Isolation and identification of bacteria from textile waste waters and evaluation of their biodegradability of textile dyes

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The textile industry has been identified as one of the major sources of water pollution in Sri Lanka because individual textile processing operations dispose large quantities of used water to the environment. The waste water from textile manufacturing industries are treated by physical, chemical and biological methods or by using a combination of the three in order to reduce pollution.

The main objective of this study was to isolate and identify different bacterial species from textile wastewater and to determine their ability to degrade textile dyes.

Twelve bacterial species were isolated from the textile wastewater samples. A special medium containing 0.03g / 100ml of a dye concentration was used to isolate the bacteria, and each isolate was identified using morphological and biochemical characters. Using these isolates their ability to reduce the colour of three textile dyes, namely Reactive Red 2, Reactive Blue 21 and Acid Blue 25 was investigated. The colour reduction was monitored by taking spectrophotometric measurements. Organisms such as *Enterobater aerogenes, Bacillus circulans, Bacillus alvei, Pseudomonas putida and Bacillus macerans* had a fair capability to reduce the colours of most of the above three dyes within a very short period of 4 – 6 days.

1. Introduction

The textile industry has made important contributions to many national economies. In Sri Lanka, the textile and garment industry has become one of the most important industrial sectors during the last few years. In 1990, the combined garment, textile and leather goods industries accounted for around 32% of the total value of the industrial production of the country (industrial population control guidelines, 1992). The textile industry manufacture finished cloth from imported raw material. These raw materials usually consist of cotton, wool and synthetics. During the production process the raw material undergo scouring (removal of foreign compounds), desizing (removal of size materials from gray goods to prepare for bleaching, dyeing etc.), mercerizing (a process given to cotton yarns and fabric to increase lustre, strength and dying ability), bleaching, dyeing etc. (UNEP, 1994). Therefore in the textile manufacturing process environmental pollution takes place as a result of the above operations (industrial pollution control guidelines, 1992). The finishing processes consume large quantities of water, resulting in substantial volume of liquid wastes which contributes significantly to water pollution in Sri Lanka.

Much of the textile wastes consist of natural impurities arising from fibre and processing chemicals. However the types of the impurities vary according to the type of raw materials and the processes used (Industrial pollution control guidelines, 1992). In addition chemical companies market a range of products such as dye formulations and colorants to the textile industry, many under trade names rather than by their chemical compositions. The discharge of coloured effluents into water streams makes water inhibitory to aquatic life. This also causes reduction in light penetration and depletion of dissolved oxygen levels in water in addition to visual pollution (Iiorng et al., 1993; Mittal et al., 1989).

Textile effluents are generally grey in colour. It has high biological oxygen demand (BOD) levels & total dissolved solids and an increased temperature. The synthetic finishing effluents which are generally low in volume may contain toxic substances especially when chemical dyes with metallic ions are used (UNEP, 1994).

To depollute these textile wastes, many different techniques have been used. Biological methods together with physical and chemical methods have been employed in many textile waste treatment plants. It has been emphasized that a combination of more than one process is generally necessary to achieve adequate removal of all contaminants rendering adverse effects to receiving water streams. Due to the wide range of dyes in use, each waste will demand a tailored economical method of treatment, making use of a combination of methods (IEC, 1985).

In the case of biological treatment, the eliminating extent (which is a measurement of break down of the dye) of dyes by activated sludge or trickling filter is small (UNEP / IE, 1994). However biological methods are used widely for its simplicity and low cost (Liu and liu, 1991). The decolouration of the textile dyes demonstrates only the transformation of the chromophoric group of a dye and it does not demonstrate the complete degradation of the compound (Spadaro *et al.*, 1992). In activated sludge systems, the degree of elimination of coloured compounds has been attributed to adsorption rather than degradation.

In nature, micro-organisms are known to play a major role in biologically degrading textile effluents (Pathiraja, 1996). The extent to which these dyes are subjected to microbial degradation depends on the solubility, ionic characters and the degree and type of substitutions that are found in the dye molecules (Jinqui & Houtain, 1991). It has been noted that through biodegradation organic loads of the textile effluent could be removed satisfactorily. A substantial reduction of the organic contents results values of pH, BOD and nitrogen to phosphorous ratios in the effluent to be maintained at suitable levels in-accordance to environmental standards. Further artificial introduction of suitable micro-organisms or altering of the environmental factors of the effluent is useful to increase the efficacy of the biological treatment processes.

