences between decolorization percentage recorded after one day and that which was recorded after seven days. Reactive red gave the highest degradation percentage and highest protein content by the two isolates.

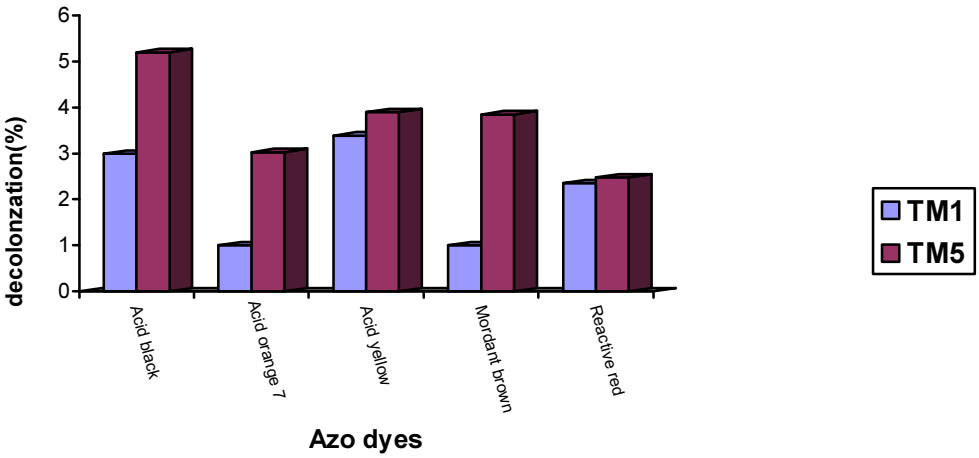

Fig.3: Determination ability of TM1& TM5 to deal with wide range of azo dyes.

After confirmatory screening experiments were performed, mineral salts media was supplemented with 1 g/l yeast extract to enhance decolorization process. Experiment was proceeded using seven azo dyes, Direct red 89, Direct blue 75, Acid orange 7, Acid yellow, acid black, Reactive red and Mordant brown. The decolorization percentage increased sharply with all dyes but the azo dyes direct blue 75 and Acid orange 7 showed the highest results, so they were selected to complete the study. The lowest result of decolorization process was recorded for Mordant brown and Acid yellow. The lowest protein content was also recorded for isolate TM1 in decolorization of acid yellow and mordant brown. The results are presented graphically in fig. (4).

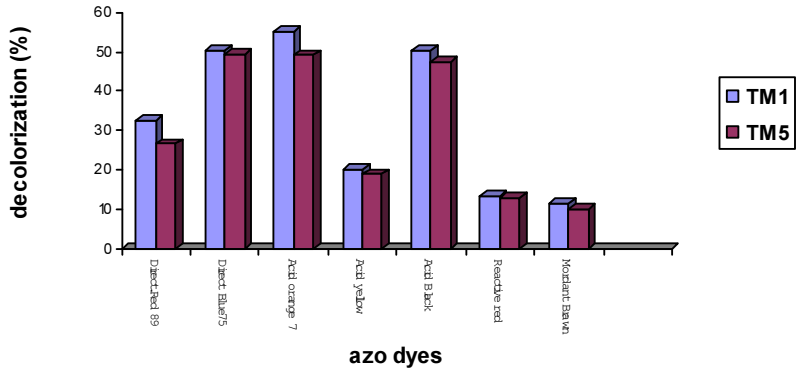

Fig. 4: Decolorization of azo dyes by TM1 and TM5 in salts medium supplemented with yeast extract 1(g/l).

III. Parameters controlling decolorization process of direct blue 75 & acid orange7 by *Com. acidovorans*-TM1and *Bur. cepacia*-TM5.

1. Effect of different initial pH values on the decolorization of acid orange7 and direct blue 75 after 24h. and 168h.:

pH value affects not only the decolorization capability, but also the color stability at pH 9 after autoclaving, where color reduction occurred at pH 9 after autoclaving in case of both the two dyes under study, but no effect in case of this particular pH on the color without autoclaving. The effect of different initial pH values (4, 6, 7, 8 and 9) on decolorization of direct blue 75 & acid orange 7 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5 was recorded after 24h. and 168h. as presented graphically in (fig.5a,b&c).The optimal pH for decolorization by both strains was pH 8. Decolorization percentage was decreased when solution pH was shifted toward the acidic side. This seems to indicate that neutral and slightly basic pH values would be more favorable for decolorization process of direct blue 75 & acid orange 7 by *Com. acidovorans*-TM1 and *Bur. Cepacia*-TM5. Results recorded for decolorization of acid orange 7 by *Com. acidovorans*-TM1 showed that decolorization percentage was decreased with the decrease in pH after 24h. and 168h., and the optimum pH was 8 but the optimum pH of growth was 7. On the other hand results recorded for decolorization of acid orange 7 by *Bur. cepacia*-TM5. Showed also decrease in color removal percentage with pH decrease but this decrease in color removal wasn't sharp and optimum growth was at pH 8. The result recorded for decolorization of direct blue 75 by *Com. acidovorans*-TM1 showed that decolorization percentage was decreased with the decrease in pH after 24h. and 168h., the optimum pH was 8 while the optimum pH of growth was 7 as in the decolorization of acid orange by the same strain. On the other side decolorization of direct blue 75 by *Bur. Cepacia*-TM5 showed also decrease in color removal percentage as pH decrease but this decrease in color removal wasn't sharp and optimum growth was at pH 8 as in the decolorization process in case of acid orange by the same strain too.

2- Effect of different incubation temperatures on the decolorization process after 24h. and 168h.

The experiments were performed at different temperatures ranged from 10°C -40°C the decolorization increased as the temperature increased (Fig.6 a,b&c). But color removal ability of both two strains decreased sharply at temperature 40◦C and further increase in temperature resulted in marginal reduction in decolorization activity of the two bacterial strains. The optimal incubation temperature for decolorization process by the two strains was 35°C. Decolorization percentage was decreased as temperature was decreased lower than 35°C or increased over this particular value. The growth of the two strains (protein content) also followed the

same behavior of decolorization, so the optimum growth of the two strains was attained at 35°C. There was no great difference between the decolorization of acid orange 7 by *Com. acidovorans*-TM1 at temperature30°C &35°C. Decolorization of direct blue 75 at temperature 10°C and 40°C was nearly the same by *Com. acidovorans*-TM or *Bur. Cepacia*-TM5. But the result recorded for decolorization of acid orange by *Com. acidovorans*-TM1 or *Bur. cepacia*-TM5 showed that the color removal at temperature 10°C was duplicated at 40°C after 24h. and 168h.

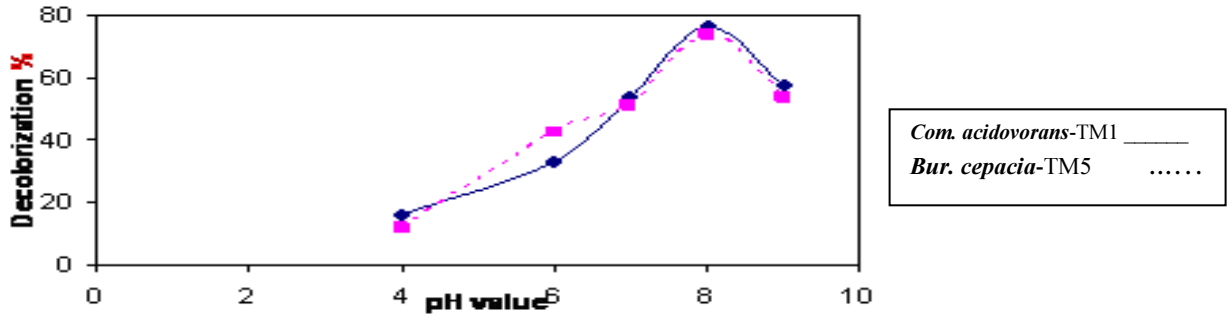

Fig 5A: Effect of pH value on decolorization of acid orange 7 by *Com. acidovorans*-TM1 and *Bur. Cepacia* TM5.

