African Journal of Biotechnology Vol. 8 (14), pp. 3261-3266, 20 July, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Turbidity and microbial load removal from river water using bioflocculants from indigenous bacteria isolated from wastewater in South Africa

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Accepted 8 April, 2009

Several serious problems associated with the use of aluminum salts as coagulants in water and wastewater treatment, including Alzheimer's disease and related health problems have necessitated the need for alternative cost effective and more environmentally acceptable coagulants. The objective of this study, therefore, was to evaluate river water turbidity and microbial load removal by bacterial bioflocculants. Turbidity removal rate ranging from 84.07 – 93.56% at 10 ppm bioflocculant concentration was obtained for all the bacterial isolates with up to 94.60% total bacterial load removal. The bioflocculants were also able to remove both Gram positive (*Staphylococcus aureus* and *Streptococcus faecalis*) and Gram-negative (*Escherichia coli* and *Klebsiella oxytoca*) bacteria used to individually spike the autoclaved river water samples, leading to complete removal of *S. aureus*, *K. oxytoca* and *E. coli* and up to 98.35% removal of *S. faecalis* in some cases. The flocculating activities (OD^{-1}) of the bacterial bioflocculants ranged between 47.9 - 161.02, 97.82 - 291.82, 138.89 - 443.45, and 106.11 - 710.88 in the river water spiked with *S. faecalis*, *S. aureus*, *K. oxytoca* and *E. coli*, respectively. Results from this study have indicated that the application of bacterial bioflocculants is a promising alternative to alum in the treatment of contaminated river water.

Key words: Alum, bioflocculant, flocculation, river water; turbidity.

INTRODUCTION

Although water is the most common and important chemical compound on earth, only 2.6% of the global water is freshwater and consequently available as potential drinking water. Availability of sufficient volume of drinking water continues to present major problems, worldwide, owing to the increasing population growth (Postel, 1997). Also, other complications of highly populated areas, such as increasing amounts of waste, wastewater, and other types of contamination, also endangered access to fresh, safe drinking water (Hunter and Quigley, 1998). This has led to the development of sophisticated techniques and systems to obtain access to new water reservoirs and to distribute water for irrigation and drinking purposes (Hammerton and Sherrat, 1972). Coagulation-flocculation followed by sedimentation, filtration and disinfection by chlorine, is used worldwide in the water treatment industry before the distribution of treated water to consumers (Ndabigengesere and Narasiah, 1998).

Today, in most industrialized countries, drinking water is ranked as food, and high standards are set for its quality and safety (Ölmez and Kretzschmar, 2009). The strict requirements for microbiological factors specify that bacterial content should be very low and that no pathogenic microorganisms should be detectable (USEPA, 1991). Guidelines and legislation state that drinking water should contain pathogenic microorganisms only in such low numbers that the risk for acquiring waterborne infections is below an accepted limit (Zhao et al., 2009). The fulfilment of these requirements demands resource protection and careful treatment of raw water, as well as accurate quality control of the treatment process (Atherton et al., 1995). Biological treatment process-

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es at sewage treatment plants could produce selective elimination and/or changes of proportion, in the bacterial populations (Mezrioui and Baleux, 1994). Moreover, the sewage effluent could modify some microbial populations in the reception waters, such as rivers, lakes, or lagoons (Sinton and Donnison, 1994). This effect could become more important where policies of water re-utilization are applied in regions with poor water resources.

River water is a widely used but often unappreciated source of water. In rural and suburban areas, 90-95% of the drinking water comes from river water (Prescott et al., 1996). Several techniques are used in the treatment of river water, with relative advantages and drawbacks (Lerch et al., 2005). Photocatalytic oxidation mediated by semi-conductor catalysts is one of the emerging advanced oxidation processes used in the treatment of river water (Meng et al., 2005) and is commonly considered capable of decomposing almost all types of organic contaminants. It has therefore been suggested for the treatment of contaminated ground waters, industrial wastewaters, and effluents of biologically treated wastewaters and polluted river water (Dillert et al., 1999; Meng et al., 2005). However, from a viewpoint of practical application, the feasibility of the photocatalytic process for the treatment of various river waters is not certain. Furthermore, the use of photocatalytic oxidation system is economically not feasible (Rodriguez et al., 1996; Crittenden et al., 1997). There is therefore a great need to develop cheap and effective river water treatment methods.