Therefore the overall objective of this study was to isolate and identify bacteria that have the ability to degrade textile dyes and to asses the efficiency of the isolates towards the breakdown of the dyes under various conditions.

2. Material and Methods

Composite samples were prepared by mixing small aliquots of water samples collected from textile industries such as Vayangoda textile mill and Dial textiles Ltd.

A special medium (Ogawa et al., 1978) with varying concentrations of Reactive Red 2 dye was used to isolate the bacteria from the textile waste water. The isolation was performed under different combinations of the dye and nutrient concentrations. Each isolated organism was identified using its morphological and biochemical characters (De Ley, et al., 1984).

The natural ability of the individual isolates to degrade the dyes were evaluated by using the same medium with a dye concentrations of 0.03g / 100 ml. The textile dyes that were subjected to the test were Reactive Red 2, Acid Blue 25 and Reactive Blue 21. The degradability of the organisms were evaluated by measuring the absorbance of the dyes at their λ_{max} values during a period of 6 days. The experiments were carried out under both aerobic and microaerophilic conditions. An investigation was also performed to evaluate the degradabilty of the isolates, when used as microbial combinations.

Investigations were also done to study the degradation of the intermediate components that are formed during the bio-degradation of Reactive Red 2 dye. Possible formation of the intermediates were studied by measuring the absorbances of the intermediates at their specific λ_{max} values (404 nm, 422 nm, ...564 nm).

Further, the pH variations in the medium that occur during the bio-degradation process were monitored with respect to individual and mixed microbial cultures.

3. Results / Findings

The number of different bacterial species isolated in the presence of Reactive Red 2 textile dye was very small. Twelve species were isolated among which *Enterobacter aerogenes Pseudomonas putida* and *Bacillus circulans* were found to be the most abundant (Table – 1).

Isolates which were allowed to degrade the dyes under aerobic conditions in broth cultures did not significantly reduce the colour of dyes Reactive Red 2, Reactive blue 21 and Acid blue 25. However the organisms showed a dense growth under this condition. Under microaerophilic conditions, a rapid removal of the colours were observed (Fig. 1). Therefore further experiments were carried out under microaerophilic conditions.

During 6 days of incubation, *Enterobater aerogenes, Bacillus circulans, Bacillus alvei, Pseudomonas putida and Bacillus macerans* showed a reduction in the absorbance of the dye Reactive Red 2 (at 580 nm) from around 0.7 to 0.1, indicating an 86% removal of the dye (Fig. 2). When mixed cultures of *Pseudomonas putida, Bacillus pumillus* and other *Bacillus spp.* were used in the presence of Acid Blue 25 dye the absorbance (at 584 nm) of the medium

dropped from around 0.7305 to 0.460 indicating a removal of around 35% of the dye. It was also found that when other combinations of mixed microbial cultures were used the degradation of the dye Acid Blue 25 was even lesser than the above (Fig. 3). Mixed microbial combinations when applied on any of the other two dyes, showed a lesser capability than the above to remove the colour of the dyes.

Organisms when grown in a liquid medium containing Reactive Red 2 dye, showed the appearance of various λ_{max} values in the medium during the incubation period, and it was in the range of 484 - 564 nm. The control experiment (the Day 0 in Fig. 4) showed two λ_{max} values 430nm and 580nm. The pure dye gave a maximum absorbance at 580nm while the medium gave at 430nm. The results given in Fig. 4-A for *Serratia liquefaciers* indicates that for Day 0 the maximum absorbances are at 430nm and 580nm. By Day 1, the absorbances had shifted and showed a broad peak in the range of 484 – 564nm, with a maximum at 544nm. The absorbances on Day 4 were very much similar to Day 1. However by Day 6 the overall absorbance values in the medium had become very low.

Enterobacter aerogenes, Klebsiella aerogenes, Bacillus circulans, Bacillus alvei and *Bacillus macerans* showed a similar pattern to the above. However the absorbances showed by other organisms in the range of 484 – 564nm did not show considerable reduction during the course of Day 1 to Day 6, indicating their inability to break down the intermediates efficiently.

Pseudomonas putida showed a specific behavior when grown on solid medium containing Acid Blue 25. It absorbed the dye into the cells from the solid medium and released the absorbed dye when introduced into an aqueous medium. However this feature was not observed when it was grown on a solid media containing either Reactive Red 2 or Reactive Blue 21.

