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Original Research

Bioflocculant Production by a Consortium of Two Bacterial Species and Its Potential Application in Industrial Wastewater and River Water Treatment

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Abstract

We assessed the bioflocculant production potentials of a consortium of two marine bacterial species belonging to the *Oceanobacillus* and *Halobacillus* genera, isolated from sediment samples of Algoa Bay in the Eastern Cape Province of South Africa. Cell free culture broth of the consortium had a flocculating activity of 98.3%, which is higher than those of pure cultures of the individual species. The purified bioflocculant was more efficient (optimum dose 0.2 $mg \cdot ml^{-1}$) in the flocculation of kaolin suspension (4 $g \cdot l^{-1}$) compared to polyelectrolyte (optimum dose 0.3 $mg \cdot ml^{-1}$) and alum (optimum dose 1 $mg \cdot ml^{-1}$), which are both commercially available coagulants. A neutral pH condition and the presence of Ca^{2+} as cation resulted in optimum activity of the bioflocculant. Also, the purified bioflocculant removed chemical oxygen demand (COD) in brewery wastewater, dairy wastewater, and river water at efficiencies of 99.7, 99.9, and 63.5%, respectively, and also reduced their turbidity by 93.9, 88.3, and 98.6%, respectively. Composition analysis revealed the bioflocculant to be mainly polysaccharide with an amorphous-crystal-like structure. FTIR spectra revealed the presence of carboxyl, hydroxyl, and amino groups in its thermo-stability test, suggesting a thermostable bioflocculant.

Keywords: *Oceanobacillus* sp., *Halobacillus* sp., consortium, bioflocculant, flocculation, wastewater

Introduction

Increasing industrialization has been accepted as an enviable choice due to its contribution to economic growth. However, it has considerably raised the rate of water pollution, especially from industrial sources, and this has become a major environmental concern [1]. The disposal of effluents without appropriate treatment could result in long-term undesirable negative impacts, especially on the environment and human health [2], thus necessitating the need for adequate treatment of wastewater before discharge into the environment.

Although in previous decades chemical treatment of wastewater was inadequate, significant research outcomes have shown that chemical coagulation is a viable treatment process for the treatment of wastewater [3]. Nowadays, the process of clarification (coagulation-flocculation and sedimentation) is employed worldwide in the water/wastewater

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treatment industry [4] as the process of coagulation-flocculation in wastewater treatment is a convenient and efficient technique for removing suspended solids (SS), colloids, and cell debris [5, 6]. This process involves the use of chemical coagulants such as ferric salts [3], aluminium sulfate, aluminium chloride, and polyacrylamide (PAM) [7]. However, these coagulants have been implicated in undesirable health conditions [8]. For example, polyacrylamide contains acrylamide monomers verified to be both neurotoxic and carcinogenic to humans [6]. On the other hand, a link between aluminium in drinking water and human neurological disorders, such as dialysis encephalopathy, was established with excess aluminium in dialysate fluid shown to be harmful to dialysis patients [9]. This health implication of chemical coagulant/flocculant has necessitated the need for safe alternatives.

The use of bioflocculants in wastewater treatment seems to constitute not only safe but also economical alternatives to physical and chemical treatments [10, 11]. Microbial bioflocculants are defined as essential polymers produced by microorganisms during growth with their flocculating activity being dependent on the characteristics of flocculants produced [12, 13]. Considering that microbial bioflocculants are basically non-toxic and hence produce no secondary pollution, they are purported to have great potential in industrial applications [8].

Literature reports indicate that a number of bioflocculants have been produced from different microorganisms such as bacteria, fungi, and actinomycetes [14, 15]. However, these reports have historically been more focused on pure culture fermentations, thus overlooking the important interactions between microbes in a mixed culture [16]. Hence, a different approach based on the use of consortia of microorganims for the production of high-quality bioflocculants has been suggested [17, 18]. A recent study by Zhang et al. [18] reported that a consortium of *Staphylococcus* sp. and *Pseudomonas* sp. produces a novel bioflocculant MM1 with high flocculating activity. Another study by Wang et al. [17] reported that the combination of *Rhizobium radiobacter* F2 and *Bacillus sphaeicus* F6 resulted in production of a bioflocculant with flocculating activity higher than that of individual pure strains.

In this study we report the bioflocculant production by a consortium of two marine bacteria belonging to the genera *Halobacillus* and *Oceanobacillus*, as well as the potential usefulness of the purified bioflocculant for the treatment of wastewater and river water.

Materials and Methods

Microorganisms

The bacteria were previously isolated from the sediment samples of Algoa Bay in South Africa and identified as belonging to the *Halobacillus*, and *Oceanobacillus* genera. The organisms were preserved in 20% glycerol at -80ºC as part of the culture collections of the Applied and Environmental Microbiology Research Group (AEMREG) at the University of Fort Hare, an Alice, South Africa.

Culture Media

The medium for cultivation of the bacterial consortium was composed of sodium carbonate (20 g), urea (0.5 g), yeast extract (0.5 g), $(NH_4)_2SO_4$ (0.2 g), $MgSO_4 \cdot 7H_2O$ (0.3 g), K_2HPO_4 (5 g), KH_2PO_4 (0.2 g), and NaCl (0.1 g) in 1 liter of filtered marine water. The initial pH was adjusted to 10, with NaOH (0.1 M) and HCI (0.1 M) as described by Zhang et al. [18].

