

Full Length Research Paper

## Textile effluent treatment by *Bacillus* species isolated from processed food

N.Murugalatha<sup>1\*</sup>, A. Mohankumar<sup>2</sup>, A. Sankaravadivoo<sup>3</sup> and C. Rajesh<sup>1</sup>

<sup>1</sup>Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore-641 028, Tamil Nadu, India.

<sup>2</sup>Department of Zoology, Chikkanna Government Arts and Science college, Tirupur- 637 025, Tamilnadu, India.

<sup>3</sup>Department of Botany, Government Arts College, Coimbatore, Tamilnadu, India

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Colour removal of industrial effluent has been a major concern in waste water treatment, especially for the waste water that originates from textile and dye stuff plant with a continuous discharge of great quantity of remaining dyes to the environment. The efficient treatment of the effluent is an eco- friendly method for the treatment of textile effluent. *Bacillus* species was isolated from the processed food-pickles and were characterized by means of biochemical reactions. Spore formers, non spore formers and their supernatant were used for the treatment. Effluent collected from the textile was diluted to 10, 50 and 90%, were subjected to biological treatment. The level of degradation rate was increased to 87.7 to 93.3% on dilution at 50 and 90% respectively when spore formers were used. The culture supernatant was able to degrade the dye at a rate of 34% with crude effluent and 98 with 90% of diluted effluent. Probably the metabolites produced by the organism were involved in the dye degradation process.

**Key words:** Textile effluent, *Bacillus* species, decolorization, aerobic degradation.

### INTRODUCTION

Synthetic dyes, high in usage, produced world wide every year which has lead the ecosystem blended by the hazardous compounds release at various stages of the operation from the dyeing industry. Textile dyes improves the human lifestyle on a positive account. But on the same side at a negative point they are affecting the environment due to the pollutants given out by them.

Dyes include a broad spectrum of different chemical structure, primarily based on substituted aromatic and heterocyclic groups such as aromatic amine, which is a suspected carcinogen, phenyl and naphthyl. The only thing in common is their ability to absorb light in the visible region. The removal of colour from waste water is often important than the removal of soluble colourless organic substance.

Contributing the major fraction of the biological oxygen demand (BOD) colour is the first contaminant to be recognized in waste water and has to be removed before discharging into water bodies or on land. Presence of colour in dye effluent gives an indication of water being polluted which will damage the receiving water, when

discharged.

The discharge of dyes into the environment impedes light penetration and then toxic to food chain organisms and to aquatic life. Degradation of azo dyes by means of physical and chemical methods in waste water are recently done which are not viable options for treating large waste streams and cost prohibitive (Do et al., 2002; Maier et al., 2004). Living system, especially micro-organism can catalyze the degradation of wastes without disruption of the environment is represented.

Bacteria offers a cheaper and environment friendlier alternative for colour removal in textile effluents (Olukanni et al., 2006). Biological treatment has been effective in reducing dye house effluents and when used properly has a lower operating cost than other remediation process. Anaerobic and aerobic reduction of azo dyes to simpler compounds have all demonstrated the ability of microbes and sludges to effectively reduce azo dyes to their intermediate structures, thus destroying the apparent colour (Chinwetkitvanich et al., 2000) and Razo-Flores et al., 1997). Reduction of dye compounds to their intermediates reduces the aesthetic pollution but a larger and more deleterious problem may be created (Wallace, 2001).

Aerobic treatment of azo dye wastes though effective,

\*Corresponding author. E-mail: [navikal123@gmail.com](mailto:navikal123@gmail.com).

is often the typical method of treatment. Recent combination of chemical and biological, physical and biological treatment has also proven to be effective (Seshadri et al., 1994; Horning, 1977). This research explores the ability of bacteria-*Bacillus sp* isolated from pickles for the treatment of a textile effluent containing indigo blue dye. The present investigation was taken up to screen the microorganisms *Bacillus sp* for decolourisation from home made pickles.

## METHODOLOGY

### Sources of the organism

Pickles of home made was collected from the local area, homogenized, appropriate dilution was made, plated on nutrient agar media, incubated at 37°C for 24 h. All isolations were done on nutrient agar using enrichment culture techniques and the organisms identified to the generic level using the Cowan and Steel (1993) Scheme. Two different isolates were considered for our present study; (1). *Bacillus sp.*, a spore former and (2). *Bacillus sp.*, a non spore former.