Aluminum salts are by far the most widely used coagulants in water and wastewater treatment. However, several serious disadvantages of using aluminum salts including Alzheimer's disease and similar health related problems associated with residual aluminum in treated waters have been identified (Yokoi et al., 1995). There is also a problem of reaction of alum with natural alkalinity present in the water leading to a reduction of pH, and a low efficiency in the coagulation process (Okuda et al., 2001; Stumm and Morgan, 1981). A significant economic factor is that many developing countries can hardly afford the high costs of imported chemicals for water and wastewater treatment. Therefore, it is desirable that other cost effective and more environmentally acceptable alternative coagulants be developed to supplement, if not replace alum, ferric salts, and synthetic polymers (Ndabigengesere and Narasiah, 1998). Hence, the objective of this study was to evaluate the efficacy of indigenous bacterial bioflocculants as an alternative to alum in decreasing both the microbial load and turbidity of river water.

MATERIALS AND METHODS

Isolation and identification of bioflocculant-producing bacteria

Bioflocculant-producing bacteria were isolated from the activated sludge collected from the Northern Wastewater Treatment Plant in Durban, South Africa. Identification was done using standard biochemical tests, the API test kit (Biomerieux) as well as the 16S rRNA gene sequence analysis as described elsewhere (Olaniran et al., 2008). The 16S rRNA gene sequences of the bacterial isolates were compared to those in the GenBank database (http://www.ncbi.nlm.nih.gov) by using BLAST search tool (Altschul et al., 1997) to determine the most similar sequences.

River water collection

The river water used in this study was collected from Palmiet River close to the University of KwaZulu-Natal (Westville Campus), Durban, South Africa using sterilized containers. The container was first rinsed with water from the source before collecting the water sample by holding the bottle at the bottom and plunging it below the water surface. The mouth of the bottle was placed opposite the water current. If there was no current, it was created artificially by pushing the bottle forward. The bottle was filled leaving about 30 mm of empty space to allow mixing during laboratory analysis (Buckalew et al., 2006).

Production and purification of bacterial bioflocculants

A 0.7 ml aliquot of the pre-culture of each organism grown in 30 ml YMPG medium for 20 h at 28°C on a rotary shaker at 220 rpm was added into a 500 ml Erlenmeyer flask containing 70 ml of production medium (0.5% yeast extract, 0.5% polypeptone, 2% ethanol, 1% glycerol, 0.05% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.2% NaCl, and 0.2% $CaCO_3$) and incubated for 3 days at 28°C. The viscous culture broth was diluted with an equal volume of distilled water and centrifuged at 2800 $\times g$ for 30 min to remove cell pellets before ethanol precipitation of the bioflocculant produced according to the method of Kurane et al. (1994). This was done by adding two volumes of cold ethanol (4°C) to the supernatant and overnight drying of the crude bioflocculant precipitate in a dessicator. Thereafter, the crude bioflocculant was purified as described by Salehizadeh and Shojaosadati (2002). Briefly, this was done by dissolving the bioflocculant in distilled water and followed by the addition of 2% (w/v) cetylpyridinium chloride solution (CPC) until no more insoluble CPC-bioflocculants complex were formed. After several hours, the precipitate collected by centrifugal separation of the CPC-bioflocculants complex was dissolved in saline solution [0.85% (w/v) NaCl], washed with cold ethanol three times and lyophilized.

Turbidity and bacterial load measurement

The turbidity of the river water was measured using a HACH 2100P turbidometer and expressed in NTU. Aliquots of 0.1 ml of appropriate dilution of the river water in each tube were spread plated on nutrient agar plates and incubated at 37°C for 24 h. The number of colonies was counted and bacterial population expressed as colony forming units per ml (Cfu/ml). Percentage removal was determined by comparing the estimated values to that of the control (river water without bioflocculant or alum). To determine the removal of specific bacterial group from the river water using the bacterial bioflocculants, the pH of the river water was first adjusted to 9 and then sterilised by autoclaving and allowed to cool. One millilitre of standardized culture (OD of 1 at 550 nm) of Gram-positive (*Staphylococcus aureus* and *Streptococcus faecalis*) and Gram-negative (*Escherichia coli* and *Klebsiella oxytoca*) bacteria were separately used to spike the river water before adding 1 ml of 10 ppm concentration of bacterial bioflocculant or alum.