The pure cultures of microorganisms brought about a gradual increase in pH from around 6.4 – 10.4 in the medium containing Reactive Red 2, during a period of 6 days of degradation. However during the same period of time the mixed microbial cultures only brought about a variation from 6.4 – 8.3.

4. Discussion

The soil of the draining channels of textile factories, were considered the most suitable natural substrate to isolate bacteria having the degradability of textile dyes. In the present study, water samples were collected from different sites of the waste water dumping marshes of textile mills. All of these samples showed very minor variations with respect to pH, diversity of organisms (Table - 1), colour (visual observation), odor etc. The major organisms isolated and identified from these samples were *Enterobacter aerogenes, Klebsiella aerogenes, Bacillus circulans, Bacillus anthracis, Bacillus alvei, Pseudomonas putida* and *Bacillus macerans* (Table - 1).

The dyes appeared to be one of the major sources of carbon available to the organisms when they were in the environment. In general natural organisms tends to utilize complex carbon compounds such as the dyes only when other simple carbon sources are limited in the environment (Hollaender, 1982). Therefore it was believed that these isolated organisms possessed alternative pathways to utilize the textile dyes.

A previous research carried out (Pathiraja, 1996) on textile dye effluents revealed that these organisms have the tendency to loose their ability to degrade the dyes when they are exposed to environments rich in glucose or other simple carbon sources. This occurrence was believed to be due to the loosing of a naturally occurring plasmid which would have contained the genetic information leading to the degradation of the dye (Hollaender, 1982). Therefore through out the present study a medium consisting a high concentration (0.03 g / 100 ml) of the Reactive red 2 dye was used to store the organisms, and periodically the ability of the organisms to degrade the dye was tested. The organisms showed a very slow growth on very high concentrations of the dyes. To avoid the slow growth rate of the organisms, initially a small fraction of glucose (0.01 g / 100 ml) was added into the medium and that allowed the organisms to get adapted in to the new environment gradually. The medium was also supplemented with the sterile effluent, as a method to supply the special micronutrients that might be needed by the organisms for their growth and active degradation of the dyes.

At the beginning of the study the degradation process was studied under aerobic conditions, where the organisms were allowed to grow in shake flask cultures. However the degradation of the dye did not occur as expected (Fig. 1). The colour of the dye, more or less remained along with a highly dense growth of the bacterial culture (visual observation). When the degradation of the dye was allowed to take place under microaerophilic conditions, i.e. by only shaking the media every 12 hrs, a very rapid and an effective removal of the dye was observed.

It is presumed that under microaerophilic conditions, the degradation of the dyes begin mainly and simultaneously at the logarithmic phase (Ogawa et al., 1978), but the growth rate of the cells are low. According to Ogawa if the shaking culture method is practically applied the removal of the biomass at the end of the treatment process can be a problem. On the other hand, in the case of the static cultures, the cells get heavily injured affecting their growth due to the localized dye concentrations in the medium.

The biodegradability of the textile dyes by the organisms were assessed by the capability of the organisms to remove the colour of the dyes from the experimental medium. An organism capable of removing the colour of a dye within a very short period of time was considered to be the most efficient organism for that textile dye.

Textile dyes are organic compounds, and many of these are aliphatic or aromatic hydrocarbons substituted by various substitutary groups like nitro, sulpho, -SH etc. (IEC,1985). Most of the microorganisms have the ability to breakdown these hydrocarbons incompletely resulting intermediate products, while using other carbon sources like glucose etc. as their energy source. This was apparent by the appearance of several $\lambda_{max.}$ values in the test medium during the time course of the experiment. However to confirm the formation of intermediates and to elucidate the above results a detailed analysis of the

medium is necessary. This can be carried out using the thin layer chromatographic technique. The lack of information on the chemical structures of the textile dyes, and the limited time available for this study, made the task of finding a suitable solvent to carry out the thin layer chromatography difficult.

The natural environment consists of mixed bacterial populations. Therefore it was assumed that mixed bacterial populations could be much efficient than an individual organism in degrading the dyes. However according to results reported in Fig. 2 and Fig. 3 individual bacteria were more capable than the mixed bacterial populations in degrading the textile dyes.

Fig. 4 shows the changes in absorbances that occurred in the test media during 6 days of degradation by individual microorganisms. In this study it was assumed that the sudden appearance of various λ_{max} values in the test medium was associated with the formation of intermediate products in the process of biodegradation. Therefore the breakdown of the textile dye and the formation and break down of the intermediates in the test medium were monitored by measuring the absorbances at their specific λ_{max} values. Some organisms are only capable of breaking down the textile dyes partially, resulting intermediate compounds. However an effective organism(s) should breakdown the textile dye and remove any intermediates that are formed as well. Therefore the most effective organism(s) finally should show very low absorbance values in the test medium, similar to that of the medium without the dye.

