Bioremediation of reactive black 5 textile dye wastewater using bacterial exopolysaccharides

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Abstract

Purpose – The purpose of this paper is to investigate the efficacy of bacterial exopolysaccharides (*Eps*) in reactive black 5 (RB5) textile dye wastewater bioremediation.

Design/methodology/approach – The *Eps* were produced by bacteria isolated from cotton gin trash soils collected from different cotton-growing regions in Kenya for comparison purposes. A broth medium reconstituted using molasses was assessed for its capacity to produce the *Eps*. RB5 textile dye wastewater was optimized for dye removal under different temperatures, times and molasses concentrations. Dye removal was studied by Lovibond-Day Light Comparator, UV–Vis spectrophotometer and FTIR.

Findings – It was found that cotton gin trash soils contained *Eps*-producing bacteria. Three of the *Eps* studied were found to have the capacity to remove at least 80% of the dye from the wastewater.

Research limitations/implications – This research did not assess the efficacy of the RB5 dye removal from the wastewater by mixtures of the *Eps*.

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Practical implications – Bioremediation of textile dye wastewater with *Eps* produced by bacteria cultured from cotton gin trash soil is significant because it will offer an effective and cleaner alternative to the chemical coagulants.

Social implications – Alternative treatment of textile wastewater with the *Eps* would result in safer water being released into the water bodies as opposed to the chemically treated wastewater that contains remnant chemicals.

Originality/value – Research on the use of *Eps* produced by bacteria isolated from cotton gin trash soils for removal of RB5 dye from textile wastewater has not been done before.

Keywords Dye wastewater, Reactive black 5, Gin trash soil, Eps, Molasses

Paper type Research paper

1. Introduction

Removal of dyes from textile wastewater presents a formidable challenge, as most of them are completely soluble in aqueous solutions, have complicated aromatic structures and are highly stable to sunlight, oxidizing agents and microorganisms (Sanayei *et al.*, 2010). Hinderance of light penetration by colors inhibits photosynthesis and depletes dissolved oxygen (DO), which affects aquatic life (Trovaslet *et al.*, 2007). Textile industries have therefore been classified among the world's most polluting, with color being considered the most obvious and undesirable pollutant (Daneshvar *et al.*, 2007). Methods such as coagulation, ozonation, electrocoagulation, adsorption, reverse osmosis and membrane filtration, are used for color removal from textile effluents (Al-Tohamy *et al.*, 2022). However, most of these methods are costly, have limited applicability and produce toxic secondary sludge. Treatment of effluents with chemical coagulants results in secondary sludge that is difficult to treat and the chemicals remain in wastewater and may cause health and environmental problems. Bioremediation on the other hand is a cleaner and effective alternative that is relatively lower in cost and generates minimal secondary sludge hence fewer damaging effects on the environment, aquatic and human life (Yin, 2010). Therefore, attention has shifted to bioremediation because of its relatively eco-friendliness (Ben Rebah *et al.*, 2018). Extracellular biological components generated by microbes, primarily polysaccharides and proteins, can precipitate or flocculate dissolved compounds or suspended particles in aqueous solutions (Wagner *et al.*, 2021).

Bio flocculation is a bioremediation process of accumulation of compounds by microbial exopolysaccharides (*Eps*). *Eps* have flocculation and biosorption properties that have prompted interest in their application in wastewater bioremediation owing to their safety and biodegradability (Singh *et al.*, 2015).

1.1 Literature review

Many microorganisms possess the ability to synthesize and excrete *Eps* macromolecules with novel chemical compositions, properties and structures. The chemical composition of *Eps* depends on the genetics of the microbial cells and the physicochemical properties of the environment in which they are developed. The environmental conditions therefore affect the key properties of *Eps* such as porosity, water content, charge, sorption, hydrophobicity and mechanical stability (Sutherland, 2001). Sorptive *Eps* are composed of charged polymers that adsorb, immobilize and entrap charged molecules (Flemming *et al.*, 2007). Removal of molecules is achieved through mechanisms such as ion exchange, complexation, surface precipitation and electrostatic interactions (Chen *et al.*, 2015). The majority of *Eps* are negatively charged because they contain functional groups such as amine, phosphate, hydroxyl, carboxyl and urinate that provide adsorption sites (Durán *et al.*, 2011). They have high molecular weight with binding sites and strong van der Waals forces that promote dye