Construction of Bacterial Consortium

Two loop-fulls of the bacterial colonies were inoculated separately into a 150 ml flask containing 50 ml of the cultivation medium and incubated with shaking (160 rpm) at 30ºC for 3 days and used as standard inoculum preparation for all subsequent experiments. To construct the bacterial consortium, 1 ml of the standard inoculum preparation of each bacterial strain was inoculated into a 150 ml flask containing 50 ml culture medium and incubated at 30ºC with shaking at 160 rpm for 3 days. At the end of the incubation period, the culture was centrifuged at 4000×g for 30 min to separate the cells. The cell-free supernatant was assessed for flocculating activity [18]. The experiments were performed in triplicate.

Measurement of Flocculating Activity

The flocculating activity test was done in accordance with the method previously described by Kurane et al. [19]. The kaolin clay suspension was 4 $g \cdot l^{-1}$ concentration. Two ml of bioflocculant solution and 3 ml of (1%) $CaCl_2$ were added to 100 ml kaolin suspension, stirred for 1 minute, held for 5 minutes, and supernatant was taken from the upper phase. A control was prepared by substituting the bioflocculant solution with deionized water and measured under similar conditions. Flocculating activity was then calculated using the following formula:

$$\textit{Flocculating activity}\ (\%) = [(A - B)/A] \times 100$$

...where A – optical density at 550 nm (OD550) of control and B – optical density at 550 nm (OD550) of a sample.

Extraction and Purification of Bioflocculant

Purification of the bioflocculant was carried out following the method described below [11, 20-22], with minor modifications. Briefly, after microbial fermentation the culture (1 l) was centrifuged at 8,000×g, for 30 minutes to remove bacterial cells. One volume of distilled water was added to the upper phase and centrifuged at 8,000×g for 15 minutes to remove insoluble substances. To the supernatant, two volumes of ethanol were added, and the mixture was stirred and left to stand at 4ºC for 12 hours. The precipitate was vacuum dried to obtain the crude bioflocculant. The crude product was directly dissolved in distilled water to yield a solution, to which one volume of the mixed solution of chloroform and n-butyl alcohol (5:2 v/v) was added.

After stirring, the mixture was left standing at room temperature (about 20ºC) for 12 hours. The upper phase was centrifuged at 3,000×g, for 15 minutes and two volumes of ethanol were added to recover the precipitate. After centrifugation at 3,000×g, for 15 minutes, the precipitate was vacuum-dried and re-dissolved in distilled water. After dialyzing against de-ionized water overnight the solution was vacuum dried to obtain a purified bioflocculant.

Chemical Analysis of Purified Bioflocculant

Measurement of total sugar content was done using the method of Dubois et al. [23] with glucose as a standard. The total protein content was determined using the Folin-Lowry method with bovine serum albumin (BSA) as a standard [24]. The presence of uronic acid content was measured by the carbazole method as described by Cesaretti et al. [25].

FTIR Analysis

The purified bioflocculant was characterized by using a Fourier Transform Infrared (FTIR) Spectrophotometer (Perkin Elmer System 2000, England). The dried bioflocculant powder was mixed with potassium bromide (Kbr), ground, and pressed into pellets for FTIR spectral measurement in the frequency range of 4000-370 cm^{-1}.

Scanning Electron Microscopic Observations

A sprinkle of bioflocculant powder was spread and fixed on the iron stub. The fixed specimen was gold-coated and examined with a JEOL-JSM-6390LV scanning electron microscope (Japan).

Thermal Stability Test

The bioflocculant solutions were incubated in water baths fixed at 100ºC for 30 minutes. Samples were drawn at appropriate time intervals and analyzed for residual flocculating activity as previously described by Gong et al. [26].

Effect of Bioflocculant Dosage on Flocculation

The jar-test experiment was used to determine the optimum dose of the purified bioflocculant for the clarification of kaolin clay suspension (4 $g \cdot l^{-1}$) at neutral pH. Different concentrations of the purified bioflocculant ranging from 0.1 to 1 $mg \cdot ml^{-1}$ were used. The rapid mixing time was 1 minutes at 180 rpm, the flocculation period was 3 min at 45 rpm, and the sedimentation was 5 min [27]. After settling, the upper phase was sampled to measure optical density.

Effects of pH and Cations on Flocculating Activity of the Purified Bioflocculant

The effect of pH and cations on flocculating activity of the purified bioflocculant examined. The pH of the bioflocculant solution varied in the range 3-11. $CaCl_2$ solution previously used as stimulating agent was replaced by various metal salt solutions of KCl, NaCl, LiCl (monovalent), $MnCl_2$, $MgCl_2$ (divalent), $AlCl_3$, and $FeSO_4$ (trivalent), and flocculating activity was measured.