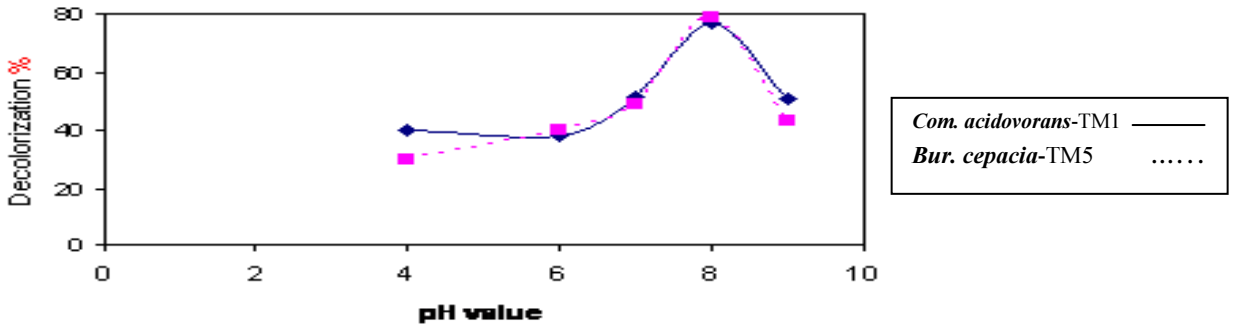

Fig. 5B: Effect of pH value on decolorization of acid orange 7 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

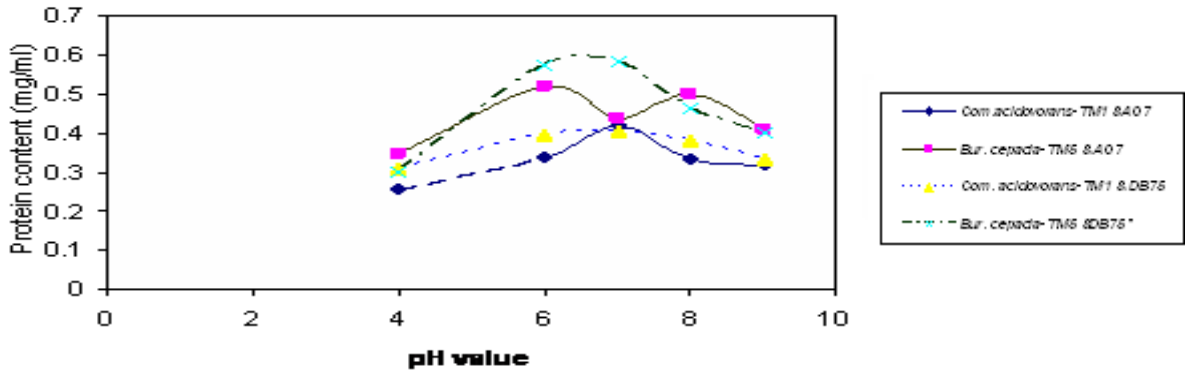

Fig. 5C: Effect of pH value on protein content of *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5 in decolorization process of acid orange7 and direct blue 75

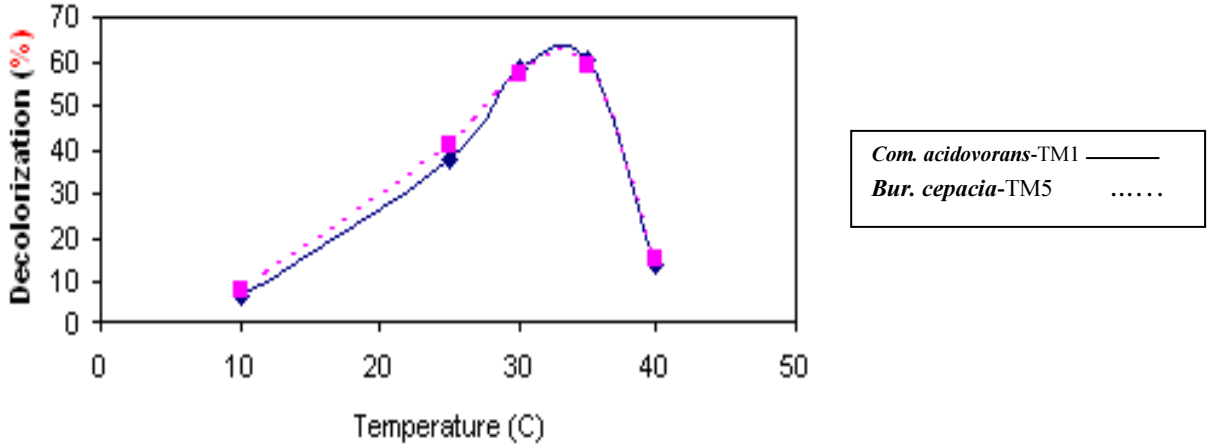

Fig. 6A: Effect of incubation temperature on decolorization process of acid orange 7 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

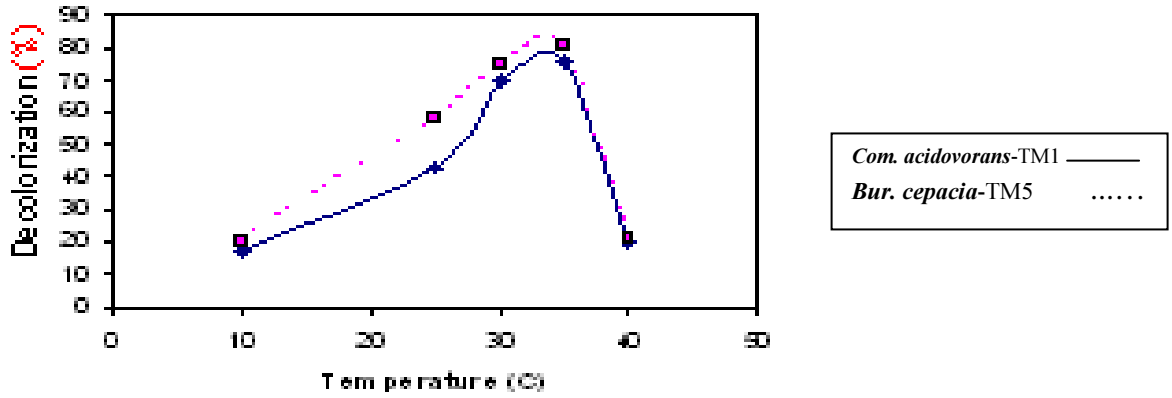

Fig. 6B: Effect of incubation temperature on decolorization process of direct blue 75 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

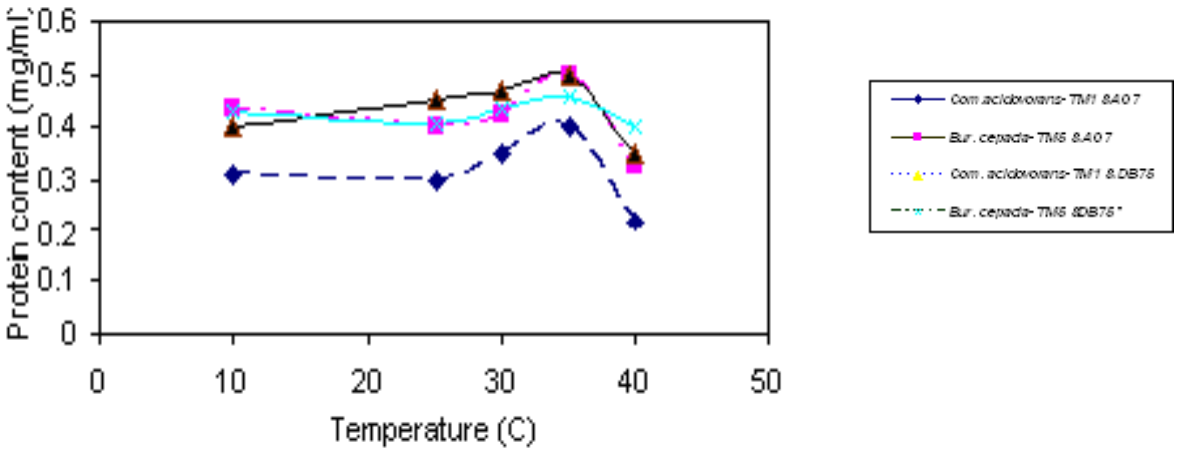

Fig. 6C: Relation between protein content and incubation temperature in decolorization process of acid orange 7 & direct blue 75 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

3-Effect of different incubation periods on the decolorization of Acid orange7 & direct blue 75 by *Com. acidovorans*-TM1 and *Bur. Cepacia*-TM5:

Decolorization of AO 7& direct blue 75 by both bacterial strains was recorded at different intervals of time under the optimum pH (pH8) and optimum temperature (35°C) (Fig.7a,b&c). The results recorded for decolorization of acid orange 7 by *Com. acidovorans*-TM1 showed that there were slight changes in color removal after six hours and 12 hours of incubation but at 24 hours the decolorization percentage increased sharply to achieve more than 60 % of the total decolorization percentage occurred at the end of incubation. The progress in color removal after 24 hours became slow and the difference between the result recorded at 120 hours and 168 hours was only 1.3 % color removal. The growth represented by protein content increased gradually not in sharp way as in decolorization process. On the other hand the decolorization percentage recorded by *Bur. cepacia*-TM5 with acid orange 7 showed detectable change in color removal percentage after 6 hours and 12 hours when compared with the change in color removal by *Com. acidovorans*-TM1 at the same interval of time. Protein content measured after 6 and 12 hours for *Bur. cepacia*-TM5 also was higher than that achieved in the same intervals by *Com. acidovorans*-TM1. The decolorization of direct blue 75 by both the two strains also followed the same pathway of the decolorization of acid orange7.