adsorption properties (Zhang *et al.*, 2009). *Eps* have been investigated for the adsorption of heavy metals and dyes in industrial wastewater (Gong *et al.*, 2008; Mohammad *et al.*, 2017). *Bacillus* strains are widely studied for *Eps* production because they are ubiquitous and are present in almost all ecosystems (Earl *et al.*, 2008). *Bacillus cereus* B-11 cultivated on molasses wastewater produced *Eps* effective in removing reactive dyes from aqueous solutions. The decolorization exhibited by 25 mL of the *Eps* in a 100 mgL^{-1} dye solution of reactive yellow K-4G and turquoise blue KN-G was 97.5% and 94.7%, respectively (Mao *et al.*, 2011). Zobaidul *et al.* (2019) reported that bacteria isolated from textile industry effluent and identified as *Alcaligenes faecalis*, AZ26, *Bacillus cereus*, AZ27 and *Bacillus sp.*, AZ28, could decolorize Novacron super black G dye. Maximum decolorization of 200 mg L^{-1} of the dye by the *Bacillus cereus*, AZ27, *Alcaligenes faecalis*, AZ26 and *Bacillus sp.*, AZ28 after 96 h of incubation time at 37°C and pH 7.0 under static conditions was 93%, 92% and 91%, respectively. Maximum absorbance wavelengths in visible spectra shifted from 600 nm to 410 nm, 378 nm and 373 nm, respectively, which was attributed to the formation of new metabolites due to biodegradation of the parent dye compound.

Bacterial *Eps* production has therefore become an area of research interest because bacteria are easy to culture. Production of bacterial *Eps* is affected by nutrient conditions, such as carbon, nitrogen, phosphorus, pH, temperature, metal ions, salt and oxygen (More *et al.*, 2014). The fermentation medium for *Eps* production can, however, account for at least 70% of the total cost (Mohammed and Wan Dagang, 2019; Küçükaşik *et al.*, 2011). Research has therefore been focused on screening novel *Eps*-producing microorganisms with good yields at low production costs (Mohammad *et al.*, 2017). Sugarcane molasses, a by-product of the sugar industry that is rich in fermentable sugars, has been used previously in an attempt to reduce the production costs of *Eps* (Mao *et al.*, 2011). The molasses contains sucrose, amongst other sugars and vitamins, which have a significant growth-stimulatory effect on microorganisms (Liu *et al.*, 2011). Sucrose was used for the culture of *B. cereus* KMS3-1 and was found to have the highest yield of *Eps* compared to other carbon sources (Krishnamurthy *et al.*, 2020).

Consequently, therefore, this study investigated the use of molasses in reconstituting *Eps* production broth medium. *Eps*-producing bacteria such as *Bacillus* are known to exist in habitats with a carbon/nitrogen medium. These bacteria have been reported to have several defense mechanisms that allow them to live in soil environments such as cotton gin trash where they feed on carbs and proteins present therein (Saxena *et al.*, 2020). Coton gin trash soil originates from plant parts such as small pieces of leaves, petiole, burs, branches and broken seeds, and constitutes about 10% of a 170 kg bale of ginned cotton (Chaudhry and Guitchounts, 2003; Velmourougane *et al.*, 2021). This waste is generally disposed of through incineration and landfilling causing environmental pollution.

The *Eps*-producing bacteria were therefore isolated from gin trash soil sampled from different cotton-growing regions in Kenya. The *Eps* were evaluated for their application in reactive black 5 (RB5) textile dye wastewater bioremediation. RB5 was selected as a model dye because it is widely used for cotton and other cellulosic fiber dyeing and constitutes at least 30% of the total world demand for the global textile dye market (Zobaidul *et al.*, 2019; Hayman *et al.*, 2023).

Bioremediation of textile dye wastewater with *Eps* produced by bacteria cultured from cotton gin trash soil is significant because it will offer a cleaner and effective alternative to the chemicals coagulants commonly used. Such an intervention will also provide an opportunity for a profitable exploitation of the massive gin trash waste which poses a disposal challenge to the ginneries. To the best of our knowledge, research on bioremediation of RB5 dye textile

wastewater with *Eps* produced by bacteria isolated from cotton gin trash has not been done before, hence the novelty of the work.

2. Materials and methods

2.1 Collection and characterization of dye wastewater

A textile dye wastewater sample was collected at a jig dyeing machine drainage point at Rivatex East Africa Limited, Kenya. Sampling was done according to standard sampling principles and guidelines outlined by the American Public Health Association (APHA, 1998). The dye bath constituted of RB5, 4%; sodium sulphate, 80 g/L; sodium carbonate, 30 g/L; and acetic acid, 1 g/L. The sample was immediately transported in a cooler box and refrigerated at 4°C to await analysis. pH and total dissolved substance (TDS) of the wastewater were tested using a BIOBASE precision pH/ion meter. Biochemical oxygen demand (BOD) was analyzed by incubating the sample at 21°C for five days using OxiTop – IDS bottles. Color concentration was analyzed by Lovibond-Day Light Comparator 2000 Unit Equipment (Code No. LSC/WW/006) and UV–Vis spectrophotometer (UV-1900 Shimadzu) at 597 nm. COD was analyzed by COD-571 Analyzer.