Wastewater and River Water Flocculation Studies

Brewery and dairy wastewaters and river water were collected from a brewery, a dairy factory, and the Tyume river respectively, using clean sterile containers. The pH, COD (chemical oxygen demand), and turbidities of the waters were measured using spectro-quant, a pH meter (Pharo 100, Merck KGaA, Germany) and a 2100P turbidimeter (HACH Company, Germany) before flocculation. The jar-test experiment was then carried out as described above at neutral pH. For comparison with chemically synthesized flocculants, the bioflocculant was replaced by polyacrylamide (PAM) and $AlCl_3$. The residual COD and turbidity were determined, and the removal efficiency was calculated as follows:

$$\textit{Removal efficiency}\ (\%) = [C_0 - C/C_0] \times 100$$

...where C_0 is the initial value and C is the value after the flocculation treatment [26].

Statistical Analysis

Statistically significant differences of the percentage bioflocculant activity among the treatment means were analyzed using the analysis of variance (ANOVA) test (Minitab Student Release 12). Microsoft excel office 2007 was used to determine means and standard deviations.

Results and Discussion

Bioflocculant Production

Flocculating efficiency is still one of the limiting factors with regard to the application of bioflocculants [18]. Determined to overcome this quandary, we examined two bioflocculant-producing bacteria belonging to the *Halobacillus* and *Oceanobacillus* genera previously isolated from the sediments of Algoa Bay, South Africa, to assess their bioflocculant production potential as a consortium. When these isolates coexist in the same environment they may form a proto-cooperation relationship, thus benefiting both and leading to a more efficient bioflocculant being produced [18, 19]. The consortium culture had a flocculating activity of about 98.3%. In our previous reports, axenic cultures of the individual bacteria had lower flocculating activities viz 76% for *Halobacillus* sp. [28] and 95% for *Oceanobacillus* sp.

The yield of the bioflocculant from the consortium culture after three days of fermentation was 11.2 g from 1 l of culture broth. In our previous study, *Halobacillus* sp. pro-

duced 0.344 g·l^{-1} bioflocculant [28], while *Oceanobacillus* sp. produced 2.4 g·l^{-1}. Hence the bioflocculant yield recovered from the consortium was quite comparable with that produced from the pure individual cultures. The yield of bioflocculant is also an important factor to consider with respect to its industrial application [18].

The bioflocculants yield by individual pure strains has been documented. A bioflocculant yield of 1.47 g·l^{-1} was produced by *Bacillus* sp. Strain F19 [29] while a yield of 2.3 and 2.27 g·l^{-1} was reported for *Vagococcus* sp. strain W31 and *Enterobacter cloacae* WD7 [12, 30]. However, Zhang et al. [18] reported 15 g·l^{-1} of purified bioflocculant produced by multiple-microorganism consortia. Our bioflocculant was 11.2 g·l^{-1} and hence may be good for application.

Characterization of Bioflocculant

Chemical analysis revealed that the bioflocculant was mostly acidic polysaccharide with little protein. Chemical analysis revealed that the proportions of the uronic acid, neutral sugar content, and total protein content were 69%, 5%, and 26% (w/w), respectively. The purified bioflocculant from the pure strain *Halobacillus* sp. Mvuyo was amorphous while that of *Oceanobacillus* sp. Pinky had a crystal-linear-like structure (Figs. 1a and b). Morphological surface structure of the freeze-dried mixed-culture bioflocculant using a scanning electron microscope (SEM) was white, with a combination of amorphous-crystal-like structure (Fig. 1c). Fig. 1d shows the bioflocculant and kaolin interaction coupled after flocculation. During this process the kaolin particles were probably adsorbed onto the binding sites of the bioflocculant, and thus larger flocs were formed as a result of this interaction, leading to rapid sedimentation due to gravity [31].

FTIR and EDX Analysis of the Purified Bioflocculant

Analysis of the functional groups in the purified bioflocculant was carried out and results are shown in Fig. 2. The infrared spectrum showed a broad stretching peak at 3,413.47 cm^{-1}, suggestive of the presence of the hydroxyl and amino groups [32]. Two peaks at 1,616.44 cm^{-1} and 1,455.96 cm^{-1} were observed, indicative of the carboxyl groups while the intense peak at 1,016.64 cm^{-1} may show C-O stretching vibration [29]. The small weak peaks at 870.92, 572.46, and 464.99 cm^{-1} may represent the presence of all sugar derivatives and also show characteristics of β-glycosidic bonds between the sugar monomers [31]. The spectrum revealed the presence of carboxyl, hydroxyl, and amino groups in the produced bioflocculant, which might have contributed to better flocculating activity. The carboxyl groups may act as binding sites for the metal ions present in surface particles, hence forming chemical bonds [32, 33]. It is also possible that the attraction force (i.e. van der Waals forces) overcame the electrostatic repulsion force when the bioflocculant approached the particle matters in suspension, thereby the bioflocculant functional groups and H^+ and OH^- on surface particles resulted in the formation of hydrogen bonds [33]. These results agree with most reported studies [17, 31, 33, 34].

