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Biodegradation of Indigo Containing Textile Effluent Using Some Strains of Bacteria

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Abstract: The ability of *Staphylococcus aureus*, *Bacteroides fragilis*, *Bacillus subtilis*, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli* and *Peptostreptococcus* spp. to reduce and stabilize textile effluents containing predominantly indigo blue was carried out. The primary objective was to reduce the colour of the effluent to an intensity that complies with the Environmental Protection Agency limits for discharge to surface water. Maximum color removal was achieved with anaerobic biodegradation during 45 days of incubation, while aerobic degradation took 90 days. It was found that *Peptostreptococcus* spp. among the anaerobes and *E. coli* among the aerobes ranked best among the bacteria strains used. In general however, aerobic microbes do not have the ability to substantially decolorize the textile effluent but the converse applies to anaerobic microbes. This result shows that the sequential use of both can completely decolorize effluents containing indigo dye.

Key words: Textile effluent, decolorization, aerobic, anaerobic degradation

INTRODUCTION

Indigo dyes are used by a wide number of industries. While textile mills predominantly use them, indigo dyes can also be found in the paper and printing, leather and cosmetics industries. It is not surprising that these compounds have become a major environmental concern[1]. Many of these dyes find their way into the environment via wastewater from textile industries and retain their color and structural integrity for a long time in the environment. Currently, much research has been focused on chemically and physically degrading azo dyes in wastewaters[1,2]. These methods are cost prohibitive and therefore are not viable options for treating large waste streams.

Biotechnology presents opportunities to detoxify industrial effluents. The treatment of textile effluents is of interest due to their toxic and aesthetic impacts on receiving waters. While much research has been preformed to develop effective treatment technologies for wastewaters containing azo dyes, no single solution has been satisfactory for remediating the broad diversity of textile wastes[1].

The anaerobic reduction of azo dyes to simpler compounds have all demonstrated the ability of anaerobic microbes and sludges to effectively reduce azo dyes to their intermediate structures, thus destroying the apparent color[2-4]. 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[1]. Anaerobic reduction using microbial sludges can be an effective and economic treatment process for removing color from textile effluents. The ability of anaerobic bacteria to reductively cleave the azo linkages in reactive dyes have been reported[5-7]. However, aerobic treatment of azo dye wastes are ineffective in most cases, but is often the typical method of treatment used of recent[8].

Biological treatment has been effective in reducing dyehouse effluents and when used properly has a lower operating cost than other remediation processes. Combinations of chemical[9] and biological or physical and biological treatment have also proven to be effective[10,11]. This research further explores the ability of some bacteria for the treatment of a textile effluent containing mainly indigo blue dye.

MATERIALS AND METHODS

Culturing of bacteria and inoculation of effluent samples: Textile effluents were collected in Kaduna, Nigeria in February 2004 from a Textile Mill that uses indigo dye in the manufacture of wax fabrics. The bacteria were cultured on different media prepared according to manufacturer's instruction[12]. The cultured anaerobes were then separately introduced into the effluent sample and other dilutions of the effluent containing 10 and 50% of the effluent using a sterilized loop carrying the

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inoculum. The jar was closed and the air evacuated via one of the two taps in the lid by means of a vacuum pump, while hydrogen was allowed to flow in through the other tap. The jar was then placed in an incubator set at 37°C and allowed to stay for 45 days in case of anaerobic degradation and 90 days in case of aerobic degradation.

At the end of the incubation periods, the colour of the degraded samples were measured using a Lovibond colour comparator[13].

RESULTS AND DISCUSSION

The results obtained from the chemical analysis of the effluent showed that it was alkaline with a pH of 9.80 and colour of 400 hazens (Table 1). *Clostridium perfringens* an obligate anaerobe was only able to decolorize the crude effluent (pH = 9.80) by 67.7% for the period of time it was incubated in the effluent (Fig. 1). The colour was reduced by a half when the effluent was diluted by 50% (pH = 8.28, colour = 235 hazens) with distilled water and at 90% dilution (pH = 8.05, colour = 47 hazens), 58.8% reduction in the colour was achieved. This suggests that the action of *C. perfringens* was not influenced by dilution of the effluent suggesting that greater performance was at relatively high pH. *Bacteroides fragilis* was able to reduce the colour of the crude effluent by 67.7% for the same period of incubation as *C. perfringens*. *Bacteroides fragilis* which respires anaerobically was found to decolorize the effluent more at increased dilution (50 and 90%), where colour reduction of 87.7 and 93.3% were achieved, respectively. However, unlike *C. perfringens*, a higher decolorization was achieved by *B. fragilis* at higher dilutions.