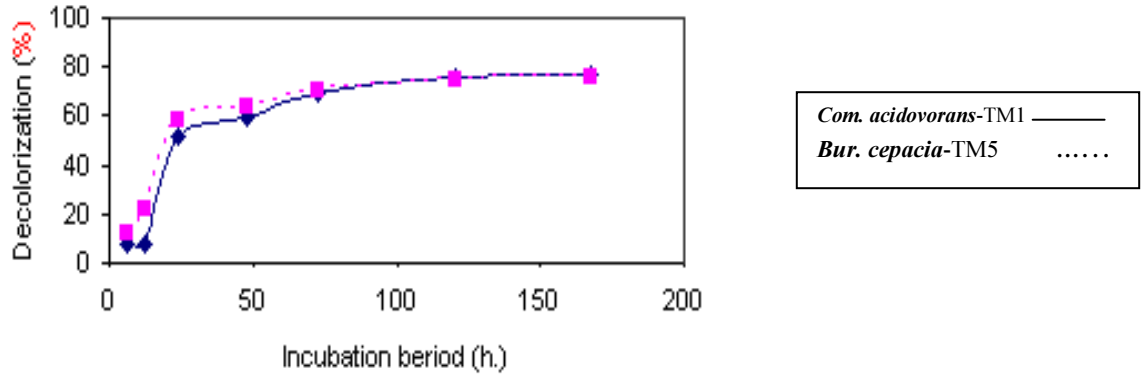

Fig. 7A: Effect of incubation period on decolorization process of acid orange 7 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

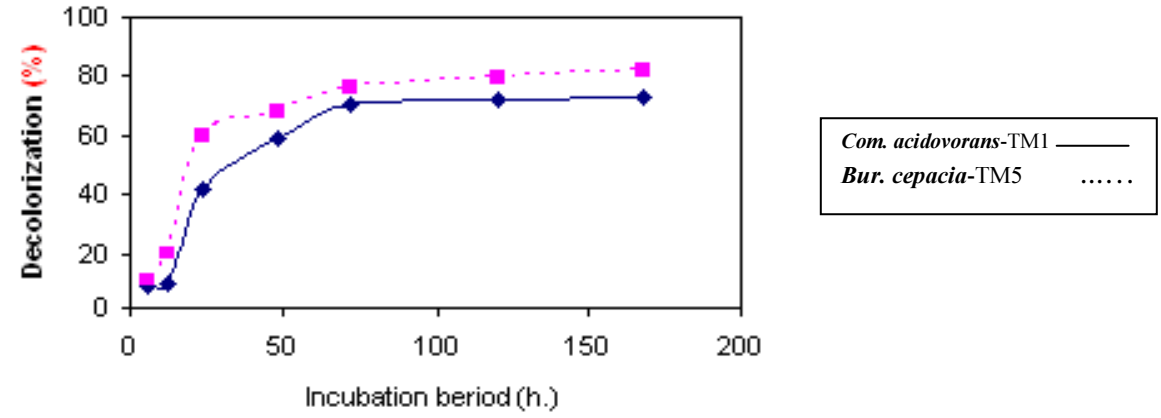

Fig. 7B: Effect of incubation period on decolorization process of direct blue75 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

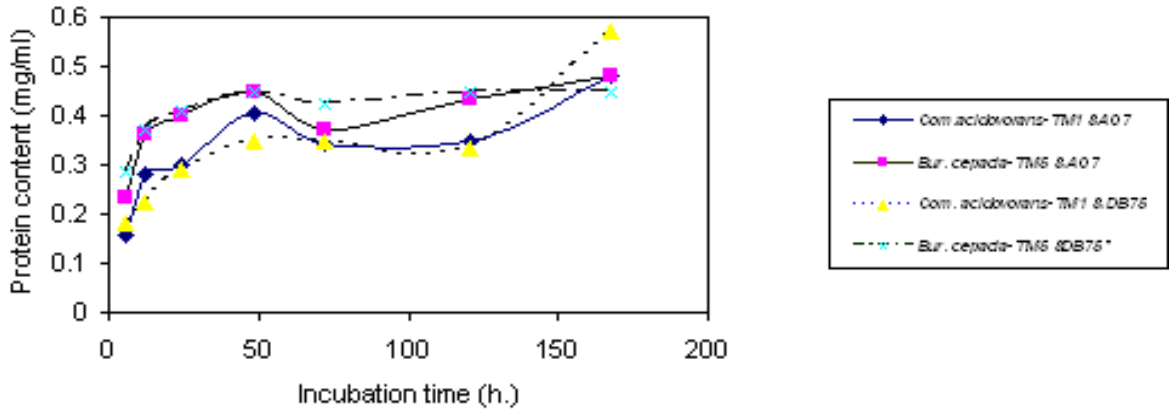

Fig.7C: Relation between protein content and incubation period in decolorization process of acid orange 7 & direct blue 75 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

4- Effect of different inocula sizes on the decolorization process of acid orange7 & direct blue 75 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5:

Results presented graphically in fig.(8 a,b&c) indicated that there was no clear relation can be detected between the inoculum size and the decolorization percentage after 24 or 120 hours for example, decolorization of acid orange 7 by *Com. acidovorans*-TM1 after 24 hours did not show detectable difference at inoculum size 1ml, 2ml, and 3ml respectively but decolorization percentage was decreased at inoculum size 0.2ml and 0.5ml and the decolorization at 0.2ml inoculum size was better than that at 0.5 ml , although the color removal achieved after 120 hours using 0.5 ml inoculum size was the highest decolorization percentage recorded in this experiment.

The decolorization of acid orange 7 by *Bur. cepacia*-TM5 after 24 hours showed that the best decolorization percentage was at inoculum size (2ml) and the lowest value was for the inoculum size (0.2ml), but results recorded after 12 hours showed that inoculum size (3ml) gave the highest color removal, other inocula sizes gave nearly the same value of decolorization except for the inoculum size (1ml) where recorded the lowest value of decolorization percentage.

As we discussed earlier there was no clear relation can be detected between the inoculum size and the decolorization percentage after 24 hours or 120 hours, this situation was additionally proved by the result recorded for the decolorization of direct blue 75 by Com. *acidovorans*-TM1 and *Bur. cepacia*-TM5.

Decolorization of direct blue 75 by *Com.acidovorans*-TM1 after 24 hours showed detectable difference at different inocula sizes. Decolorization using inoculum size (0.5) was the best, followed by decolorization% using inoculum size(0.2) and the lower result was recorded for the inoculum size(3ml). But decolorization of direct blue 75 by *Com. acidovorans*-TM1 after 120 hours indicated that the highest color removal was recorded at 0.2 ml inoculum size.

The relationship between decolorization percentage and inoculum size is still absent in the decolorization of direct blue 75 by *Bur. cepacia*-TM5 where it was found that the best decolorization after 24 hours was recorded for the inoculum size (2ml) but the best result recorded after 120 hours was for the inoculum size (1ml).

Protein content recorded for the decolorization of acid orange 7 & direct blue 75 by *Com. acidovorans*-TM and *Bur. cepacia*-TM5 had the same behavior, so we can't establish clear relation between protein content and inoculum size or decolorization percent but in general, the values of protein content of the two strains at any inoculum size with direct blue 75 were higher than those of the two strains at any inoculum size with acid orange 7.

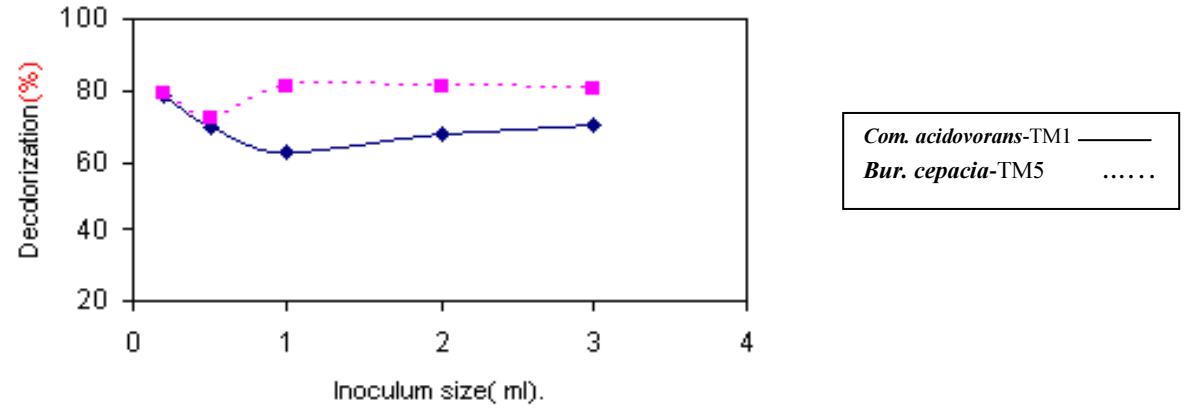

Fig. 8A: Effect of inoculum size on decolorization process of acid orange 7 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