Further characterization of the bioflocculant with elemental dispersive x-ray (EDX) analysis showed the pres-

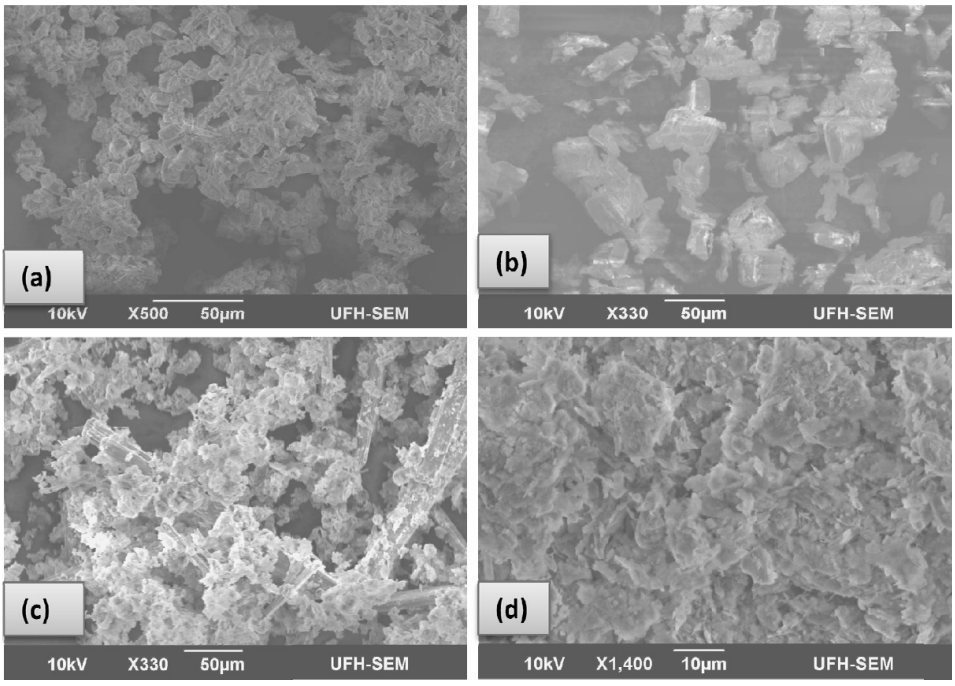

Fig. 1. Scanning electron microscope images of (a) purified powder bioflocculant from *Halobacillus* sp. Mvuyo, (b) purified biofloflocculant from *Oceanobacillus* sp. Pinky, (c) purified bioflocculant from mixed culture of *Halobacillus* sp. Mvuyo and *Oceanobacillus* sp. Pinky, and (d) kaolin suspension flocculated by bioflocculant from mixed culture.

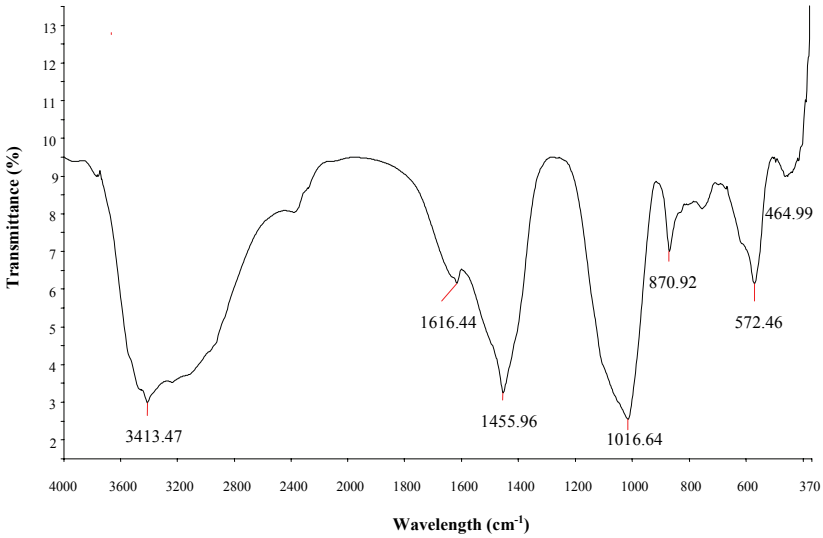

Fig. 2. Fourier Transform Infrared Spectrum of purified bioflocculant.

ence of carbon, nitrogen, oxygen, and phosphate as 7.18, 7.60, 36.9 and 4.67% weight elements while sulphur was present as 0.91% minor element on the surface of the bioflocculant. Similarly, elemental analysis of the bioflocculant produced by *Enterobacteraerogenes* were carbon, nitrogen, oxygen, and sulphur as 39.8, 0.8, 52.6, and 0.2%, respectively, with no phosphorus detected [35]. Yim et al. [34] reported elemental analysis of bioflocculant p-KG03 as proportions of carbon, hydrogen, nitrogen, and sulfur at 32.2, 3.9, 0.52, and 10.3%, respectively. Our results consequently substantiate the FTIR analysis findings, thus validating the presence of the above-mentioned functional groups in the bioflocculant.