In comparison *Serratia liquefaciens*, *Enerobacter aerogenes*, *Klebsiella aerogenes*, *Bacillus circulans*, *Bacillus alvei*, and *Bacillus macerans* appeared to be effective in degrading the dye, and most other intermediates that are formed in the medium. The literature reveals that *Pseudomonas* sp., *Aeromonas hydrophila* (ogawa et al., 1978), *Bacillus cereus* and *Streptomyces* sp. (Horn et al., 1992) have the ability to degrade the textile dyes. However when considering the isolated organisms only a few had the ability to degrade the dye partially or completely, but all of them were capable of growing in the culture medium containing very high concentrations of all the three dyes.

Furthermore a change of the pH in the medium along with the growth of the organisms was one of the notable features in this study. Here it was very clear that the microbial combinations had a better control over the pH in the medium than the individual microorganisms.

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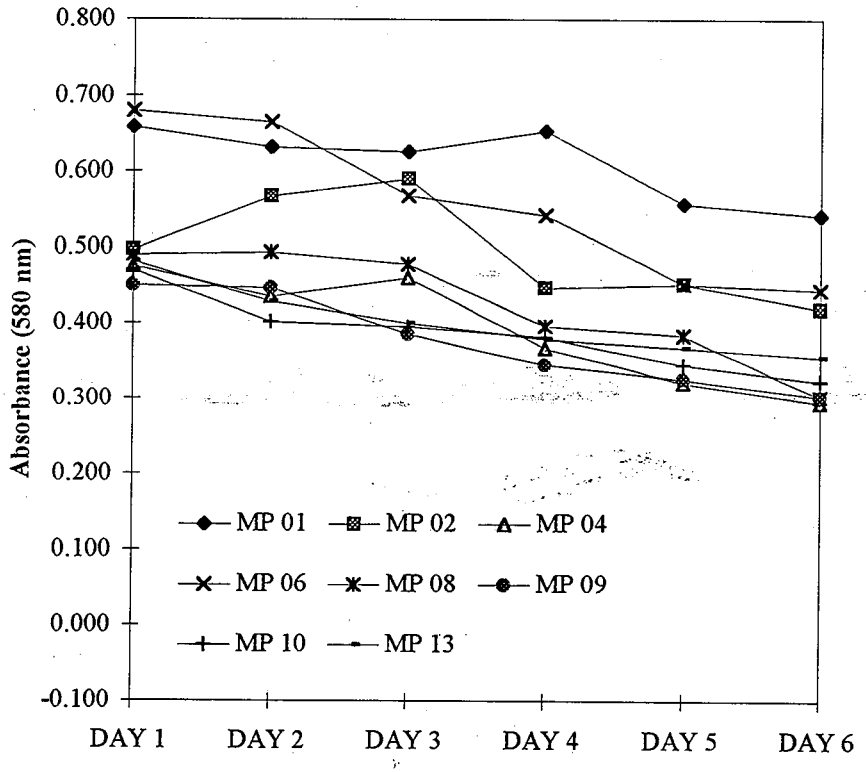
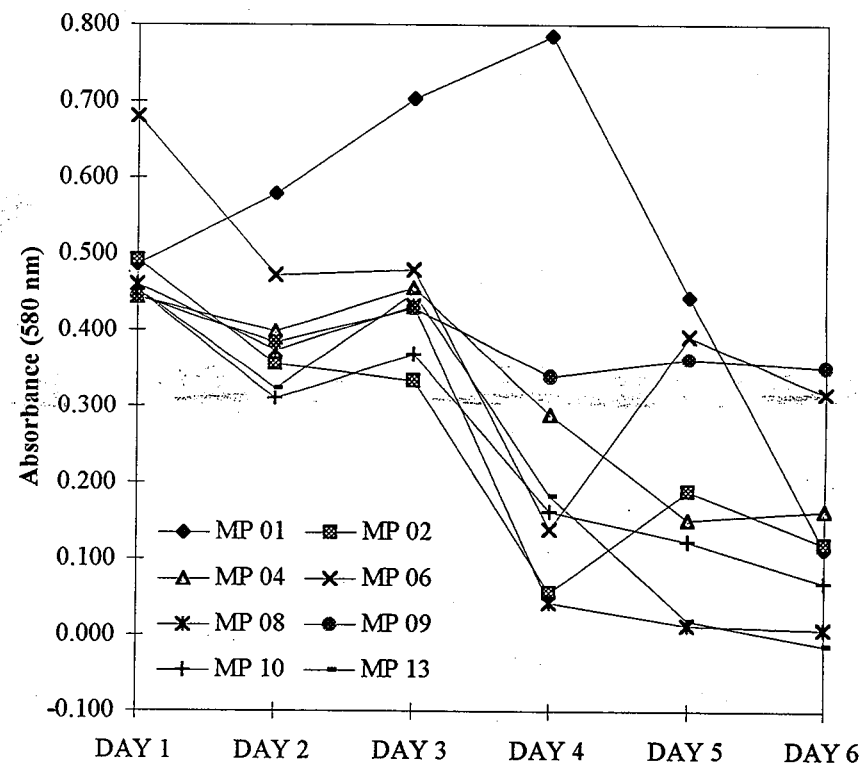
Table - 1 Different species of bacteria isolated from the textile waste water

