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Full Length Research Paper

Textile effluent biodegradation potentials of textile effluent-adapted and non-adapted bacteria

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Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and the dwindling supply has made the treatment and reuse of industrial effluents an attractive option. Textile effluents are of concern because they colour the drains and ultimately the water bodies. They also diminish the water quality. The ability of microorganisms to degrade and metabolize a wide variety of compounds has been recognized and exploited in various biotreatment processes. This study investigated the potential of bacteria isolated from textile industries wastewater and drains (textile effluent adapted bacteria) and isolates from a municipal landfill (effluent non-adapted bacteria). We discovered effluent adapted strains of *Acinetobacter, Bacillus* and *Legionella* with potentials for colour removal and strains of *Acinetobacter, Bacillus and Pseudomonas* with chemical oxygen demand (COD) removal activities. Only strains of *Bacillus* with potentials for use in colour and COD removal were isolated from the landfill. Plasmid screening did not reveal the presence of plasmids in the isolates. Thus the involvement of extra-chromosomal genes is not suggested. In conclusion, as a preliminary step in the development of textile effluent biotreatment using indigenous microbes, we have discovered some strains with potency to decolourize and/or remove COD.

Key words: Textile effluent, dyes, biodegradation, decolourization, COD removal.

INTRODUCTION

Industrialization is vital to a nation's economy because it serves as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment. Many of these products are problematic because of persistence (low biodegradability) and/or toxicity.

The textile industries produce effluents that contain several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes (Cooper, 1995.). In many Nigerian cities, the textile factories daily discharge millions of litres of untreated effluents in the forms of wastewater into public drains that eventually empty into rivers (Olayinka and Alo, 2004). This alters the pH, increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and gives the rivers intense colourations (Ajayi and Osibanjo, 1980). The use of these water resources is limited and the ecosystem is affected.

Several methods are used in the treatment of textile effluents to achieve decolourization. These include physiochemical methods such as filtration, specific coagulation, use of activated carbon and chemical flocculation. Some of these methods are effective but quite expensive (Do et al., 2002; Maier et al., 2004). Biotreatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluents. The ubiquitous nature of bacteria makes them invaluable tools in effluent biotreatment.

The chemical nature of dyes varies, but azo dyes are the most widely used. The oxidative decolourizations of dyes of several classes have been reported and azo dyes were found to be the most recalcitrant compounds. (Maeir et al., 2004). The decolourization of azo dyes has been found to be effective under anaerobic conditions.

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However, the anaerobic degradation yields aromatic amines which are mutagenic and toxic to humans and cannot be metabolized further under the conditions which generated them (Chung and Stevens, 1993; Do et al., 2002). In activated sludge treatments of dye effluents, reactive azo dyes and aromatic amino derivatives are a non-biodegradable class of compounds which can even inhibit activated sludge organism (Maeir et al., 2004). It is thus important to explore the possibilities of isolating efficient aerobic degraders for use in decolourization and biotreatment of textile effluents.

In a bid to exploit the biodegradation abilities of our indigenous microbial flora for remediative purposes, we isolated and screened organisms for the ability to decolourize and/or reduce the COD of textile effluents. The isolates were also screened for plasmids in order to determine the contribution of extra-chromosomal genetic factors to the degradative ability. The study is aimed at discovering isolates with the potential for use in biological treatment of textile effluents.

MATERIALS AND METHODS

Materials

All chemicals used are of analytical grade. The reactive dyes used: Orange P3R, Yellow P3R, Blue H5R, Violet P3R, Brown P5R, Black V3R, Orange P2R were kindly donated by one of the textile companies whose effluent was used as a source of effluent adapted bacteria.

Sterilization techniques

All glasswares were washed with detergent, rinsed thoroughly with distilled water and oven sterilized at 80°C for 2.5 h. All polypropylene tubes and tips used as well as media and solutions prepared were sterilized by autoclaving at 121°C for 15 - 25 min. Inoculations were done with flame sterilized loops and all experiments were performed wearing sterile disposable hand gloves.

Sources of organisms

Textile effluent-adapted bacteria were isolated from effluent samples collected from the discharge and drainage pipes of three textile industries in the Oshodi/Isolo Local Government area of Lagos, Nigeria. The textile effluent non-adapted bacteria were isolated from soil samples taken from a municipal landfill. All isolations were done on nutrient agar using enrichment culture techniques and the organisms identified to the generic level using the Cowan and Steel Scheme (1993).

Preparation of simulated effluent

A stock dye solution was prepared with 80 mg of each of the seven dyes used per L; giving a concentration of 5.6 g dyes mix/L. To prepare the simulated effluent the stock solution was supplemented with modified minimal medium (Mills et al., 1978) to get a final concentration of 56 mg/L.