Thermal Stability test of the Bioflocculant

Thermal stability of the purified bioflocculant produced by the combination of strains *Halobacillus* sp. Mvuyo and *Oceanobacillus* sp. Pinky was examined at 100ºC for a 30 minute period and compared to the bioflocculants produced by the individual strains. Fig. 3 shows the thermal stability of the bioflocculant produced by the mixed culture compared to that of individual pure strains. The bioflocculant from the combination retained more than 80% flocculating activity after 30 minutes incubation at 100ºC. Hence the bioflocculant was deduced to be thermally stable. The notable decline in flocculating activity with time may be attributed to the partial denaturation of the protein component in bioflocculant structure. On the other hand, the high flocculating activity and thermal stability may be attributed to the nature of the bioflocculant, i.e. mainly polysaccharide and the interaction therein [17]. Wang et al. [17] also reported a similar finding regarding thermal stability of bioflocculant CBF-F26 produced from a mixed culture of *Rhizobium radiobacter* F2 and *Bacillus sphaeicus* F6.

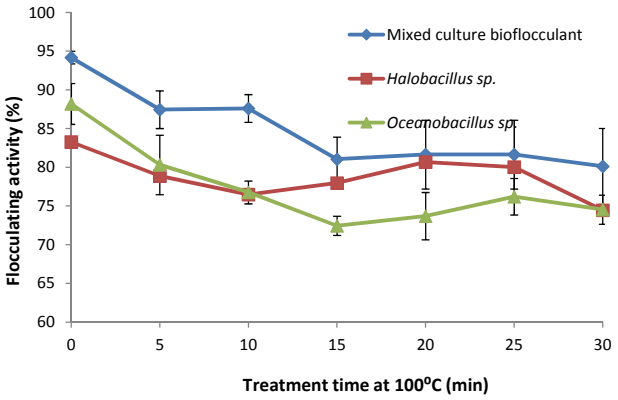

Fig. 3. Thermal stability of purified bioflocculants.

Effect of Bioflocculant Concentration

Dosage is still among the vital parameters considered when determining the optimum conditions for the performance of coagulant/flocculant in the process of coagulation-flocculation [36]. Insufficient dosage or over-dosage may lead to reduced performance in flocculation [36]. Hence, it became essential to establish the optimum bioflocculant dose, as this could help minimize costs and achieve better performance in treatment processes. Fig. 4 shows the relationship between turbidity reduction in kaolin suspension and the bioflocculant dosage. The bioflocculant could achieve about 90% reduction in turbid-

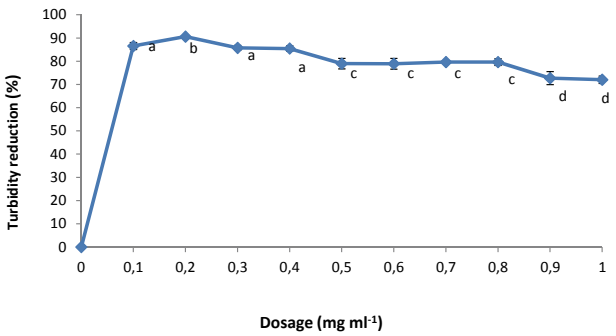

Fig. 4. Results of jar-test studies of the purified bioflocculant. Percentage flocculating activity with different letters (a, b, c, and d) are significantly ($p<0.05$) different.

ity at a low dosage of 0.2 mg·ml^{-1}. Thereafter, there was a decrease in turbidity reduction, which may be attributed to excess bioflocculant being adsorbed on the colloidal surfaces resulting in restabilization of colloids and thus blocking the sites available on the particle surface for the development of interparticle bridges [37].

Effect of pH on Flocculation

The mechanisms of how pH affects the process of bioflocculation are not quite apparent. However, a number of documented studies show that pH impacts the flocculating activity of microbial flocculants [9]. In this study, the effect of pH on flocculating activity was assessed at a bioflocculant concentration of 0.2 mg·ml^{-1} at a pH range of 3-12. Fig. 5 shows that initially the flocculating activity increased with increases in pH, attaining a maximum flocculating activity (94.2%) at neutral pH followed by a steady drop at alkaline pH conditions. The lowest flocculation activity of 25.7% was recorded at pH 12 (Fig. 5). The decline may be as a result of hydroxide ion (OH^-) interfering with the complex formation of bioflocculant and kaolin particles, hence the kaolin particles were restabilized [30]. Similar results were reported for PGA bioflocculant produced by *Bacillus licheniformis* [38]. The effect of pH on flocculating activity varies with the type of bioflocculants under investigation. Flocculation by pKG03 bioflocculant was higher under acidic conditions [34], while the bioflocculant produced by *Bacillus* sp. PY-90 produced optimal activity at pH 4.0 [39]. The bioflocculant studied herein achieved high flocculation efficiency under neutral conditions, making it suitable for field application.

Effect of Cations

Cations are usually used in the process of flocculation to enhance the efficiency and increase the adsorption of bioflocculants onto the suspended particles, thereby decreasing the negative charge of the particles and the bioflocculant [33]. In this study, the effect of cations was examined using NaCl, KCl, LiCl, $CaCl_2$, $MgCl_2$, $MnCl_2$, $FeCl_3$, and $AlCl_3$ as cation sources. Both monovalent (Li^+, K^+, Na^+) and divalent (Ca^{2+}, Mn^{2+}, Mg^{2+}) cations, including

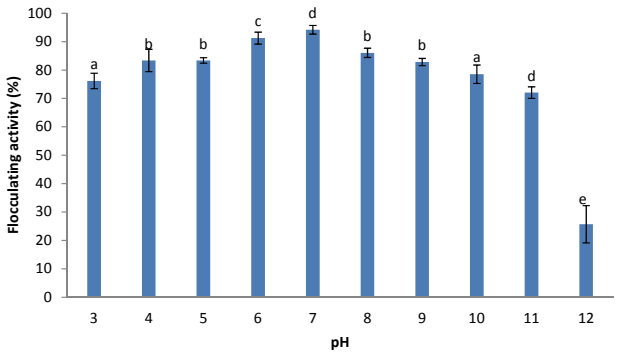

Fig. 5. Effect of pH on flocculation. Percentage of flocculating activity with different letters (a, b, c, and d) are significantly ($p<0.05$) different.