Determination of bioflocculants' flocculating activity in river water spiked with different bacterial species

One millilitre of different concentrations of bacterial bioflocculants (10-50 ppm) was added to 49 ml of river water spiked with 1 ml of standardized (OD of 1 at 550 nm) culture of each test bacterium. The test tube was mixed vigorously for 30 s and then left to stand, without shaking, for 2 h. The turbidity of the sample supernatant (A) and a control experiment without the bioflocculant or alum (B) were measured at 550 nm with a spectrophotometer (LKB Ultrospec II). The flocculating activity of the bacterial bioflocculant and alum was expressed as follows:

$$\text{Flocculating activity} = \frac{1}{A} - \frac{1}{B}$$

For alum, the procedure was carried out as described above but instead of bacterial bioflocculants, different concentrations of alum (10-50 ppm) were added.

RESULTS

Identification of bioflocculant-producing bacterial isolates

The identity of the bioflocculant-producing bacteria used in this study based on the various biochemical tests and the analysis of their 16S rRNA gene sequences is shown in Table 1. These isolates were selected for use based on their bioflocculant-producing ability in comparison to the other isolates.

Turbidity and bacterial load removal from river water by the bacterial bioflocculants

The effect of bacterial bioflocculants and alum on the turbidity and microbial load removal of river water is depicted in Figure 1. Turbidity removal rates ranging from 84.07 – 93.56% at 10 ppm; 66.52 – 94.00% at 20 ppm; 46.96 – 95.70% at 30 ppm; and 19.26 – 96.00% at 50 ppm bioflocculant concentration were obtained for all the bacterial isolates (Figure 1a). The highest turbidity removal rate was observed using bioflocculant produced by isolate D1 and the least by those produced by isolate R2 at all the concentrations tested (Figure 1a). These values compared favourably well with a removal rate of up to 90.74% obtained using alum at 10 ppm concentration. Similarly, bacterial load removal by the bioflocculant ranged between 77.93 and 94.60%, with the highest removal obtained using bioflocculant from isolate A17 at 30 ppm (Figure 1b). Bioflocculant from isolates D1 and A22 at 10 ppm resulted in higher bacterial removal from the river water, compared to alum at the same concentration (Figure 1b). In all cases, an increase in bioflocculant and alum concentration resulted in increasing bacterial load removal, except for isolate A17 with optimum bacterial load removal at 30 ppm bioflocculant concentration.

Table 1. Identity of the bioflocculant-producing bacterial isolates used in this study.

Isolate code	Identity based on 16S rRNA gene sequence analysis
E1	*Bacillus subtilis*
D1	*Exiguobacterium acetylicum*
R2	*Klebsiella terrigena*
A22	*Staphylococcus aureus*
A17	*Pseudomonas pseudoalcaligenes*
A14	*Pseudomonas plecoglossicida*