### Effluent treatment with isolated bacteria

#### Collection of effluent

The effluent was collected from the dyeing industry near Tirupur and was stored under appropriate conditions for further use.

#### Determination of BOD of effluent

The BOD bottle was filled with the sample (effluent from dyeing industry) up to the rim and 2 ml of MgSO<sub>4</sub> and alkaline iodide azide solution was added and closed, the solution was mixed by inverting the bottle, then it is checked for the formation of brown precipitate. 2 ml of sulphuric acid was added along the sides of the bottle to dissolve the precipitate formed. From this 100 ml was taken and titrated against sodium thiosulphate taken in the burette. The color change of the solution to yellow was noted after which 2 ml of starch solution was added. The titration was continued by adding thiosulphate solution in drops till the disappearance of blue colour. The experiment was repeated to obtain concordant value. The BOD bottle was filled with the sample and incubated for 5 days and the OD values were calculated to find BOD value.

$$\text{BOD} = \text{DO}_b - \text{DO}_a$$

DO<sub>bbn</sub> = Dissolved oxygen of sample before incubation, DO<sub>a</sub> = Dissolved oxygen of sample after incubation.

BOD procedure was repeated for the sample which was inoculated with respective cultures. The values were noted and tabulated.

#### Determination of biodegradation activity

##### Potential decolourization of the stimulated effluent by each isolate was investigated

The crude effluent and diluted effluent to 10, 50 and 90% with distilled water was taken as samples. Into 20 ml stimulated diluted

effluent,  $2 \times 10^5$  CFU of the isolate was added and poured into transparent bottles (200 mg/L starch and 250 mg/L yeast extract were added as co-substrates) and cocked with sterile cotton wool. After 14 and 90 days decolourization was measured. Decolourization was determined by measuring the absorbance of the stimulated effluent at the effluent pre-determined  $\lambda$  max (485 nm) and the absorbance of the treated stimulated effluent. The percentage decolourization was calculated as

$$\left[ \frac{(A_0 - A_t)}{A_0} \right] \times 100\%$$

A<sub>0</sub>-absorbance of the stimulated effluent

A<sub>t</sub>-absorbance of the treated stimulated effluent 14 days post microbial inoculation.

### Effluent treatment with supernatant of bacteria

Nutrient broth was prepared in a conical flask sterilized. It was allowed to cool. A loop full of bacterial culture was added to nutrient broth. It was incubated respectively. The following day the broth was dispensed into sterile centrifuge tubes centrifuged at 10,000 rpm for 15 min. The pellet was discarded and the supernatant was collected in a sterile beaker. To the 100 ml sample (effluent) 5 ml of supernatant is added using sterile pipette for bacteria. These flasks were also incubated accordingly to note the color change.

### Plasmid screening

About 0.1 ml of the bacterial culture was dispensed into in eppendorf tube and centrifuged at 10000 rpm for 15 - 20 min and the supernatant was discarded. To the pellet 350  $\mu$ l of STET buffer was added and mixed well. Centrifuged at 10,000 rpm for 10 min, pellet was removed using sterile tooth pick then added 40  $\mu$ l of 3 M sodium acetate and 420  $\mu$ l of isopropanol. Centrifuge at 10,000 rpm for 10 min. Finally the pellet was suspended in 20  $\mu$ l of distilled water or TE buffer and electrophoresis on agarose gel to find the presence of plasmid DNA.

## RESULTS

A total of ten isolates were obtained belonging to the genus *Bacillus* from the source pickle and were named as HB1, HB2, and HB3 respectively. Gram reactions of the isolates were observed in which all the isolates were gram positive. They were found to be rod shaped and grew under aerobic conditions. Few were able to ferment carbohydrates and almost all strains hydrolyzed starch, catalase positive and were oxidase negative. The isolates HB1, HB5, HB8 and HB10 were able to produce intracellular structures like spores.