Table 1. Effect of metal ions on flocculating activity of purified bioflocculant.

Cations	Flocculating activity (%)
K^+	74.8±2.74^a
Li^+	82.2±0.43^b
Na^+	73.8±0.42^b
Mg^{2+}	83.6±3.62^{a,c}
Mn^{2+}	86.9±2.47^{a,c}
Ca^{2+}	90.6±0.50^c
Al^{3+}	86.6±2.80^{a,c}
Fe^{3+}	47.8±6.45^d

Percentage flocculating activity with different letters (a, b, c, and d) are significantly ($p<0.05$) different.

Table 2. Characteristics of brewery, dairy wastewater, and river water.

Parameter	Brewery wastewater	Dairy wastewater	River water
pH	5.62	7.5	7.2
COD (mg·l^{-1})	8213	4813	92
Turbidity (NTU)	750	1382	174

The values are means of triplicates data

Al^{3+}, stimulated flocculating activity to a greater degree compared to the trivalent (Fe^{3+}) cation (Table 1). Ca^{2+} was more effective and enhanced the formation of bigger flocs when compared to others, hence it was chosen as the coagulant aid for this study. Cations enhanced the process of coagulation-flocculation by neutralization and destabilization of residual negative charges of functional groups in the bioflocculant and by forming bridges that attach particles in kaolin suspension [6]. Various cations show significant effects on different bioflocculants. According to Zhang et al. [6], various cations Ca^{2+}, Al^{3+}, and Mg^{2+} enhanced flocculating efficiency of xn11 + xn7, but not the addition of K^+, Na^+, and Fe^{3+}. The flocculation by bioflocculant CBF-F26 produced by a mixed culture of *Rhizobium radiobacter* F2 and *Bacillus sphaeicus* F6, could be stimulated by the presence of Ca^{2+}, Zn^{2+}, Fe^{2+}, Al^{3+}, and Fe^{3+} [17].

Flocculation of Real Wastewaters

The process of coagulation-flocculation is a critical physicochemical treatment step in the treatment of industrial wastewater to reduce both the suspended colloidal substances responsible for wastewater turbidity and organic matter that contributes to the BOD and COD content of the water [1]. The utilization of coagulants/flocculants in water and wastewater treatment may enable destabilization of particulate matters, resulting in the formation of flocs and, consequently, improved sedimentation [1]. However, this

Table 3. Flocculation of real wastewaters and river water.

Flocculant used	Wastewater/water	Dosage (mg·ml^{-1})	Turbidity removal (%)	COD reduction (%)	Flocculation efficiency (%)
Test bioflocculant	Brewery	0.2	93.9±1.2	99.7±0.0	92.0±1.4
	Dairy		88.3±1.8	99.9±0.0	91.0±1.8
	River		98.62.3	70.8±2.3	96.0±2.6
Polyacrylamide	Brewery	0.3	85.4±0.1	99.5±0.0	66.1±0.4
	Dairy		72.5±1.6	98.0±0.2	67.3±1.1
	River		48.8±7.2	68.8±1.7	70.8±0.6
$AlCl_3$	Brewery	1	87.0±0.1	98.0±0.0	75.1±0.3
	Dairy		71.4±0.5	98.6±0.2	67.0±0.1
	River		33.1±9.9	70.0±2.7	57.4±0.1

The values are means of triplicates data±Standard deviation.

may vary with the quality of water/wastewater being treated. The bioflocculant produced by the consortium culture of *Halobacillus* sp. Mvuyo and *Oceanobacillus* sp. Pinky exhibited high flocculating efficiency for kaolin suspension, hence it was subsequently tested on wastewaters and river water. The jar test experiment on the treatment of brewery, dairy wastewater, and river water was performed with the test bioflocclant and two conventional flocculants (i.e. polyacrylamide and aluminium chloride) and the initial turbidity, COD of the brewery, dairy wastewater, and river water are shown in Table 2. Table 3 shows the results of the effectiveness of the bioflocculant in comparison to polyacrylamide and aluminium chloride in turbidity removal and COD reduction efficiency. After the bioflocculant-enhanced flocculation, turbidity reduction achieved for brewery, dairy wastewaters, and river water was 93.9, 88.3, and 98.6%, respectively, with the respective COD reduction of 99.7, 99.9, and 70.8%. The effectiveness of turbidity removal may be attributed to the polymer-floc interaction of the bioflocculant, leading to increased aggregation of particles. The attraction between:

(i) the bioflocculant
(ii) mediating factor Ca^{2+}
(iii) the negatively charged particles was strengthened as earlier suggested by Lee et al. [27].