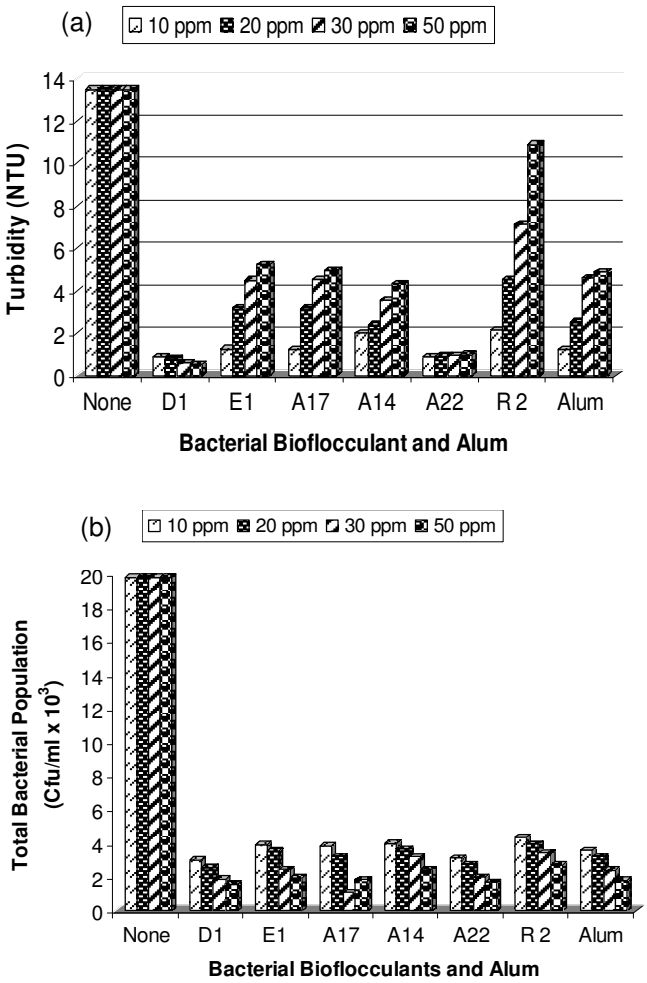

Figure 1. Removal of (a) turbidity and (b) microbial load in river water by the different bacterial bioflocculants.

Effect of bioflocculants on river water spiked with bacterial cultures

In the experiment to determine the ability of the bioflocculant to remove specific bacterial type from the contaminated river water, 100% removal of *S. aureus*

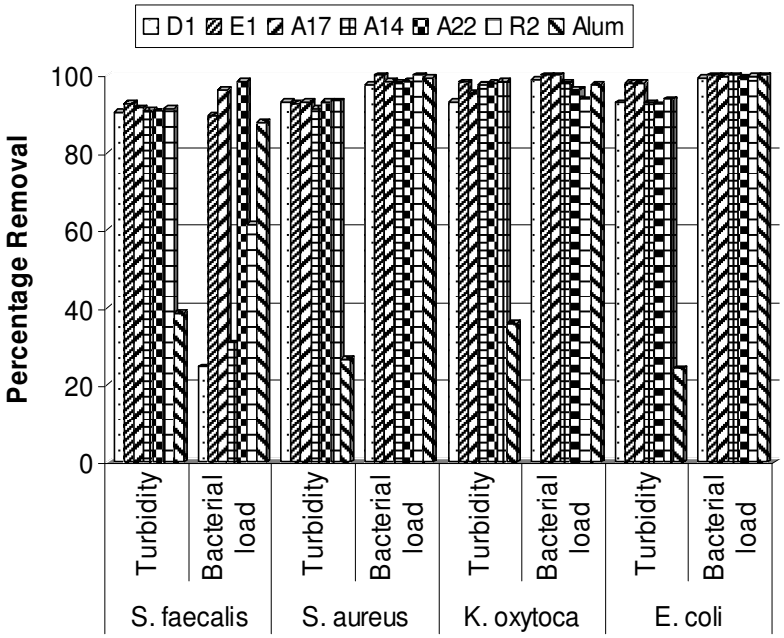

Figure 2. Turbidity and Bacterial load reduction in river water spiked with different Gram positive and Gram negative bacteria using 10 ppm bacterial bioflocculant and alum.

was obtained with bioflocculants from isolates E1 and R2; *K. oxytoca* by those from isolate E1; and *E. coli* by those from isolate E1 and alum (Figure 2). Similarly, the highest removal rate of 98.35% was obtained with bioflocculant from isolate A22. The least removal of *S faecalis* was observed in the river water sample with only 24.73, 30.77 and 61.54% obtained using bioflocculants from isolates D1, A14 and R2, respectively (Figure 2). A generally higher turbidity removal rate from the river water spiked with the different organisms was observed for all the bacterial bioflocculants, compared to alum at the same concentration (Figure 2). The flocculating activities (OD^{-1}) of the bacterial bioflocculants ranged between 47.90 - 161.02, 97.82 - 291.82, 138.89 - 443.45, and 106.11 - 710.89 in the river water spiked with *S. faecalis*, *S. aureus*, *K. oxytoca* and *E. coli*, respectively (Table 1).

DISCUSSION

The bacterial bioflocculants investigated in this study were able to reduce both turbidity and bacterial load from the contaminated river water to a varying degree, with higher bacterial load removal rate observed with increasing concentrations of the bioflocculants. However, a lower turbidity removal rate was observed with increase in bioflocculant concentrations in most cases. This is because the optimum amount of flocculants in the suspension causes the microorganisms and the fine particles to aggregate and settle. However, when the optimum concentration is exceeded, the aggregated particles can re-disperse and this disturbs particle-settling (Chan and Chiang, 1995). This has been attributed to an increase in the repulsive energy between the flocculants and the microorganisms, which causes hindrance in floc formation (Mishra et al., 2004). It is worth noting that there was no significant reduction in pH of the river water (pH 7.38) after the addition of the bioflocculants as the final pH ranged between 6.55 - 6.92, compared to alum which resulted in an acidic pH of 4.14 (Results not shown). This makes bacterial bioflocculant preferable in the practical terms as no further chemical addition is necessary in order to correct the pH of the finished water. The reduction in pH is as a result of alum hydrolysis, resulting in H^+ production (Nordstrom and May, 1989; Stumm and Morgan, 1981). Faust and Aly (1998) showed that alum was not effective in removing bacteria within the range of 5–10 ppm, with a removal of 99.7% achieved with 50 ppm, which is closer to the observation in this study.