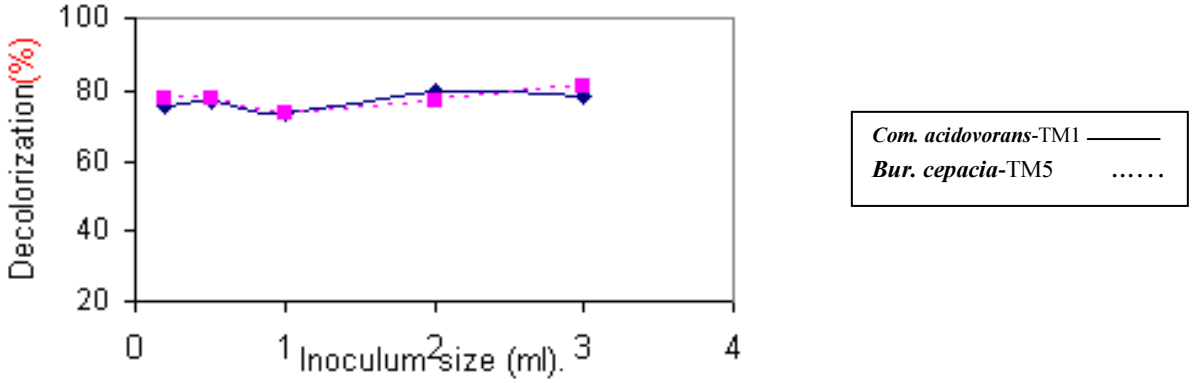

Fig. 8B: Effect of inoculum size on decolorization process of direct blue75 by *Com. acidovorans*-TM1and *Bur. cepacia*-TM5.

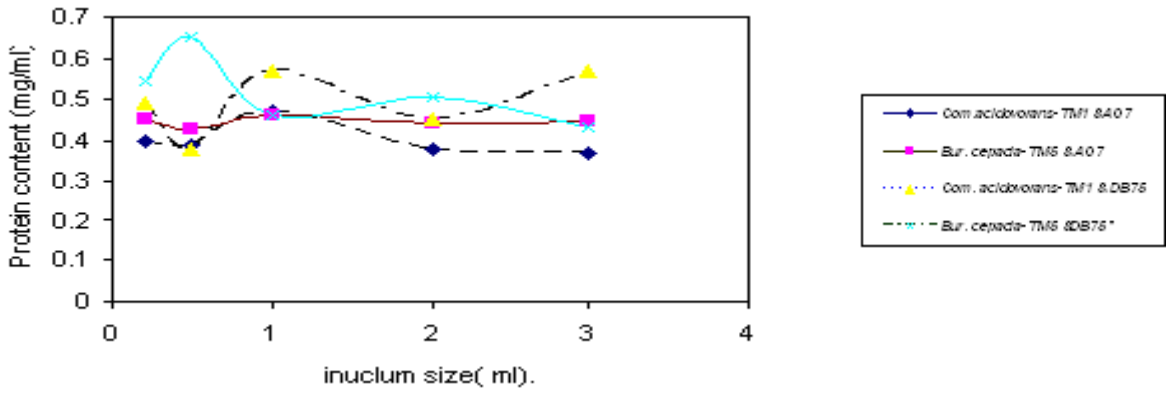

Fig. 8C: Relation between protein content and inoculum size in decolorization process of acid orange 7 & direct blue 75 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

5- Effect of different carbon sources on the decolorization of acid orange7 & direct blue 75 by *Com. acidovorans*-TM1 and *Bur. Cepacia*-TM5:

Textile industrial effluent that is deficient in carbon content and biodegradation without any extra carbon source was very difficult. Therefore, different co-substrates such as sucrose, glucose, maltose, starch, dextrin, fructose, mannitol and lactose (0.5 g/l), were supplemented in the medium and decolorization of all the two dyes (acid orange7 & direct blue 75) was studied individually by *Com. acidovorans*-TM1 and *Bur. Cepacia*-TM5. Results presented graphically in Fig.(9 a&b), revealed that the decolorization of acid orange7 & direct blue 75 was absolutely dependent on the presence of carbon source. Results recorded for the decolorization of acid orange 7 by *Com. acidovorans*-TM1 showed that the best decolorization percentage was recorded in presence of starch as a carbon source, followed by fructose then maltose. Dextrin and mannitol recorded the lowest decolorization percentage. The highest protein content was achieved with glucose and fructose. The lowest protein content was recorded by adding mannitol as a carbon source as the same in decolorization process. Decolorization of acid orange 7 by *Bur. cepacia*-TM5 showed that the best decolorization percentage was recorded in the presence of starch as a carbon source, followed by maltose then glucose. Fructose and lactose recorded the lowest decolorization percentage. The highest protein content was achieved with glucose and fructose as in the decolorization of the same dye by *Com. acidovorans*-TM1. The lowest protein content was recorded when mannitol was used as a carbon source in decolorization process.

Starch also was the best co-substrate by using a carbon source in decolorization of direct blue 75 by *Com. acidovorans*-TM1 & *Bur. cepacia*-TM5 followed by glucose then fructose in case of decolorization by *Com. acidovorans*-TM1 and glucose followed by fructose in case of decolorization by *Bur. cepacia*-TM5. The highest protein content was achieved with fructose and dextrin in case of decolorization by *Com. acidovorans*-TM1 and fructose then maltose in case of decolorization by *Bur. cepacia*-TM5. The lowest protein content was recorded by adding sucrose was used as a carbon source in decolorization of direct blue 75 by each of the two strains respectively.

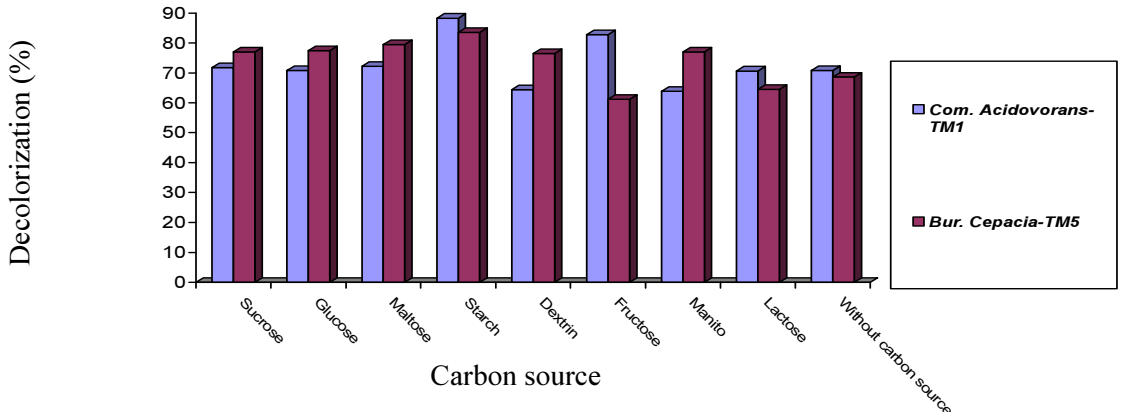

Fig. 9A: Effect of different carbon sources on decolorization of acid orange by *Com. acidovorans* -TM1 and *Bur. cepacia*-TM5.

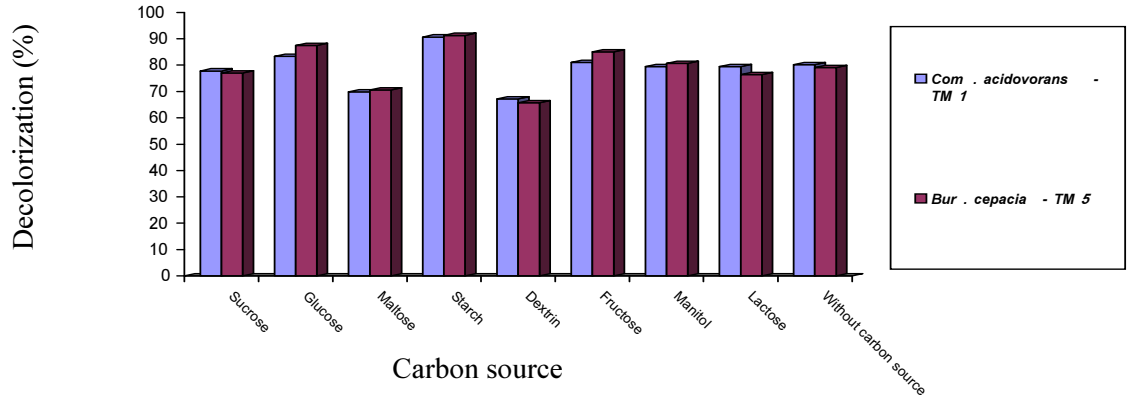

Fig. 9B: Effect of different carbon source on decolorization of direct blue 75 by *Com. acidovorans*-TM1 and *Bur. cepacia* -TM5.