Table 1: Some quality parameters of crude textile effluent

Parameters	
pH	9.80
Conductivity (dS m^{-1})	133.20
Colour (Hazens)	400*
Dissolved Oxygen (mg L^{-1})	8.60
Biological Oxygen Demand (mg L^{-1})	15.40
Alkalinity (mg L^{-1})	480.00
Hardness (mg L^{-1})	364.00
Sulphide (mg L^{-1})	270.00

* 10% and 50% dilution gave colour of 235 and 47 hazens respectively

Table 2: Freidman Rank Test for the different bacteria used

Type of bacteria	Freidman rank
Anaerobic	
Bacteriodes fragilis	2.00
Peptostreptococcus spp.	2.33
Clostridium perfringens	1.67
Aerobic	
Bacillus cereus	1.33
Bacillus subtilis	1.67
Staphylococcus aures	3.00
Escherichia coli	4.00

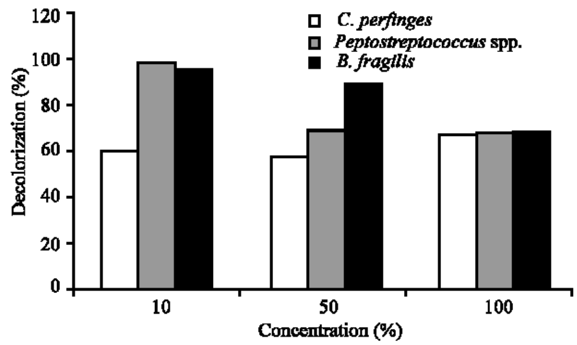

Fig. 1: Percentage decolorization at different concentration of effluent by anaerobic bacteria

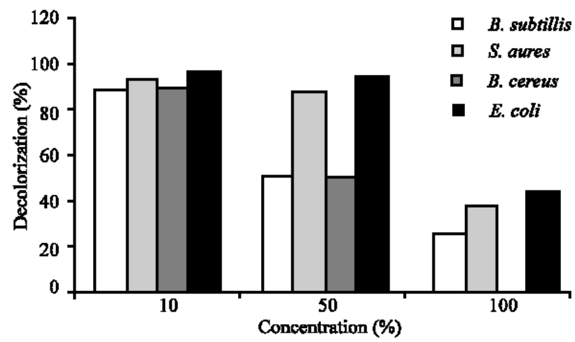

Fig. 2: Percentage decolorization at different concentration of effluent by aerobic bacteriz

Peptostreptococcus spp. reduced the colour of the crude sample by 67.5% as *B. fragilis*. On dilution of the effluent an almost colourless effluent was obtained at 90% dilution amounting to 96.7% decolorization. *Bacteroides fragilis* appeared to have a similar mode of action on the effluent, except that *Peptostreptococcus* spp. decolorized the effluent to a much greater extent than *B. fragilis*.

Among the aerobic bacteria used in this study *Straphylococcus aureus* was able to reduce the colour of the crude effluent 37.5% after 90 days of incubation in the crude effluent. This strain of bacteria appears to be less active at higher pH of the effluent as evident in the results obtained when the effluent was diluted by 50 and 90%, respectively (Fig. 2). *Bacillus subtilis* reduced the colour of crude effluent by 25% and the activity of the bacteria increased as the effluent was diluted. *Bacillus cereus* was not able to reduce the colour of the crude effluent within 90 days of incubation as evident in the lack of colour change. *Bacillus cereus* is a spore forming obligate aerobe and could form spores under this unfavourable conditions hence reducing its metabolic activities to a minimum. This may explain why *B. cereus* was not able to degrade the crude effluent, because of the environmental conditions of the effluent sample. However, with increased dilution of the effluent the bacteria became more active in the decolorization of the effluent. *Escherichia coli* is a typical coliform that carries out aerobic respiration and are capable of carrying out nitrate respiration anaerobically[12]. *Escherichia coli* was able to reduce the colour the crude sample by 48.4%, while for 50 and 90% dilution the effluent, 95 and 97% decolorization was achieved, amounting to almost complete decolorization.

Because aerobic microbes cannot reduce azo linkages, their ability to destroy dye chromogens at short period of time is less than the anaerobic bacteria. The wastewater pH appeared to affect the proper functioning of both anaerobic and more so the aerobic organisms because the bacterial cultures were unable to proliferate properly in the crude effluent sample[14]. This shows that type of bacteria or consortium used for dye biodegradation may affect the reduction rate.

Non-parametric ranking of the efficiency of the individual bacteria used were performed using the Freidman Rank Test. From Table 2 it was found that *Peptostreptococcus* spp. performed better than the other two bacteria used in anaerobic decolorization of the effluent while *E. coli* was ranked best among the aerobic bacteria used. The result obtained shows that sequential use of *Peptostreptococcus* spp. and *E. coli* should effectively decolorize an indigo-rich textile effluent. However, because *B. fragilis* performed about equally with *Peptostreptococcus* spp. at a higher pH, it could serve as an alternative to be used with *E. coli*.

CONCLUSIONS

Anaerobic and aerobic treatment of the textile effluent were both effective in reducing the wastewater color. However, anaerobic treatment generally produced the greatest color removal at a much shorter time. *Peptostreptococcus* spp. and *B. fragilis* because of their relatively higher pH-tolerance can be used together with *E. coli* for the decolorization of textile effluents containing indigo dye.

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