BOD of the effluent on the initial day and on the fifth day was observed for the crude effluent and treated effluent and calculated. The BOD value was found to be reduced to 5.6. Biodegradation with the isolates and with their supernatant showed colour removing activities in the diluted effluents between 40.74 and 47.73%. The colour of the crude effluent was reduced to 25 - 35% after 14 days incubation. But complete degradation of dye was not possible within 90 days of incubation in crude effluent as evident in the lack of colour change. When dilution of

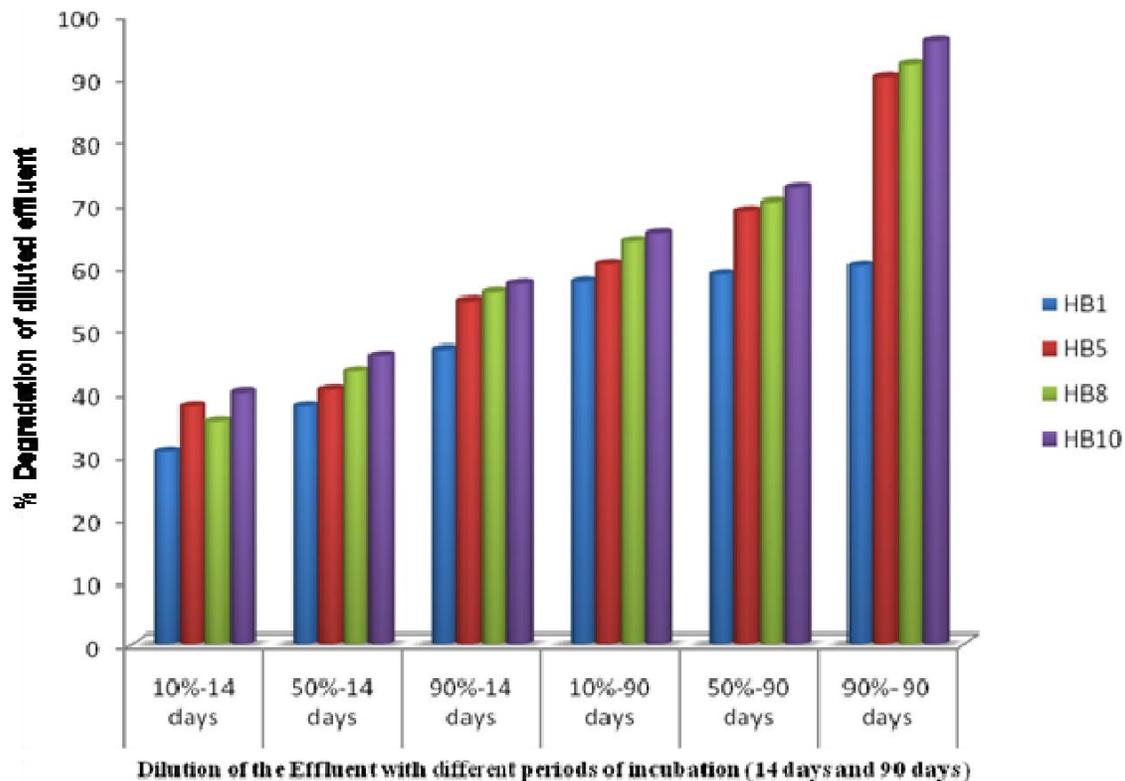


Figure 1. Endospore forming Bacillus treated effluent.

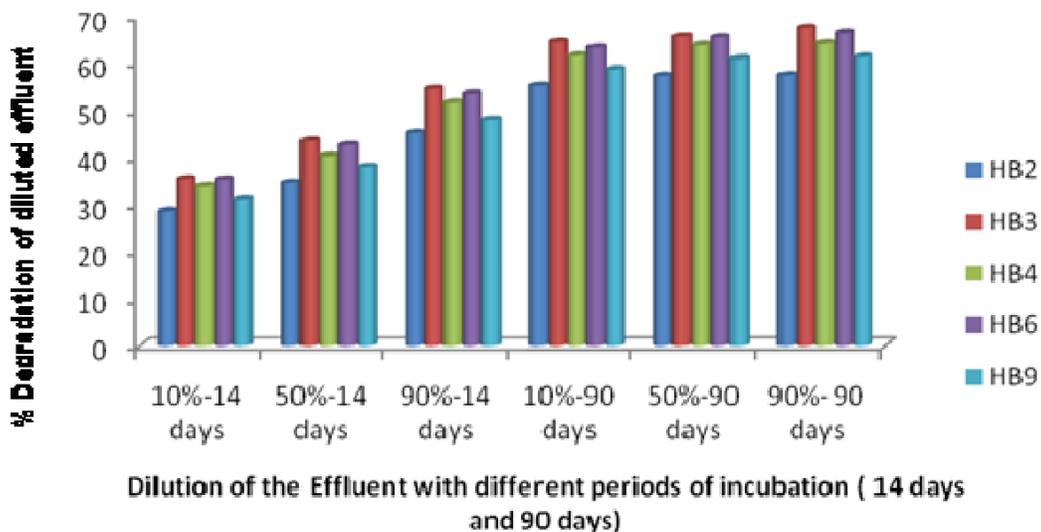


Figure 2. Non-endospore former Bacillus treated effluent.

the effluent was increased the decolourization of effluent was high by increase in activity of bacteria.