Different bacterial isolates used to spike the river water were randomly flocculated by the bioflocculants (Table 2), with a high microbial load and turbidity removal of river water observed for all the bacterial bioflocculants and alum. Bacterial load removal obtained in this study corroborates the report of Bitton (1994) that removal of bacteria, although variable may exceed 90% during the flocculation process, while coagulation removes 74-99.4% of *E. coli* and coliforms. Kurane et al. (1986) reported that the bioflocculant produced by *Rhodococcus erythropolis* could efficiently flocculate all suspended solids in aqueous solutions tested and had a wide flocculating activity against both organic and inorganic

Table 2. Flocculating activity of the different bacterial bioflocculants and alum against river water spiked with specific bacterial groups.

Bacterial bioflocculant	Flocculating activity (OD^{-1})			
	S. faecalis	***S. aureus***	***K. oxytoca***	***E. coli***
A22	161.015(1.235)	291.824(5.215)	138.887(0.355)	365.371(5.127)
A17	114.481(1.888)	97.815(0.374)	190.031(2.122)	322.624(1.747)
D1	86.363(0.980)	149.053(1.771)	248.623(2.083)	557.856(3.111)
R2	82.074(0.900)	144.990(0.537)	443.446(4.180)	401.249(1.559)
A14	47.904(1.395)	177.561(2.171)	232.905(4.001)	710.876(9.160)
E1	56.923(0.234)	104.109(0.369)	175.245(0.493)	106.109 (0.300)
Alum	34.813(0.902)	36.603(0)	30.961(0)	16.185(0.179)

Values in parenthesis represent standard deviation from triplicate data.

materials as well as microorganisms such as *E. coli* and alcohol yeast. Takagi and Kadowaki (1985) also reported that bioflocculant from *Paecilomyces* had the ability to flocculate all suspended solids from organic materials such as microorganisms to inorganic materials such as aluminium oxide.

Apart from the aesthetically displeasing appearance that high turbidity and/or colour impart to water, it also provides adsorption sites for biological organisms and interferes with disinfection. Hence, the maximum turbidity of 1 NTU is allowable in drinking water (USEPA, 1991). Excessive turbidity is often associated with unacceptable tastes, odours, and colour in water and may represent a health concern where heavy metal ions, pesticides or waterborne disease causing organisms, including bacteria, viruses, and parasites may attach to the suspended particles (Vigneswaran and Visvanathan, 1995). The high turbidity observed with the use of alum as coagulant (Figure 2) can be due to the production of aluminium hydroxide precipitate in water. Besides being voluminous, the alum sludges are gelatinous, acidic, and difficult to dewater and dispose in the environment (Ndabigengesere and Narasiah, 1998), thus making bioflocculant a better alternative in wastewater treatment.

Alum is a widely used coagulant in wastewater treatment. However, medical reports indicated that aluminum might induce Alzheimer's disease, while residual aluminum concentrations in treated water can also impose health problems apart from the production of large amounts of sludge (Letterman and Driscoll, 1988). Therefore, the use of high concentrations of alum in the treatment of river water must be avoided (Zouboulisa et al., 2004). The use of bacterial bioflocculants in the areas of wastewater and drinking water treatment, downstream processing, and food and fermentation industry is well anticipated due to their relative harmlessness towards humans and the environment. As shown from the results from this study, the application of bacterial bioflocculants in the treatment of river water is a promising alternative to using alum. However, more studies are required for the practical application of the bioflocculants in the treatment of contaminated water.

ACKNOWLEDGEMENTS

This research was funded by the National Research Foundation (NRF) of South Africa. The first author is grateful to LIFE*lab*, DAAD (Germany) and University of KwaZulu-Natal for the award of MSc bursaries for this study.

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