Code No	Isolated Species
MP 01	*Serratia liquefaciers*
MP 02	*Enterobacter aerogenes*
MP 03	*Pseudomonas putida*
MP 04	*Klebsiella aerogenes*
MP 05	*Bacillus subtilis*
MP 06	*Bacillus pumilus*
MP 07	*Bacillus megaterium*
MP 08	*Bacillus circulans*
MP 09	Unknown *Bacillus* spp
MP 10	*Bacillus alvei*
MP 11	*Bacillus licheniformis*
MP 13	*Bacillus macerans*



[A]

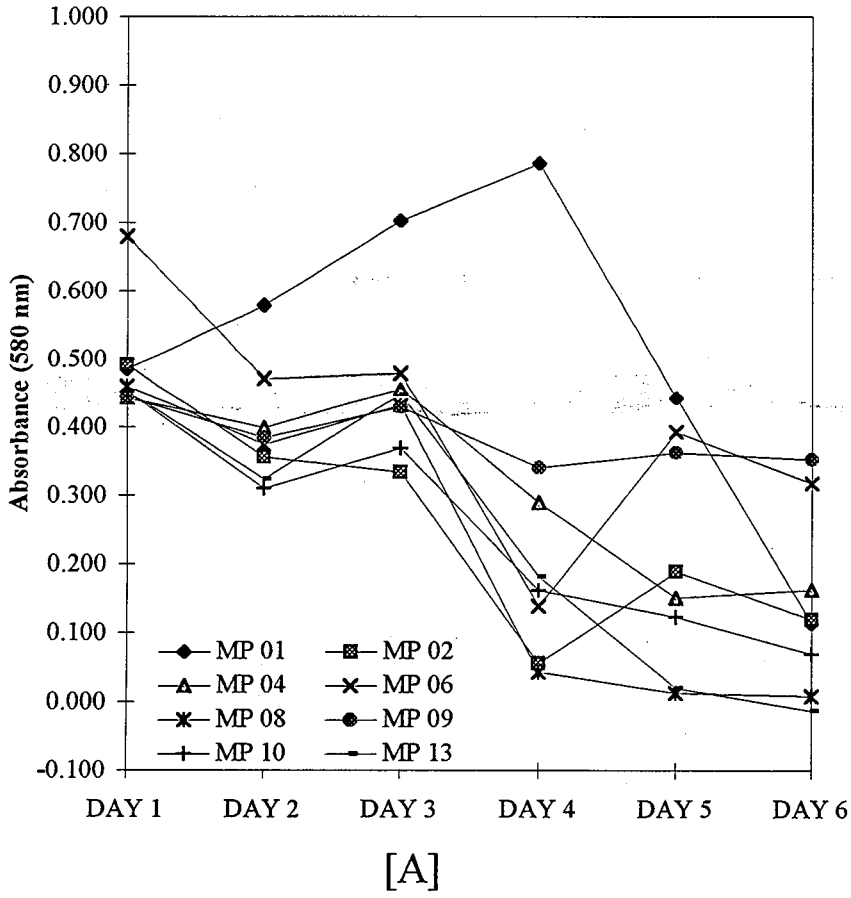


[B]

Fig : 1 [A] Reactive 2 dye eleminating capability of the isolated microorganisms in shaking cultures