Zhang et al. [18] reported maximum removal efficiencies of COD of 79.2% in indigotin printing and dyeing wastewater by MMF1 bioflocculant. In addition, Gong et al. [26] reported COD and turbidity removal of 64.1-80.7% and 91.8-93.7%, respectively, in agricultural wastewater using the bioflocculant produced by *Serratia ficaria*. On the other hand, the removal efficiencies of turbidity and COD for swine wastewater treated with bioflocculant (xn11 + xn7) were 91% and 42%, respectively [6].

In this study, the bioflocculant produced by the consortium was marginally effective in COD and turbidity removal in wastewaters (brewery and dairy) and river water when compared to conventional flocculants (Table 3). Based on these findings, it is evident that the bioflocculant achieved significant improvement in COD and turbidity reduction and improved efficiency when compared to polyacrylamide and aluminium chloride.

Conclusions

The bioflocculant produced from the mixed culture of *Halobacillus* sp. Mvuyo and *Oceanobacillus* sp. Pinky showed remarkable flocculating efficiency. The hydroxyl, carboxyl, and amino groups were present in its structure. The bioflocculant was thermally stable and could achieve good turbidity and COD removal efficiencies in brewery, dairy, and river water when compared to conventional flocculants. Hence, the bioflocculant may be a strong alternative candidate to commercial flocculants.

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References

1. SARKAR B., CHAKRABARTI P.P., VIJAYKUMAR A., KALE V. Wastewater treatment in dairy industries – possibility of reuse. Desalination, **195**, 141, **2006**.
2. LIN J., HARICHUND C. Industrial effluent treatments using heavy-metal removing bacterial bioflocculants. Water SA. **37**, 265, **2011**.
3. TANIK A., GENCELI E.S., EKDAL A. Chemical treatability of dairy wastewater. J. Environ. Management and Health., **13**, 63, **2002**.
4. BUTHELEZI S.P., OLANIRAN A.O., PILLAY B. Turbidity and microbial load removal from river water using bioflocculants from indigenous bacteria isolated from wastewater in South Africa. African J. Biotechnol., **8**, 3261, **2009**.
5. GHOSH M., GANGULI A., PATHAK S. Application of a novel biopolymer of *Salmonella* from poultry wastewater. Environ. Technol., **30**, 337, **2008**.