2.2 Gin trash collection and isolation of Eps-producing bacteria

Gin trash soil samples were collected from Kitui, Baringo and Meru Ginneries in Kenya. The soil from the first seed cotton cleaner machine was collected into sterile plastic jars using sterile hand gloves. The coning and quarter sampling method was used (Campos, 2017). All the samples were transported in a cooler box and immediately refrigerated at 4°C to await analysis.

The gin trash soil samples from each of the three sources were thoroughly mixed, and 1 g of each of the soil samples was weighed and separately mixed with 9 mL of normal saline and vortexed for 1 min (Sheetal and Arpita, 2016). The soil suspension was then serially diluted up to 10^{-6} by sequentially transferring 1 mL of each of the soil suspensions into 9 mL of normal saline and vortexed again as adapted from Gowsalya *et al.* (2014). The samples were left at room temperature (25°C) in a sterile biosafety cabinet for 48 h. Nine different solid media were used to culture and isolate the bacteria: yeast extract agar (YEA), yeast extract + glucose (YEG), nutrient agar (NA), nutrient agar + glucose (NA+G), malt extract agar (MEA), yeast extract + peptone + dextrose (YEPD), peptone + dextrose agar (PDA), tryptic soy agar (TSA) and sabaroud dextrose agar (SDA). Aliquots of 100 μL from all six dilutions of each of the three soils were individually inoculated in triplicate on Petri dishes containing the nine different media, spread over the surface with a sterile glass rod and incubated at 37°C for 24 h and respective pure colonies isolated. Colonies were examined visually, and those with a glistening, opaque, mucoid, slimy appearance were selected for *Eps* production (Hereher *et al.*, 2018).

2.3 Screening of Eps with activity on reactive black 5 dye wastewater using a reconstituted broth

Each of the isolates with *Eps*-production capacity was screened for their ability to remove RB5 dye from textile wastewater. A broth reconstituted with molasses, 10 g/L, soya, 5 g/L, yeast extract, 3 g/L and magnesium sulphate, 0.2 g/L (MSYM) was used to produce the *Eps*. Each of the isolates was inoculated into 250 mL of the broth in duplicate and then incubated at 30°C for 72 h on an orbital shaker (INCU-Line ILS6) at 150 rpm. pH was maintained at 7–8, being the most optimal, as previously reported by Hereher *et al.* (2018). After the incubation, each of the samples was refrigerated at 4°C for 24 h, then mixed with 5 mL of 10% (v/v) trichloroacetic acid (TCA) and left again to stand for 2 h at 4°C to allow for purification of proteins and salts (Vivek *et al.*, 2016). Respective cell- and protein-free supernatants were then obtained by centrifugation of each of the samples at 6,000 rpm for 30 min (Hereher *et al.*, 2018).

Respective 8 mL of the cell- and protein-free supernatants were individually but separately dosed in duplicate with 1 mL of presterilized dye wastewater (diluted in a 1:500 ratio). All the samples were then incubated at 30°C for 72 h on the shaker at 150 rpm while maintaining pH between 7 and 8. Respective optical densities of the samples were then obtained by a UV–Vis spectrophotometer at 597 nm. A control sample of uninoculated broth mixed with the dye wastewater was prepared in the same manner. Percentage dye removal was calculated using equation (1) as adopted from Kumar *et al.* (2017):

$$\% \text{ color removal} = \frac{A_{c-A_i}}{A_c} \times 100. \quad (1)$$

Where A_i and A_c represent the absorbance of the inoculated and uninoculated control samples, respectively.

The isolates whose *Eps* gave at least 60% dye removal capacity were selected for optimization.