Degradation activity was compared with the spore formers and non spore formers. The latter showed better degradation level than the previous one (Figures 1 and 2). The level of degradation rate was increased to 72.3 to

95.63% on dilution at 50 and 90% respectively in case of spore forming isolates when compared to the non spore formers. HB10 spore formers showed the highest degradation level than any other spore formers (Figure 1). In case of non spore formers thought the level of degradation was very less. HB3 showed the highest

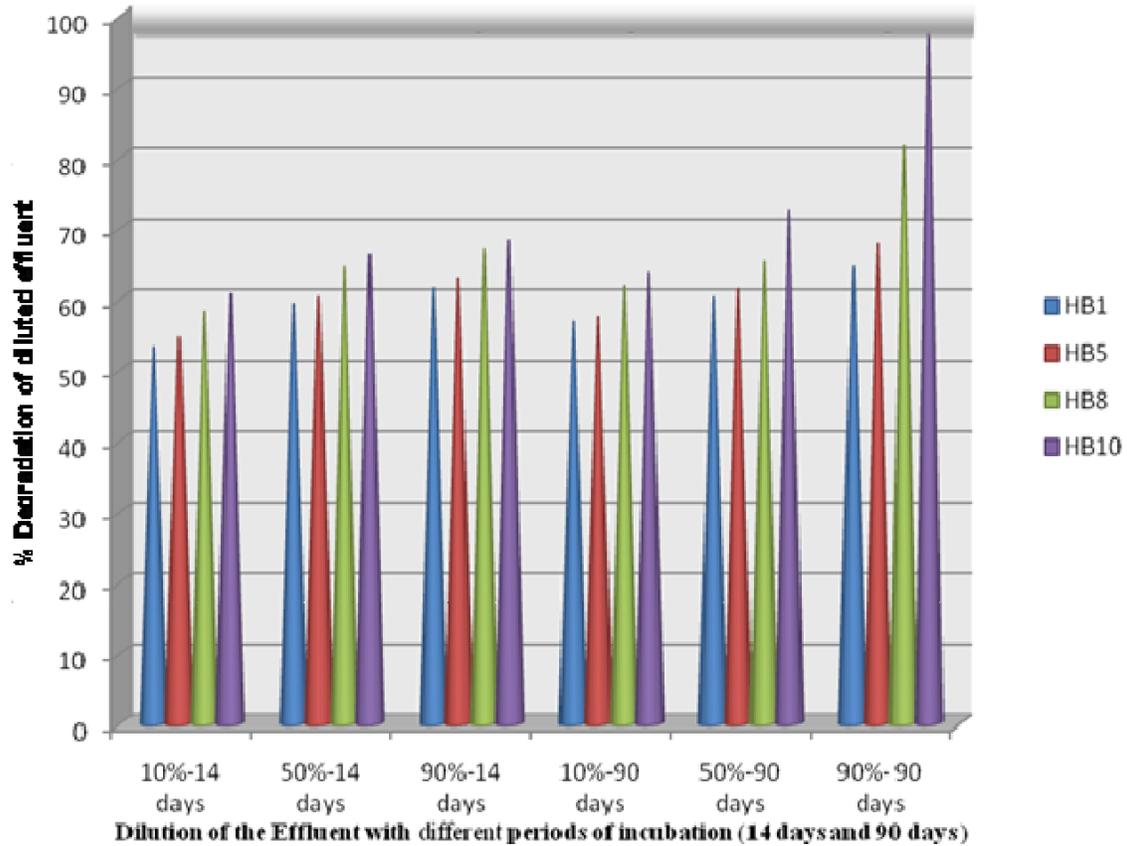


Figure 3. Effluent treatment using culture supernatant of endospore formers.