Determination of biodegradation activity

Potential decolourization and COD removal of the simulated effluent by each isolate were investigated. Into 20 ml simulated effluent 2 x 10^5 cfu of the isolate was added in 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 days decolourization and COD removal were measured. Decolourization was determined by measuring the absorbance of the simulated effluent at the effluent pre-determined λ max (485 nm) and the absorbance of the treated simulated effluent. The % decolourization was calculated as $[(A_o - A_t)/A_o]$ x 100%, Where A_o is absorbance of the simulated effluent and A_t the absorbance of the treated simulated effluent 14 days post microbial innoculation. The COD of the simulated and treated effluents were determined by a standard spectroscopic method (APHA, 1980).

Plasmid screening

The isolates were screened for plasmids using the plasmid isolation technique described by Kado and Liu (1981) followed by electrophoresis on 0.8% agarose gels. The gels were stained with ethidium bromide and viewed on a UV transilluminator.

RESULTS

Source and identity of isolates

A total of 24 isolates were obtained; eighteen organisms belonging to the genera, *Bacillus, Acinetobacter, Legionella, Staphylococcus* and *Pseudomonas* were isolated from the textile effluents (effluent-adapted bacteria) while six isolates belonging to the genus *Bacillus* were isolated from the landfill site (effluent non-adapted isolates). The sources and identity of the isolates are shown on Table 1.

Biodegradation

The majority of the effluent adapted isolated showed colour-removing activities between 40.74 and 47.73% while the others had activities of between 17.91 and 36.69%. For the effluent adapted bacteria, isolates of *Acinetobacter, Legionella* and *Bacillus* may be potentially useful in decolourization processes, whereas the six non-adapted *Bacillus* isolates seem to be potentially useful (Table 2). The non-adapted isolates had decolourization activities of between 40.25 and 46.63% (Table 2). This was similar to that of the majority of the effluent adapted organisms.

The non-adapted *Bacillus* isolates had a relatively higher mean % COD removal when compared to the adapted microbes of the same species (Table 3) or to the mean % COD removal for all the adapted isolates (Table 4). Only effluent-adapted isolates of *Acinetobacter, Pseudomonas* and *Bacillus* species have relatively high COD

Table 1: Source and identity of bacteria isolates.

LAB. #	Grams reaction	Shape	Aerobic growth	MacConkey growth	Lactose fermentation	Endospore	Motility	Catalase	Oxidase	Identity (Genus)
T1	-	S	+	+	+	-	-	+	-	*Acinetobacter*
T2	-	S	+	+	+	-	-	+	-	*Acinetobacter*
T3	-	R	+	+	-	-	+	-	+	*Legionella*
T4	+	R	+	+	+	+	+	+	-	*Bacillus*
T5	-	R	+	+	-	-	+	+	+	*Pseudomonas*
T6	+	S	+	+	+	-	-	+	-	*Staphylococcus*
T7	+	R	+	+	+	+	+	+	-	*Bacillus*
T8	+	R	+	+	-	+	-	+	-	*Bacillus*
T9	+	S	+	+	+	-	-	+	-	*Staphylococcus*
T10	-	R	+	+	+	+	-	+	w	*Bacillus*
T11	+	R	+	+	+	-	+	+	-	*Bacillus*
T12	-	S	+	+	+	-	-	+	-	*Acinetobacter*
T13	-	R	+	+	+	+	-	+	-	*Bacillus*
T14	+	R	+	+	-	+	-	+	-	*Bacillus*
T15	+	R	+	+	+	-	-	+	-	*Bacillus*
T16	-	R	+	+	-	+	-	+	-	*Bacillus*
T17	+	R	+	-	*ND*	+	+	+	-	*Bacillus*
T18	+	R	+	-	*ND*	-	+	+	-	*Bacillus*
N1	+	R	+	+	+	*ND*	+	+	-	*Bacillus*
N2	+	R	+	+	+	*ND*	+	+	-	*Bacillus*
N3	+	R	+	+	+	*ND*	+	+	+	*Bacillus*
N4	+	R	+	+	+	*ND*	+	+	-	*Bacillus*
N5	+	R	+	-	*ND*	*ND*	+	*ND*	-	*Bacillus*
N6	+	R	+	-	*ND*	*ND*	+	*ND*	-	*Bacillus*

+ Positive
- Negative
w weak reaction
ND Not determined
R Rod shape
S Spherical
T Textile adapted strains
N Non – adapted strains

removal activities while all the non-adapted isolates have high COD removal capabilities (Table 3).

Plasmid screening

Plasmids were not detected in any of the isolates from the effluent adapted or non-adapted sources.