[B] Reactive 2 dye eleminating capability of the isolated microorganisms in stand still culutres

MP01 - *Serratia liquefaciers* ; MP02 - *Enterobacter aerogenes* ; MP04 - *Klebsiella aerogenes*
MP06 - Unknown Bacillus spp ; MP08 - *Bacillus circulans* ; MP09 - Unknown Bacillus spp
MP10 - *Bacillus alvei* ; MP13 - *Bacillus macerans*



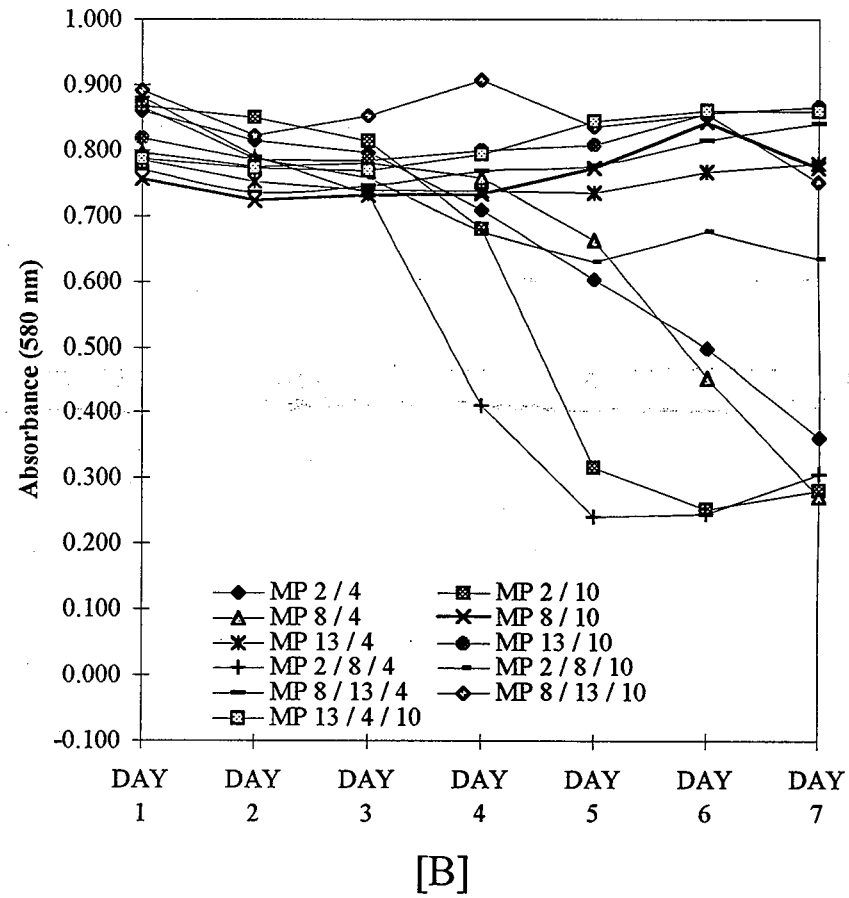


Fig : 2 [A] Reactive Red 2 Dye eleminating capability of the isolated microorganisms in stand still culutres

[B] Reactive Red 2 Dye eleminating capability of the mixed microbial cultures in stand still condition

MP01 - *Serratia liquefaciers* ; MP02 - *Enterobacter aerogenes* ; MP04 - *Klebsiella aerogenes*
MP06 - Unknown Bacillus spp ; MP08 - *Bacillus circulans* ; MP09 - Unknown Bacillus spp
MP10 - *Bacillus alvei* ; MP13 - *Bacillus macerans*