2.4 Optimization of reactive black dye removal

Dye removal by the screened *Eps* was optimized using the MSYM broth containing varying molasses concentrations. A full factorial design of three variables; molasses concentrations, temperature and incubation time at three different levels each (K^N), was used to optimize the dye wastewater bioremediation. Molasses was varied from 10 g/L to 15 g/L and 20 g/L. The amounts of yeast extract and magnesium sulphate were retained as previously used in Section 2.3. MSYM broth media were prepared, inoculated with each of the selected isolates and incubated under conditions shown in Table 1. Cell and protein-free *Eps* supernatants were extracted and optimized for their dye removal capacity under the temperature and time conditions shown in Table 1. Dye removal efficiency for each of the *Eps* was assessed as per the procedure described in Section 2.3. Glucose, 5 g/L, peptone, 2.5 g/L, yeast extract, 4.5 g/L, magnesium sulphate, 0.5 g/L (GPYM) broth was simultaneously prepared and studied alongside the MSYM for comparison purposes.

Bacteria isolates whose *Eps* were found to have at least 80% dye removal efficiency were selected for characterization. Table 1 shows 3^3 full factorial dye removal experimental designs as generated by MATLAB 17 software.

2.5 Characterization of Eps-treated dye wastewater

Samples of the dye wastewater, 250 mL each, were further treated by mixing with 50 mL of the *Eps*-containing supernatants found to give at least 80% dye removal capacity. The samples were incubated at the optimal conditions determined under Section 2.4, The *Eps*-treated dye wastewater samples were then analyzed for color removal by Lovibond-Day Light Comparator, UV–Vis spectrophotometer and FTIR.

2.6 Molecular characterization of bacteria isolates

The isolates whose *Eps* met the optimal dye removal threshold were characterized through molecular tests by 16S rRNA sequencing for identification. The DNA of the isolates was extracted using standard protocols. The confirmation of DNA was done using agarose gel electrophoresis on 1% agarose gel. A fragment of the 16S rRNA gene was amplified by 27F (5-GAGTTTGATCCTGGCTCA-3′) and 1492 (R-TACG GYTACCTTGTTACGACTT) universal primers. The PCR reaction was performed at 94°C for 2 min, followed by 35 amplification cycles at 94°C for 45 s, 55°C for 60 s, 72°C for 60 s and a final extension at 72°C for 10 min, followed by termination at 4°C. The PCR amplicon was purified to remove contaminants.

Experiment no.	Temperature (°C)	Time (h)	Molasses (g/L) in (MSYM) broth
1	30	24	10
2	34	24	10
3	38	24	10
4	30	48	10
5	34	48	10
6	38	48	10
7	30	72	10
8	34	72	10
9	38	72	10
10	30	24	15
11	34	24	15
12	38	24	15
13	30	48	15
14	34	48	15
15	38	48	15
16	30	72	15
17	34	72	15
18	38	72	15
19	30	24	20
20	34	24	20
21	38	24	20
22	30	48	20
23	34	48	20
24	38	48	20
25	30	72	20
26	34	72	20
27	38	72	20

Table 1.
3^3 Full factorial experiment design

Source: Authors' own work

Forward and reverse DNA sequencing reactions of PCR amplicons were carried out with forward primers and reverse primers using the BDT v3.1 Cycle Sequencing Kit on the ABI 3730xl Genetic Analyzer. The consensus sequence of the 16S rRNA gene was generated from forward and reverse sequence data using aligner software.

3. Results and discussion

3.1 Dye wastewater characterization

Table 2 shows the physicochemical properties of the textile dye wastewater sample in comparison to the water quality regulations set by the National Environment Management

Parameter	Value	NEMA limits
pH	10.23	6–9
Color concentration (Hazen)	>250	<40
Total dissolved solids (TDS) (mg/L)	81.575	2,000
COD (mg/L)	1,486	1,000
BOD (mg/L)	350	500

Table 2.
Physicochemical properties of the dye wastewater in comparison with NEMA limits

Source: Authors' own work

Authority (NEMA) [Environmental Management and Co-Ordination Act (Water Quality) Regulations, 2006].

pH, color and COD of the dye wastewater were found to be higher than the set limits.

3.2 *Isolation of Eps-producing bacteria*

Figure 1 shows some of the selected colonies with *Eps*-production capacity, characterized by glistening, mucoid, slimy colonies as reported by Hereher *et al.* (2018). The isolated *Eps*-producing bacteria colonies were mainly cream and white in color.

Out of the nine solid media used, YEA, PDA, NA+G and NA were found to be the best in supporting the culture and isolation of the majority of the *Eps*-producing bacteria from the soil sample collected from Meru Ginnery. The majority of the *Eps*-producers cultured and isolated from the Kitui and Baringo soil samples were supported by YEPD and YEA, and PDA and YEA, respectively. SDA supported the growth of the least number of *Eps*-producers across the three gin trash soil samples.