6. ZHANG C., CUI Y., WANG Y. Bioflocculant produced from bacteria for decolorization, Cr removal and swine wastewater application. Sustain. Environ. Res., **22**, 129, **2012**.
7. GURUMOORTHY A.V.P., KHAN K.H. Polymers at interfaces: Biological and non-biological applications. Recent Res. Sci. Technol., **3**, 80, **2011**.
8. LIAN B., CHEN Y., ZHAO J., TENG H., ZHU L., YUAN S. Microbial flocculation by *Bacillus mucilaginosus*: applications and mechanisms. Bioresource Technol., **99**, 4825, **2008**.
9. MA F., ZHENG L.N., CHI Y. Applications of biological flocculants (BFs) for coagulation treatment in water purification: turbidity elimination. Chem. Biochem. Eng. Q., **22**, 321, **2008**.
10. VIJAYALAKSHMI S.P., RAICHUR A.M. The utility of *Bacillus* subtilis as a bioflocculant for fine coal. Colloid Surface B., **29**, 265, **2003**.
11. UGBENYEN A., COSA S., MABINYA L., BABALOLA O.O., AGHDASI F., OKOH A. Thermostable bacterial bioflocculant produced by Cobetia spp. isolated from Algoa Bay (South Africa). Int. J. Environ. Res. Public Health., **9**, 2108, **2012**.
12. GAOJ., BAO M., XIN Y., LIU Q., ZHANG Y. Characterization of a bioflocculant from a newly isolated *Vagococcus* sp. W31.J. Zhejiang University Sci. B., **7**, 186, **2006**.
13. COSA S., MABINYA L.V., OLANIRAN A.O., OKOH O.O., BERNARD K., DEYZEL S., OKOH A.I. Bioflocculant production by *Virgibacillus* sp. Rob isolated from the bottom sediments of Algoa Bay in the Eastern Cape, South Africa. Molecules. **16**, 2431, **2011**.
14. ZHANG J., LIU Z., WANG S., JIANG P. Characterization of a bioflocculant produced by the marine myxobacterium Nannocystis sp. NU2. Appl. Microbiol. Biot., **59**, 517, **2002**.
15. SHENG Y., ZHANG Q., SHENG Y., LI C., WANG H. Screening and flocculating properties of bioflocculant-producing microorganisms. Sci. Technol., **13**, 289, **2006**.
16. NAVARRETE-BOLANOS J.L., SERRATO-JOYA O., BOTELLO-ALVAREZ E., JIMENEZ-ISLAS H., CARDENA-MANRIQUEZ M., CONDE-BARAJAS E., RICO-MARTINEZ R. Analyzing microbial consortia for biotechnological processes design. Trends Com. Curr. Res. Ed. Topics. Appl. Microbiol., 437, **2007**.
17. WANG L., MA F., QU Y., SUN D., LI A., GUO J., YU B. Characterization of a compound bioflocculant produced by mixed culture of *Rhizobium radiobacter* F2 and *Bacillus sphaeicus* F6. World J. Microb. Biot., **27**, 2559, **2011**.
18. ZHANG Z., LIN B., XIA S., WANG X., YANG A.Production and application of a novel bioflocculant by multiple-microorganism consortia using brewery wastewater as carbon source. J. Environ. Sci., **19**, 667, **2007**.
19. KURANE R., HATAMOCHI K., KIYOHARA T., HIRAO M., TANIGUCHI Y. Production of a bioflocculant by Rhodococcus erythropolis S-1 grown on alcohols. Biosci. Biotech. Bioch., **58**, 428, **1994**.
20. CHANG W.C., SOON A.Y., IN H.O., SANG H.P. Characterization of an extracellular flocculating substance produced by a planktonic cyanobacterium, *Anabaena* sp. Biotechnol. Lett., **20**, 643, **1998**.
21. CHEN H., ZHANG J.F., JIANG P.J., YANG S.L., LIU Z.L. Composition and characterization of microbiological flocculant SC06. Environ. Chem., **21**, 360, **2002**.
22. MABINYA L.V., COSA S., MKHWETSHANA N., OKOH A.I. *Halomonas* sp. OKOH – a marine bacterium isolated from the bottom sediment of Algoa Bay – produces a polysaccharide bioflocculant: partial characterization and biochemical analysis of its properties. Molecules. **16**, 4358, **2011**.
23. DUBOIS M., GILLES K., HAMILTON J.K., REBERS P.A., SMITH F. Colorimetric method for determination ofsugars and related substances. Anal. Chem., **28**, 350, **1956**.
24. LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J. Protein measurement with the Folin Phenol reagent. J. Biol. Chem., **193**, 265, **1951**.
25. CESARETTI M., LUPPI E., MACCARI F., VOLPI N. A 96-well assay for uronic acid carbazole reaction. Carbohyd. Polym., **54**, 59, **2003**.
26. GONG W., WANG S., SUN X., LIU X., YUE Q., GAO B. Bioflocculant production by culture of *Serratia ficaria* and its application in wastewater treatment. Bioresource Technol., **99**, 4668, **2008**.
27. LEE S.H., SHIN W.S., SHIN M.C., CHOI S.J., PARK L.S. Improvement of water treatment performance byusing polyamine flocculants. Environ. Technol., **22**, 653, **2001**.
28. COSA S., MABINYA L.V., OLANIRAN A.O., OKOH A.I. Production and characterization of bioflocculant produced by *Halobacillus* sp. Mvuyo isolated from bottom sediment of Algoa Bay. Environ. Technol., iFirst, 1, **2012**.
29. ZHENG Y., YE Z.L., FANG X.L., LI Y.H., CAI W.M. Production and characteristics of a bioflocculant produced by *Bacillus* sp. F19. Biol., **99**, 7686, **2008**.
30. PRASERTSAN P., DERMLIM W., DOELLE H., KENNEDY J.F. Screening, characterization and flocculating property of carbohydrate polymer from newly isolated *Enterobacter cloacae* WD7. Carbohyd. Polym., **66**, 289, **2006**.
31. XIONG Y., WANG Y., YU Y., LI Q., WANG H., CHEN R., HE N. Production and characterization of a novel bioflocculant from *Bacillus licheniformis*. Appl. Environ. Microbiol., **76**, 2778, **2010**.
32. KUMAR C.G., JOO H.S., KAVALI R., CHOI J.W., CHANG C.S. Characterization of an extracellular biopolymer flocculant from a haloakalophilic *Bacillus* isolate. J. Microbiol. Biotechn., **20**, 837, **2004**.
33. DENG S.B., BAI R.B., HU X.M., LUO Q. Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch wastewater treatment. Appl. Microbiol. Biot., **60**, 588, **2003**.
34. YIM J.H.S., KIM J., AHN S.H., LEE H.K. Characterization of novel bioflocculant, p-KG03, from a marine dinoflagellate, Gyrodiumimpudicum KG03. Bioresource Technol., **98**, 361, **2007**.
35. LU W., ZHANG T., ZHANG D., LI C., WEN J., DU L. A novel bioflocculant produced by *Enterobacter aerogenes* and its use in defecating the trona suspension. Biochem. Eng. J., **27**, 1, **2005**.
36. HASSAN M.A.A., LI T.P., NOOR Z.Z. Coagulation and flocculation treatment of wastewater in textile industry using chitosan. J. Chem. Nat. Res. Engineer., **4**, 43, **2009**.
37. LIANG Z., HAN B., LIU H. Optimum conditions to treat high-concentration micro-plate slime water with bioflocculants. Mining Sci. Technol., **20**, 0478, **2010**.
38. BAJAJ I.B., SINGHAL R.S.Flocculation properties of poly (γ-Glutamic Acid) produced from *Bacillus* subtilis isolate. Food Bioprocess Technol. **4**, 745, **2011**.
39. YOKOI H., NATSUDA O., HIROSE J., HAYASHI S., TAKASAKI Y. Characteristics of a biopolymer flocculant produced by *Bacillus* sp. PY90. J. Ferment. Bioeng., **79**, 378, **1995**.