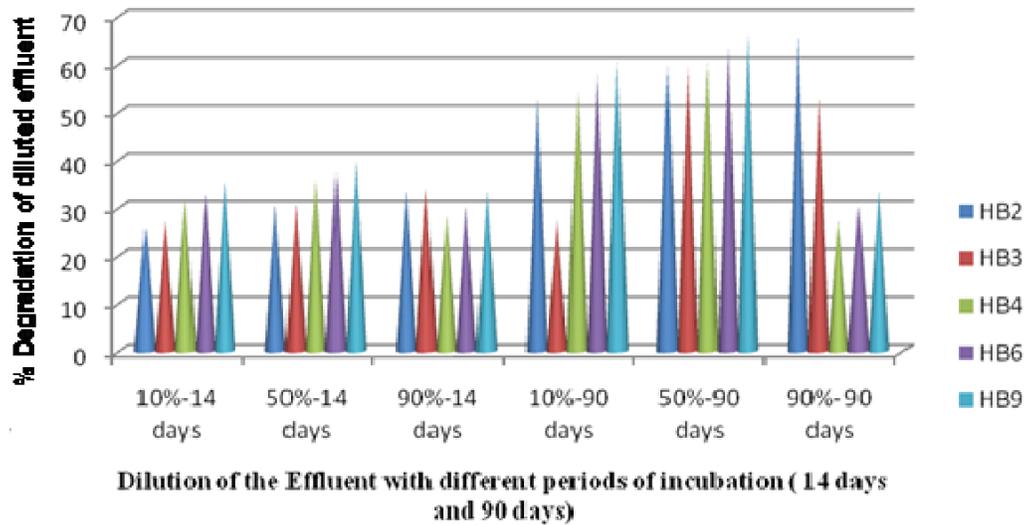


Figure 4. Effluent treatment using culture supernatant of non-endospore formers.

degradation level at 90% dilution after 90 days (Figure 2). The culture supernatants of non endospore formers and endoformers also shared the same results as above. The supernatants of endospore formers degraded the dye at

very better level than compared to the non endospore formers (Figure 3). Culture supernatants of non-endospore showed better degradation during initial days at 10 and 50% rather than at 90% dilution (Figure 4). When the

culture supernatant was used for the dye degradation, the crude extract reduction rate was found to be 34%. Probably the metabolites produced by the organism were involved in the dye degradation process. Plasmids were also detected from the isolates. Dye degrading genes may be of plasmid oriented or chromosomal oriented which can be our future work.

## DISCUSSION

During the dying process a substantial amount of dyes and other chemicals are lost in waste water. Estimates put the dye loses between 10 - 15% (Vaidya and Datye, 1982). Dye is generally not toxic to the environment but the colour water bodies may hinder high penetration there by affecting the aquatic life and limiting the utilization (Ajaiyi and Osibanjo, 1980). Color removal of industrial effluent has been a major concern in waste water that originates from textile and dye stuff plant with a continuous discharge of great quantity of remaining dyes to the environment. The efficient treatment of the effluent is an eco-friendly method for treatment of textile effluent. The degradation of molecules of dyes in the environment by microorganisms is likely to be slow, which means that it is possible for high levels of dye to persist and potentially accumulate. Due to the low degradability of the dyes, conventional biological treatment process is inefficient in treating dye waste waters. Biological decolorization is employed under either aerobic or anaerobic environment. A number of reports discourage the azo dye decolorization by microorganism under anaerobic conditions as it leads to the formation of corresponding aromatic amines. The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms. Over the past decades, many microorganisms are capable of degrading azo dyes, including bacteria, fungi and yeast.

In our present study the *Bacillus* isolates from home made pickle played a major role in degrading the effluent. Upon dilution and inoculation with isolated microorganisms, both spore and non-spore forming organisms were able to show the dye degradation at a maximum level within 90 days of incubation at a highest dilution when compared to 14 days of incubation under aerobic conditions. Ajibola et al. (2005) has stated that *Bacillus subtilis* reduced the colour of the crude effluent by 25%. Effluent when diluted, the activity of the bacteria increase the rate of decolourisation. Endospore forming isolates showed its high level of degradation both when whole cells were used and when the supernatant was

used. Spore forming obligate aerobes under unfavorable conditions could form spores which might reduce its metabolic activities to a minimum (Ajibola et al., 2005). *Bacillus cereus* was not able to degrade crude effluent because of the environment conditions of the effluent sample. Increase in dilution of the effluent activated the bacteria more in decolorization (Ajibola et al., 2005). In the current investigation, aerobic effluent treatment has involved in decolorization of the dye though it has been stated that anaerobic effluent treatment has given a better reduction of dyes in previous research work. Effective treatment was obtained with spore forming *Bacillus* when compared to non- spore forming organisms.

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