6- Effect of different nitrogen sources on the decolorization of acid orange7 & direct blue 75 by *Com. Acidovorans*-TM1 and *Bur.cepacia*-TM5:

Number of organic and inorganic sources of nitrogen was used in this experiment. Results presented graphically in fig(10a&b), indicated that, peptone gave the best decolorization percentage and highest protein content in the decolorization of direct blue 75 or acid orange 7 by each of the two strains respectively. Amm. oxalate came in the second order in the decolorization of each of both dyes by both strains except in case of decolorization of direct blue 75 by *Com. acidovorans*-TM1 and amm.molebdate was nearly the same level of color removal in decolorization of direct blue 75 by *Com. acidovorans*-TM1. The lowest color removal was attained by using $(NH_4)_2HPO_4$ as nitrogen source in the decolorization of acid orange 7 by each of the two strains individually. While lowest color removal in case of direct blue 75 was attained by adding potassium nitrate in decolorization by both of the two strains individually.

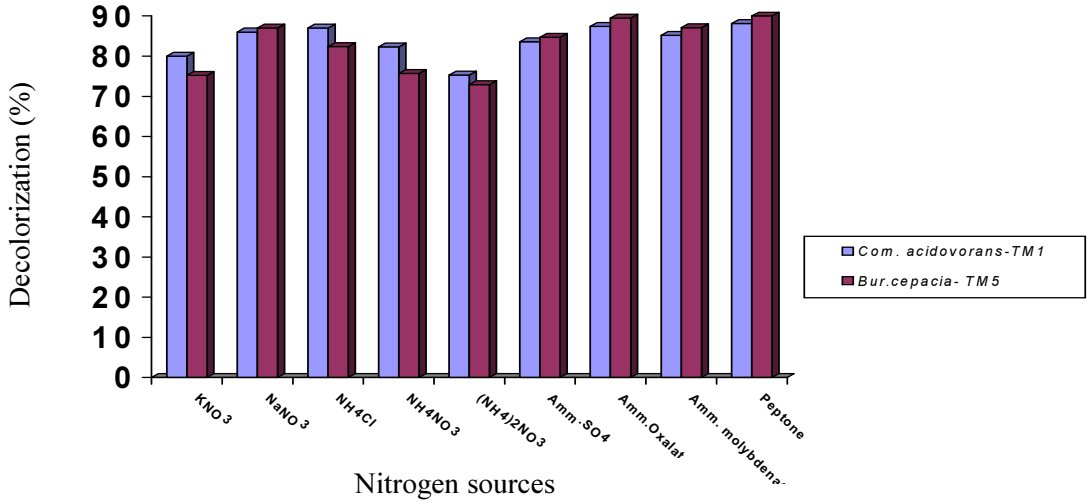

Fig. 10A: Effect of different nitrogen sources on decolorization of acid orange by *Com. acidovorans* - TM1 and *Bur. cepacia* -TM5.

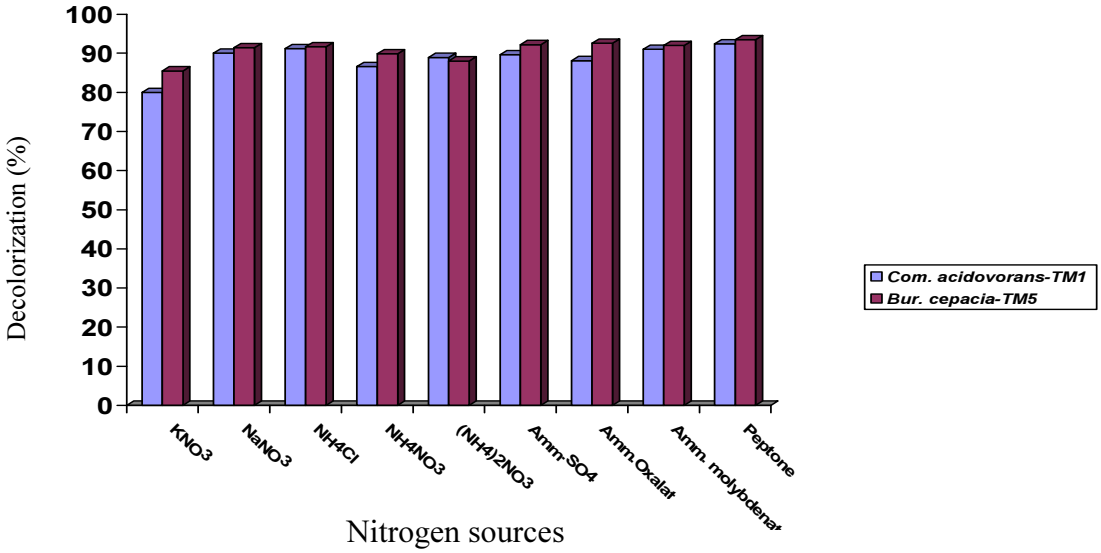

Fig. 10B: Effect of different nitrogen sources on decolorization of direct blue 75 by *Com. acidovorans* -TM1 and *Bur. cepacia* -TM5.

7- Effect of different incubation conditions on the decolorization of acid orange7 & direct blue 75 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

Depicts the color removal efficiencies throughout the 5 120h incubation period under anaerobic, aerobic and static conditions for azo dye acid orange7 & direct blue 75. More than 90% of the color was removed under anaerobic and static conditions in case of both the two dyes were decolorized by *Com. acidovorans*-TM1 or *Bur. cepacia*-TM5 while at the same time interval 50 and 55% decolorization was observed under aerobic (shaking) incubation. In other words, the efficiency of color removal by *Com. acidovorans*-TM1 or *Bur. cepacia*-TM5 in anaerobic incubations was higher than that obtained in aerobic incubation.

The strain *Bur. cepacia*-TM5 was able to decolorize nearly 90% of acid orange 7 and direct blue 75 incubation under shaking for one day then it was allowed to complete the incubation time under static condition. While *Com. acidovorans*-TM1 was able only to decolorize 51% of acid orange 7 and 63% of direct blue 75 at the same conditions presented graphically in fig. (11a&b).

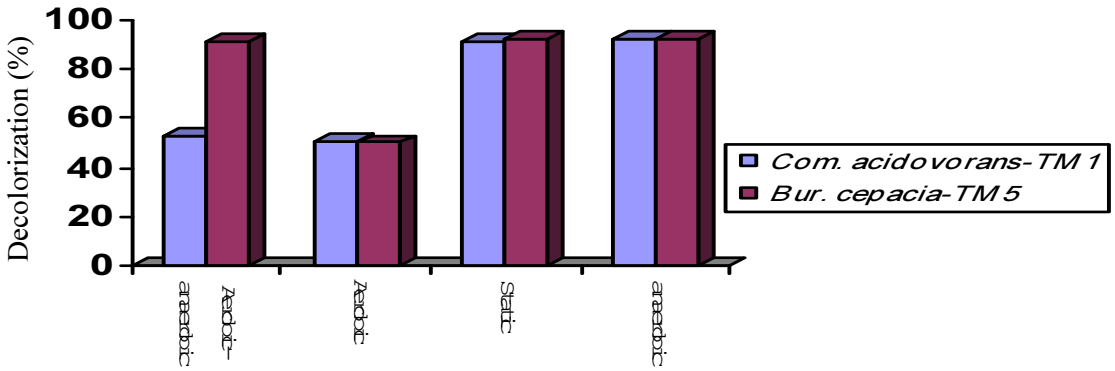

Fig. 11A: Effect of incubation condition on decolorization of direct blue 75 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

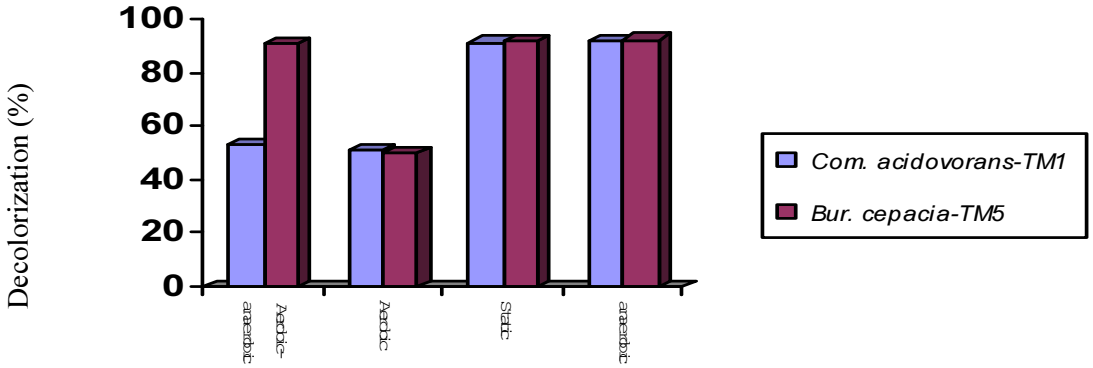

Fig. 11B: Effect of incubation condition on decolorization of acid orange7 by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5.