DISCUSSION

The textile industries are multi-chemical utilizing concerns of which dyes of various types are of importance. During the dyeing process a substantial amount of dyes and other chemicals are lost in the waste water. Estimates put the dye losses at between 10–15% (Vaidya and Datye, 1982). Though not generally toxic to the environment, dyes colour water bodies and may hinder light penetration thereby affecting aquatic life and limiting the utilization (Ajayi and Osibanjo, 1980; Goncalves et al., 2000). It has been reported that a typical textile effluent contains a dye mass concentration of 10–50 mg/L (Clarke and Anliker, 1980). However, the human eye can detect levels as low as 0.005 mg/L of reactive dyes in a clear river (Pierce, 1994). In our study a simulated effluent with a dye mass of 56 mg/L was used. The simulated effluent was supplemented with starch and yeast extract to provide nutrients for biomass maintenance and to enhance biodegration (Do et al., 2002, Padmavathy et al., 2003).

Table 2. Biodegradation of simulated textile effluent under aerobic condition.

Lab. #	Identity (Genus)	COD mgl^{-1}			% Decolourization
		Initial	Final	% Removal	
T1	*Acinetobacter*	1038	474	54.35	47.61
T2	*Acinetobacter*	1038	575	44.61	46.75
T3	*Legionella*	1038	745	28.23	46.50
T4	*Bacillus*	1038	788	24.08	47.24
T5	*Pseudomonas*	1038	602	42.00	36.69
T6	*Staphylococcus*	1038	632	39.11	35.58
T7	*Bacillus*	1038	618	40.46	34.72
T8	*Bacillus*	1038	800	22.93	31.29
T9	*Staphylococcus*	1038	814	21.58	17.91
T10	*Bacillus*	1038	832	19.85	35.83
T11	*Bacillus*	1038	511	50.77	36.69
T12	*Acinetobacter*	1038	614	40.85	47.73
T13	*Bacillus*	1038	726	30.06	44.79
T14	*Bacillus*	1038	625	39.79	43.07
T15	*Bacillus*	1038	415	60.02	46.99
T16	*Bacillus*	1038	378	63.58	44.54
T17	*Bacillus*	1038	452	56.45	40.74
T18	*Bacillus*	1038	432	58.38	46.50
N1	*Bacillus*	1038	420	59.54	44.05
N2	*Bacillus*	1038	414	60.12	44.29
N3	*Bacillus*	1038	520	49.90	42.82
N4	*Bacillus*	1038	495	52.31	40.25
N5	*Bacillus*	1038	432	58.38	45.64
N6	*Bacillus*	1038	466	55.11	46.63

Table 3. Mean biodegradative activities of the isolated genera.

Genus	N	% COD removal [#]	% Decolourization[#]
Acinetobacter	3	46.60 ± 4.03	47.36 ± 0.31
Bacillus			
T - Strains	11	42.40 ± 4.92	41.13 ± 1.69
N - Strains	6	55.89 ± 1.70	43.95 ± 0.91
**Pseudomonas*	1	42.00	36.69
**Legionella*	1	28.23	46.50
Staphylococcus	2	30.35 ± 8.79	26.75 ± 8.86

T Textile effluent adapted
N Non – adapted
[#] values in Mean ± SEM
*1 strain each

This study discovered effluent adapted strains of *Acinetobacter, Bacillus* and *Legionella* with potentials for colour removal and strains of *Acinetobacter, Bacillus and Pseudomonas* with potential use for COD removal (Table 2). The municipal landfill site soils yielded strains of bacillus with potentials for use in colour and COD removal (Table 2). This may be due to the significant exposure of these organisms to a myriad of chemicals and materials some of which contain dyes which are deposited in the landfill which may cause a release of dyes to the soil.

The results suggest that the non-adapted bacillus species have a relatively higher potential use than the textile effluent adapted isolates (Table 4). Reports however indicate that though several microorganisms may

Table 4. Mean biodegradative activities of textile effluent adapted and non-adapted bacteria.

Strain	N	% COD removal	% Decolourization
T - Strains	18	40.95 ± 3.35	40.62 ± 1.85
N – Strains	6	55.89 ± 1.70	43.95 ± 0.91

seem to have a potential for dye degradation, very few strains can withstand the conditions of dyeing effluents (Maeir et al., 2004); thus the effluent-adapted strains may be better candidates for potential bioremediative uses. However, our result does not indicate the involvement of extra-chromosomal genes in the degradative activity of the isolates.

In conclusion, as a preliminary step in the development of textile effluent biotreatment processes involving indigenous microbes, we have discovered textile effluent adapted strains of *Acinetobacter* and *Bacillus*, and effluent non-adapted *Bacillus* species with potential use in effluent treatment.

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