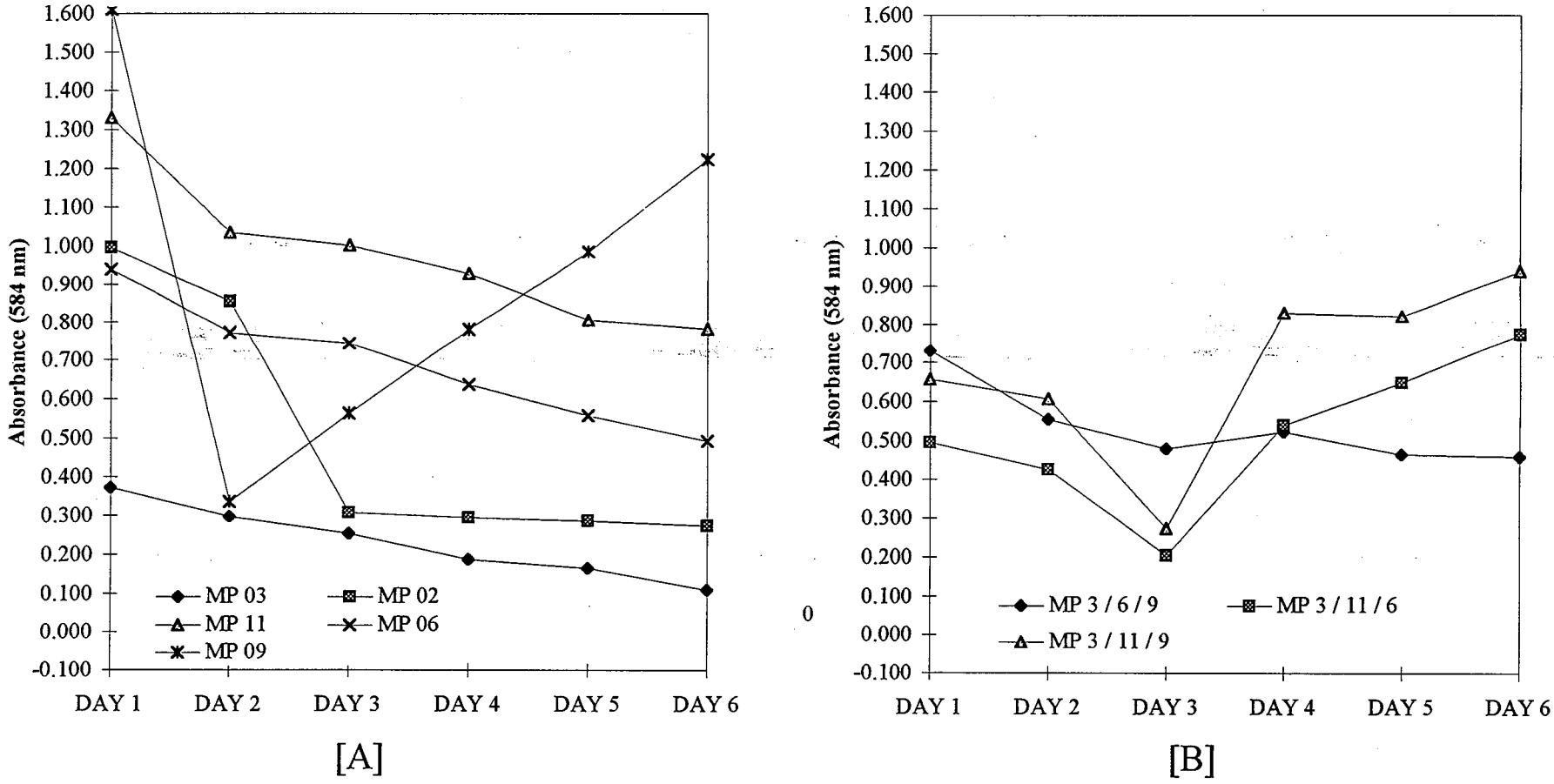
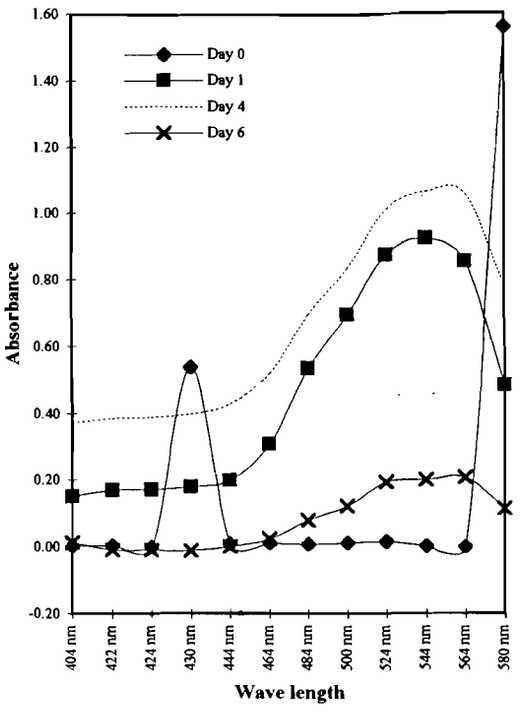


Fig : 3 [A] Acid Blue 25 Dye eleminating capability of the isolated microorganisms in stand still culutres