8- Effect of different yeast extract concentrations on the decolorization of acid orange7 & direct blue 75 by *Com.acidovorans*-TM1 and *Bur.cepacia*-TM5:

Result recorded concerning the effect of yeast extract concentration on the decolorization of the two dyes by *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5 showed there was clear effect of yeast extract concentration on the decolorization, but in general decolorization increased slightly as yeast extract concentration increased while the absence of yeast extract completely caused decline in color removal ability by the two strains. The results presented graphically in fig (12a&b).

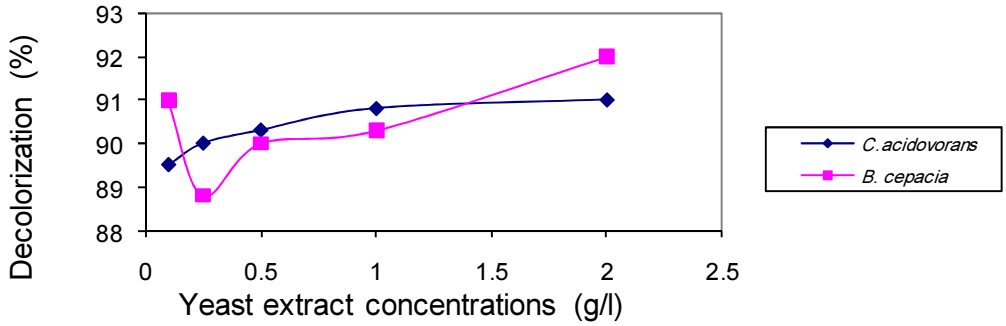

Fig. 12A: Effect of different concentration of yeast extract on decolorization of acid orange 7 by the two isolates *Com. acidovorans*-TM1 & *Bur. cepacia*-TM5.

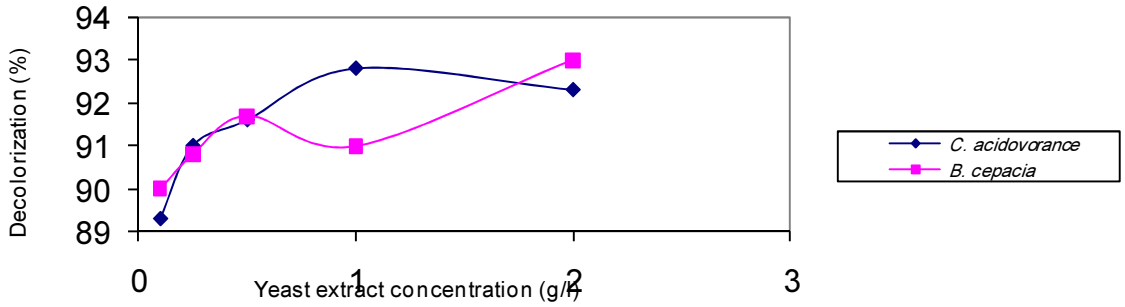

Fig. 12B: Effect of different concentration of yeast extract on decolorization of direct blue 75 by the two isolates *Com. acidovorans*-TM1 & *Bur. cepacia*-TM5.

We can summarize the optimum condition resulted from the previous experiments as follow table (2).

Table 2: A summery for the best conditions resulted from the study for decolorization of acid orange 7 and direct blue 75 by *Com. acidovorans-TM1 & Bur. cepacia-TM5.*

Condition	*Com. acidovorans TM1*	*Bur. cepacia TMS*
pH	8	8
Temperature	35	35
Incubation period	120 hours	120 hours
Carbon source	Starch	Starch
Nitrogen source	peptone	peptone
Incubation condition	Anaerobic	Anaerobic

9- Effect of using *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5 as consortium in Decolorization of acid orange 7 and direct blue 75.

The effect of using of the two strains *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5 in decolorization of acid orange 7 and direct blue 75 were proceeded compared with decolorization using single culture. Results presented graphically in fig. (13) showed that decolorization percentage raised slightly by using a consortium of both bacterial strains. The consortium removed 94 (%) of acid orange 7 and 95.5 (%) of direct blue 75.

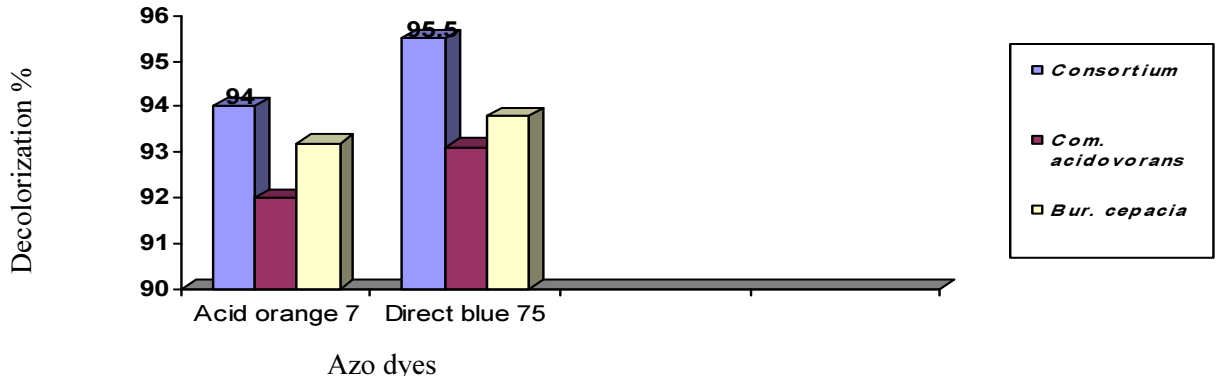

Fig. 13: Effect of using *Com. acidovorans*-TM1and *Bur. cepacia*-TM5 as consortium in decolorization of acid orange 7 and direct blue 75.

DISCUSSION

Textile dyeing industries is one of the fastest growing fields and major export oriented industrial sectors in Egypt especially in El-Mahalla Elkobra. Azo dye production is more than 1 million tons per year in the world and during dying processes, about 40% of this huge amount of azo dyes ends up in wastewaters. In addition, about 40-65 L of textile effluent is generated per kg of cloth produced. On the other hand the release of these compounds into the environment presents serious problems of pollution related to both aesthetic reasons and their toxicity (Mezohegyi *et al*., 2007).

Colored-dye-wastewater treatment and decolorization presents an arduous task. Wide ranges of pH, salt concentrations and chemical structures are often added to the complication. There are many reports on the use of physicochemical methods for color removal from dyes containing effluents (Churchley, 1994; Vandevivere *et al.,* 1998; Swaminathan *et al*., 2003; Behnajady *et al.*, 2004; Wang *et al.,* 2004; Golab *et al.,* 2005; Lopez-Grimau and Gutierrez, 2005). Extensively used coagulation/ flocculation techniques produce large amounts of sludge, which require safe disposal. Adsorption to a certain extent, membrane filtration techniques lead to secondary waste streams which need further treatment. These

constraints have led to the consideration of advanced oxidation processes (AOP) and biological methods as attractive options for the treatment of dye-containing wastewaters. AOP are defined as those processes that use strong oxidizing agents (H_2O_2, Fenton's reagent) or heterogenous photocatalysts such as TiO_2, ZnO_2, Mn and Fe in the presence or absence of an irradiation source. These involve mainly the generation of (OH) radical for the destruction of refractory and hazardous pollutants (Vandevivere *et al.,* 1998; Alaton *et al*., 2002; Al-Kdasi *et al*., 2004). These methods do not produce solid waste. However both AOP and membrane filtration methods are energy and cost intensive. Among the most economically viable choices available for effluent treatment/decolorization and the most practical in terms of manpower requirements and running expenses to adopt and develop, appear to be the biological systems. Fungi, bacteria and yeast decolorization are a promising alternative to replace or supplement present treatment processes.

The main object of the present work was an investigation of isolation; purification and screening of bacterial isolates have the ability to decolorize and mineralize models of azo dyes and optimize this ability for application in textile wastewater treatment technology. In this regard, ten bacterial isolates were isolated from different wastewater treatment plants, most of them exposed to drainage of colored textile effluent in addition to domestic effluent. These bacterial isolates were selected after growing at 30 °C and pH 7 in mineral salt medium supplemented with yeast extract and mixture of azo dyes at rotary shaker for 24 hours, then this step was repeated in order to permit the bacterial species to have the ability to survive in presence of dye to dominate.