[B] Acid Blue 25 Dye eleminating capability of the mixed microbial cultures in stand still condition

MP02 - *Enterobacter aerogenes* ; MP03 - *Pseudomonas putida* ; MP06 - *Bacillus pumilus*

MP09 - Unknown Bacillus spp ; MP11 - *Bacillus licheniformis*



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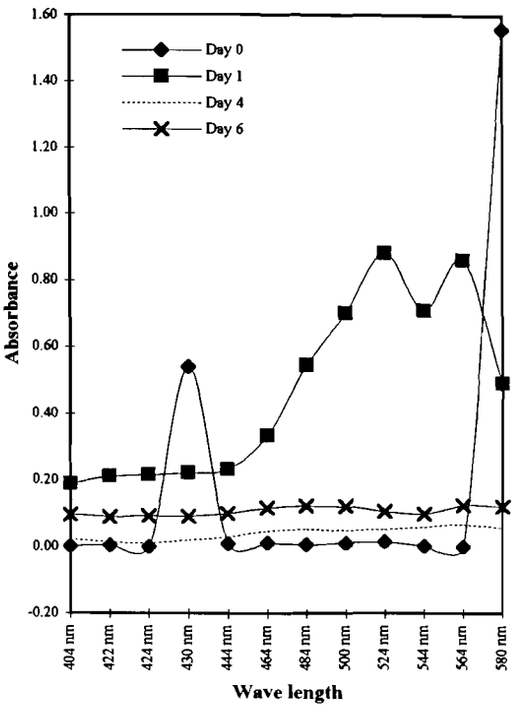

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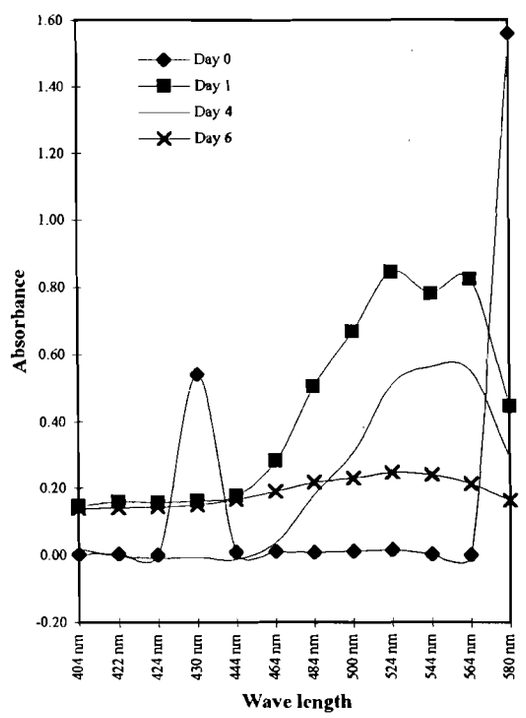

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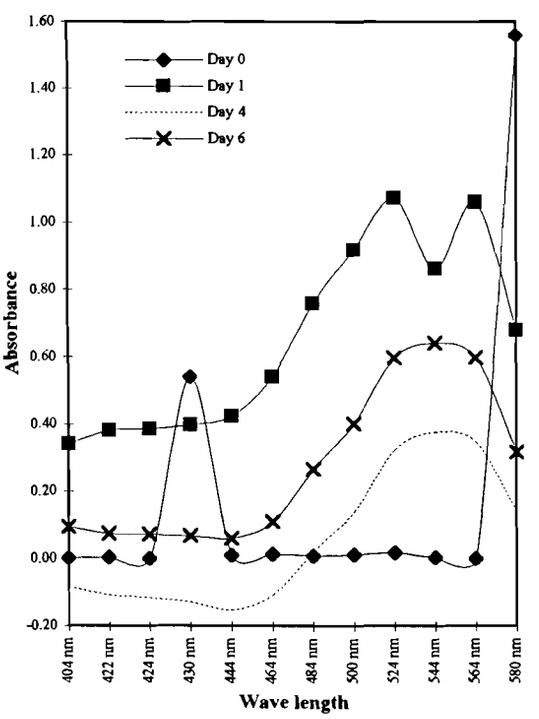

[D]

Fig : 4 Relationship between specific wave length's and the absorbance of
[A] MP01 - *Serratia liquefaciers* ; [B] MP02 - *Enterobacter aerogenes*
[C] MP04 - *Klebsiella aerogenes* ; [D] MP06 - Unknown *Bacillus* spp