A screening test for the ability of this isolates to utilize azo dyes as a sole carbon source was established to select the most potent organisms and exclude that decolorization may occur due to adsorption only. This technique was used by Asad *et al*., (2007).where the ability of halophilic and halotolerant bacterial isolates to utilize remazole blacke B as sole carbon source was used to select the most effective isolates. The screening was done in broth and solid mineral salt media containing different individual dyes to select beside effective organisms, the dyes which would be used to complete the study. Screening test was done three times on different groups of azo dyes to support the selection of organisms that have the ability to utilize different azo dyes as sole carbon sources.

This trial resulted in the fact that only two bacterial isolates TM1 and TM5 were considered to be the best decolorizers of most azo dyes used in screening. The selected two bacterial isolates were isolated from Elmahalla Elkopra wastewater treatment plant which were exposed to drainage of textile industry, this means that TM1 and TM5 were adapted bacterial isolates. Olukanni *et al*., (2006) by studying the textile effluent biodegradation potentialities of textile effluent-adapted and non adapted bacteria, it was found that there were no great difference in decolorization ability between adapted and non adapted bacteria. In addition plasmids were not detected in any of the isolates from the effluent adapted or non adapted sources.

All bacterial isolates pellets retained its original color and did not become deeply colored because of adsorbed dyes. This indicate that, color removal was due to degradation not only adsorption Chen *et al*., (1999). The low protein content and low color removal percentage observed in screening tests were attributed to the fact that azo dyes are deficient in carbon content and biodegradation without any extra carbon sources is very difficult, so optimization experiments were started by supplementation of the menial salt medium containing dyes with yeast extract. The color removal percentage of most dyes increased sharply after addition of yeast extract and this is in accordance with other reports (Chen *et al.,* 2003; Dong *et al.,* 2003; Kodam *et al.,* 2005; Moosvi *et al.,* 2005 and Asad *et al*., 2007). Metabolism of yeast extract is considered essential for regeneration of NADH, which is the electron donor for azo bond reduction (Asad *et al*., 2007).

The low decolorization percentage of azo dyes Mordant Brawn, Reactive red and Acid yellow may due to the fact that decolorization of azo dyes is affected by permeability of cell membrane depending upon the molecular weights and intramolecular hydrogen bond between the azo and hydroxyl groups (Yatome *et al*., 1981). Since the two bacterial isolates TM1 and TM5 were the most potent decolorizers of azo dyes under study, they were selected purposely for their identification as well as optimization of their color removal ability of the two azo dyes Acid orange 7 and Direct blue 75 which selected also to complete the study

representing the other azo dyes because they gave the highest color removal in screening experiments and represent the most usable groups of azo dyes. Identification trials indicated that, they are related to genus *Comamonas* and *Burkholderia*.(previously *Pseudomonas*) therefore they were suggested of being *Com. acidovorans*-TM1 and *Bur. cepacia* - TM5. Although there are not many studies on using *Comamonas* sp. and *Burkholderia* sp. in decolorization of textile wastewater containing azo dyes but the history of these two species in bioremediation specially degradation of compounds related to unites which azo dyes are constructed (as nitro aromatics) indicate that using of *Comamonas* sp and *Burkholderia* sp in this field is promising.

Burkholderia sp. strain AK-5 utilized 4-aminophenol (This compound is an intermediate in the degradation of hydroxyacetanilide and azo dyes) as the sole carbon, nitrogen and energy sources. A pathway for the metabolism of 4-aminophenol in strain AK-5 was proposed based on the identification of three key metabolites by gas chromatography-mass spectrometry analysis. Strain AK-5 converted 4-aminophenol to 1,2,4-trihydroxybenzene via 1,4-benzenediol. 1,2,4 Trihydroxybenzene 1,2-dioxygenase cleaved the benzene ring of 1,2,4-trihydroxybenzene to form maleylacetic acid. The enzyme showed a high dioxygenase activity only for 1,2,4-trihydroxybenzene, with *Km* and *V*max values of 9.6 _M and 6.8 _mol min_1 mg of protein_1, respectively Takenaka *et al.,* (2003).

Also nitroaromatic which compounds are widely used as dyes, were depredated by *Burkholderia* sp for example 4-Methyl-5-nitrocatechol (4M5NC) monooxygenase (DntB) from *Burkholderia* sp. strain DNT catalyzes the second step of 2,4-dinitrotoluene degradation by converting 4M5NC to 2-hydroxy-5-methylquinone with the concomitant removal of the nitro group Leungsakul *et al*., (2006).

The *Pseudomonas* are an extremely large, versatile and adaptable class of microorganisms and it is not surprising that *Pseudomonas* species have featured prominently in research involving the degradation of xenobiotic azo compounds. The biodegradation of azo dyes by *Pseudomonas cepacia* 13NA was investigated by Ogawa *et al*. (1986) using the dyes, CI Acid Orange 12, CI Acid Orange 20 and CI Acid Red 88. A three-stage continuous culture system (with the flasks arranged in series) was found to be more successful as a one stage system, with respect to the rate of degradation of the dyes. This was attributed to: (a) An abundance of nutrients in the first stage of the cultivation system which resulted in a rapid increase in the bacterial population, increasing the number of potential azo degrading bacteria; and (b) A consequent paucity of nutrients in the second and third culture flasks which forced the microorganisms to assimilate the azo dyes for their growth, i.e. the azo dyes were degraded under nutrient limited conditions.

These results are unusual as a pre-requisite for degradation of azo dyes is usually that a supplemental carbon source is provided to sustain the metabolic activity of the azo reducing microbial population (Wuhrmann *et al,* 1980). Ogawa and Yatome, (1990) aimed to develop a waste-water clarification technology for removal of dyes and other organic substances in a single operation using *P. cepacia* 13NA. The mode of treatment chosen was a multi-stage rotating biological contactor, with discs on which *P. cepacia* 13NA was immobilized with K-carrageenan gel. A multi-stage reactor was chosen due to the findings of Ogawa *et al.,* (1986). The researchers discovered that the natural organic substances in the effluent were preferentially assimilated and consequently, significant biomass increase was achieved. However, little dye degradation occurred at this stage and it was realized that the dyes were degraded only by starved cells. For this reason, the first reactor was suited for growth of the microorganisms because of the rich nutrient quality of the effluent, while the third reactor was suited for the catabolism of the dyes because of poor nutrient quality. The researchers concluded that it may be possible to keep both the growth of the microbes and the degradation of the dyes high, by periodically changing the path of solution in the waste-water treatment plant. The dyes treated were C.I. Acid Red 88, C.I. Direct Blue 6 and PAAB and a retention time of 20 h was chosen as the dyes were known to be resistant to biodegradation. However, the overall biodegradation rate of the dyes was poor and it was concluded that a longer retention time was necessary to enhance the rate of elimination. Yatome *et al*., (1990) investigated the degradation of several azo dyes by cell-free extracts from the azo reducing microorganism *P. stutzeri* to determine the roles of dye redox potential and hydrophobic character in the rate of azo reduction.

They concluded that both parameters are useful in helping to gain an understanding of the relationship between the ease of degradation of dyes and their structure but that no definite rules for degradability of azo dyes could be formulated. Yatome *et al.* (1991) reported that the rate of degradation of azo compounds by whole cell culture of *P. stutzeri* was limited by the relative ease at which the compounds could permeate the cell.

Com. acidovorans also were used in azo dye decolorization. Oxspring *et al*., (1996) reported the decolorization of the Reactive dye, Remazol Black B, by an immobilized microbial consortium, consisting primarily of *Alcaligenes faecalis* and *Com. acidovorans*, in an up flow anaerobic filter. Over 95% of Remazol Black B, at initial concentrations of 0.5 g/l, was decolorized within 48 h, producing metabolites that were probably aromatic amines. Chen *et al*., (2003) studied the evaluation of effective diffusion coefficient and intrinsic kinetic parameters on azo dye biodegradation using phosphorylated polyvinyl alcohol PVA-immobilized cell beads contain mixed culture of *Aeromonas hydrophila, Com. testosteroni,* and *Acinetobacter baumannii*.

Temperature control is very important for any bacterial process, since growth and production of enzymes are usually sensitive to high temperature (Sani *et al.,* 1992; Babu and Satyanarayana 1995). It must be noted that, the optimum temperature for production of an enzyme (in this case azo reductase enzyme) does not always coincide with that for growth (Sodhi *et al*., 2005).

In the present study the optimum incubation temperature for maximum color removal percentage for the two azo dyes Acid orange 7 and Direct blue 75 was 35 °C when decolorized by any of the two strains *Com. acidovorans*-TM1 or *Bur. cepacia*TM5. Results recorded indicated that this temperature was also the optimum for growth. This result is in complete accordance with the study by (Asad *et al*., 2007) where the optimum temperature for decolorization of remazol black B by *Halomonas aquamarina* was also 35 °C . The decolorization percentage and growth represented by protein content increase with temperature increase until 35 °C, further increase in temperature resulted in marginal reduction in decolorization activity of bacterial strains because the reduction in cell growth and the enzyme azo reductase may be deactivated. Mezohegyi *et al.,* (2007) investigated the anaerobic reduction of azo dye Acid Orange 7 (AO7) in a continuous up flow packed bed reactor (UPBR) containing biological activated carbon (BAC) and the temperature was adjusted at 35ºC. This result also is more related to many results those reported by many authors, where Dafale *et al*., (2008) found that, 37°C was the optimal temperature for decolorization of remazol black-B (RB-B) by a bacterial consortium containing *Pseudomonas aeruginosa*. In contrast to the present results, HU *et al*., (1994) incubated *Pseudomonas luteola* at 28°C to obtain maximum decolorization power of textile wastewater. Hefang *et al*., (2004) investigated the effect of temperature on the decolorization of azo dye Direct fast scarlet 4BS by microbial consortium consisting of white rot fungus and *Pseudomonas* isolates and they found that under optimum neutral pH condition and over a range of 20–40°C, the immobilized cells showed high activity of decolorization. At 30°C the immobilized beads became soft slowly with bulgy volume and consequently shorten their life at 35ºC, so the optimal operational temperature was 30 ºC in this study.

pH is among the other most important factors for any microbial activity . Each microorganism possesses a pH range for its growth and activity of metabolite production with a optimal value in between the range. The pH of culture medium plays a critical role for the optimal physiological performance of microbial cells and the transport of various nutrient components across the cell membrane. Thus, the pH of the decolorization medium has a marked effect on the cell growth and enzyme production. In the present study the optimum incubation pH for maximum color removal percentage for the two azo dyes acid orange 7 and direct blue 75 was pH 8 when decolorized by any of the two strains *Com. acidovorans*-TM1 or *Bur. cepacia*-TM5. The results recorded indicate that this pH was not the optimum for growth. The optimum pH for growth was pH 7. the decolorization at pH 8 was suitable for decolorization of textile wastewater. It should be mentioned that most of collected textile wastewater samples used in this study were slightly alkaline so in economic view decolorization at pH 8 is an advantages.

Decolorization of azo dyes in alkaline medium was recorded by many studies, Asad *et al*., (2007) found that decolorization rate of remazol black B

increased as pH increased. Also Dafale, *et al*., (2008) found that, the specific decolorization rate increased with increasing pH from 5 to 7, which remained approximately the same for pH 7–8. This seems to indicate that neutral and slightly basic pH values would be more favorable for decolorization process of remazole black B by a bacterial consortium containing *Pseudomonas aeruginosa*. In contrast to the present results pH 7 was the optimum pH for the decolorization of reactive red 195 by *Enterobacter* sp and the decolorization percentage decreased as pH increased (Kalyanee *et al*., 2008).

The results of pH and temperature are supported by the fact that di azo dization and coupling (the process used in manufacturing of azo dye) proceed in low temperature and acidic medium. So it is logical that the reverse reaction will be catalyzed by high temperature and alkaline pH to the value which the enzyme responsible for this process becomes inactive.

In a trial to determine the best incubation period for decolorization of acid orange 7 and direct blue 75 by both the two strains *Com. acidovorans*-TM1 *and Bur. cepacia*-TM5, the decolorization activity was measured after 6, 12, 24, 48, 72, 120, and 168 hours, the best decolorization results were recorded at 120 hours in all cases and the increase in decolorization activity after 120 hours was not affective. The decolorization of the two azo dyes under study by *Com. acidovorans*-TM1 was started after 24 hours while decolorization by *Bur. cepacia*-TM5 was started after 12 hours, this indicates that *Bur. cepacia*-TM5 can adapt with the new medium containing azo dyes faster than *Com. acidovorans*-TM1, this indicates also when screening protein content results which indicate that in most cases the protein content of *Bur. cepacia*-TM5 is more than that formed by *Com. acidovorans*-TM1. Hu, (1994) studied the decolorization of reactive azo dyes by transformation with *Pseudomonas luteola* and he found that most loss of color occurred after incubation for 7 days in total. Also (Olukanni *et al*., 2006) measured decolorization % and COD removal % after 14 days of incubation when he studied textile effluent biodegradation potentiality of textile adapted and non adapted bacteria. The effect of inoculum size on the end point of decolorization of acid orange 7 and direct blue 75 was studied and it was found that in general decolorization percentage increases as inoculum size increase in all cases but this increase is not effective.

In a trial to study the effect of introducing some carbon sources on the decolorization process of acid orange 7 and direct blue 75 by the selected most potent two bacterial strains *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5, it was found that decolorization percentage increased after addition of most carbon sources but decreased after addition of other sources of carbon when compared with the control. But the most promising results for decolorization were obtained when starch was used as a carbon source although starch was not the optimum co-metabolite for growth.

The increase in decolorization percentage after addition of carbon sources is attributed to the fact that the dyes are deficient in carbon content and biodegradation without any extra carbon sources is difficult (Padmavathy *et al*., 2003). The decrease in decolorization percent after addition of some carbon sources and the ability of some carbon sources to induce growth without increase in decolorization may attributed to that, the sugars may inhibit the decolorization of azo dyes because its effect as catabolite repression (Chang *et al*., 2001).

Presence of starch as the best co-metabolite in decolorization of azo dyes was supported by many studies Padmavathy *et al*., (2003) found that starch was the best carbon source in azo dye bio degradation from synthetic waste water under aerobic co-metabolite conditions also Georgiou *et al*., (2005) suggested the use of potato-starch industrial wastes to increase the decolorization of textile waste water in large scale. Also starch was added by Olukanni *et al*., (2006) in studying the textile effluent biodegradation potentialities of textile effluent-adapted and non-adapted bacteria. In contrast to the present study, glucose was used as a carbon source in decolorization of reactive azo dyes by *Pseudomonas luteola* (Hu, 1994). Also Asad *et al*., (2007) used glucose in decolorization of remazol black B by halotolerant and halophilic isolates.

Concerning the effect of the addition of different nitrogen sources for the purpose of decolorization of the two azo dyes acid orange 75 and direct blue 75 by the two strains *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5, it was found that organic nitrogen source peptone was the best inducer for the decolorization of the two dyes by the two strains. In contrast to the case of carbon source, peptone was

the best nitrogen source for growth in addition to decolorization. Presence of peptone as the best nitrogen source was proved by many azo dyes bioremediation studies. Chen *et al*., (1999) found that peptone gave the best color removal percentage for azo dye Red RBN by *Proteus mirabilis* and the substitution of inorganic nitrogen (NH_4Cl) for peptone gave poor cell growth and low color removal. Also HU, (1994) used medium containing peptone as a nitrogen source in decolorization of reactive azo dyes by *Pseudomonas luteola*. In contrast to this results inorganic nitrogen sources (NH_4Cl) was used in anaerobic treatment of azo dye Acid Orange 7 under fed-batch and continuous conditions Me´ndez-Paz *et al*., (2005).

In a trial to study the effect of types of incubation (shaking, static, anaerobic and sequence aerobic-anaerobic) condition on the decolorization of Acid orange 7 and Direct blue 75 by the two strains *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5, it was found that: decolorization percentage decreased under shaking incubation (aerobic condition) with the two dyes by the two strains although cell growth was the best under shaking conditions in all cases. The decolorization percentage obtained under anaerobic and static conditions were nearly equal in all cases. The difference between behavior of two strains *Com. acidovorans*-TM1 and *Bur. cepacia*-TM5 in dealing with the two azo dyes were recorded under sequential aerobic-anaerobic incubation where color removal was nearly the same in case of *Bur. cepacia*-TM5 but decreased sharply in case of *Com. acidovorans*-TM1. These results were in complete accordance with many studies. Isik and Sponza, (2003) studied, the color removal efficiencies throughout the 9 days of the incubation period under anaerobic, aerobic and microaerophilic conditions for azo dye Direct Black 38 (DB 38) decolorized by *E. coli,* more than 72% of the color was removed within 3 days under anaerobic conditions while at the same time 24 and 63% decolorization was observed under aerobic and microaerophilic incubations, respectively. In the same study, the decolorization assay during 5 days of the incubation period under different dissolved oxygen levels for Direct Black 38 and congo red was investigated using *Pseudomonas* sp. The initial color removal efficiency was zero and the extent of color removal 100% at the end of 3 days incubation under anaerobic conditions for congo red dye. 76% and 45% color removal efficiency were obtained under microaerophilic and aerobic conditions at the end of 3 days of incubation under anaerobic conditions for DB 38 dye. Similarly 99 and 49% color removal efficiency was obtained under anaerobic and aerobic/microaerophilic conditions for congo red dye. Asad *et al*., (2007) studied the decolorization of remazol black B by halotolerant and halophilic isolates under shaking, static and anaerobic conditions and found that, the anaerobic culture condition was best for decolorization process but was not very different from the static culture. Optimal growth of *Bacillus cereus* DC11 cells was achieved under well aerobic conditions, but high decolorizing activity appeared under anaerobic conditions (Deng *et al*., 2008).

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