Source: Authors' own work

Figure 1.
Samples of mucoidal and slimy *Eps*-producers bacterial isolates

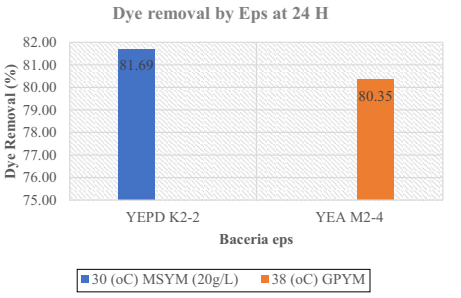
3.3 Reactive black 5 dye wastewater treatment using Eps produced in reconstituted broth

Six *Eps* produced by the isolates cultured in the molasses, 10 g/L, soya, 5 g/L, yeast extract, 3 g/L and magnesium sulphate, 0.2 g/L (MSYM) broth gave at least 60% dye removal efficiency. The six *Eps* were hence selected for optimization of the RB5 dye removal from the textile wastewater.

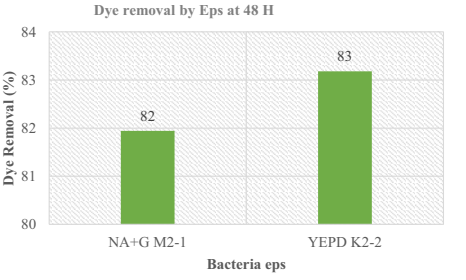
3.4 Optimization of dye wastewater treatment with bacterial Eps

Eps produced by the six isolates found to have the capacity to remove at least 60% of the RB5 dye were optimized by using the experimental design shown in Table 1. Figure 2(a)–(c) illustrates the activity of the *Eps* with at least 80% dye removal capacity after 24 h, 48 h and 72 h, respectively. At least 80% dye removal capacity was realized from the following *Eps*:

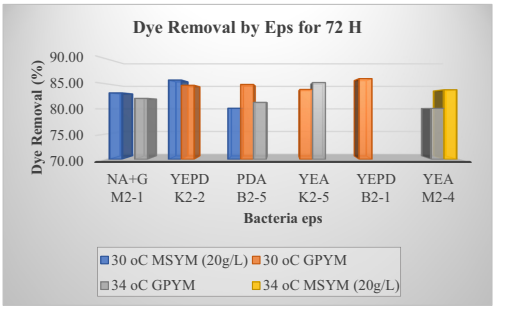
- YEPD K2^{-2} and YEAM2^{-4} *Eps* produced in the MSYM broth that contained 20 g/L molasses at 30°C for 24 h, as well as the *Eps* produced in the GPYM broth at 38°C for 24 h, as shown in Figure 2(a).
- NA+G M2^{-1} and YEPD K2^{-2} *Eps* produced in the MSYM broth that contained 20 g/L molasses at 30°C for 48 h as shown in Figure 2(b).
- NA+GM2^{-1}, YEPD K2^{-2} and PDA B2^{-5} *Eps* produced in the MSYM broth that contained 20 g/L molasses at 30°C for 72 h.



(a)



(b)



(c)

Figure 2.
Eps with at least 80% dye removal capacity for 24 h (a), 48 h (b) and 72 h (c)

Source: Authors' own work

- YEAM2^{-4} *Eps* produced in the MSYM broth that contained 20 g/L molasses at 34°C for 72 h.
- PDA B2^{-5}, YEPD K2^{-2}, YEAK2^{-5} and YEPD B2^{-1} *Eps* produced in the GPYM broth at 30°C for 72 h.
- All the *Eps* produced in the GPYM at 34°C for 72 h except those of YEPD K2^{-2} and YEPD B2^{-1}, as shown in Figure 2 (c).

NA+G$_2^{-1}$, PDA B$_2^{-5}$ and YEPD K$_2^{-2}$ *Eps,* produced in the medium that contained 20 g/L molasses, could remove at least 80% of the dye as opposed to those produced in the media that contained 10 g/L and 15 g/L due to the increased carbon content required by the bacteria for *Eps* production. This finding agrees with that reported by Krishnamurthy *et al.* (2020), that sucrose is an appropriate carbon source for *Eps* production. Razack *et al.* (2013) reported that 2% molasses sucrose yielded 2.66 g/L *Eps* compared to maltose and fructose, which yielded 1.42 and 0.96 g/L, respectively, from *Bacillus subtilis* after incubation at 30°C for 48 h.

Hereher *et al.* (2018) also reported that, out of the nine carbon sources they screened for *Eps* production, 4.5% sucrose was found to be the most efficient by giving the highest *Eps* yield of 45 g/L at 25°C after 96 h of incubation of *Micrococcus roseus*. Comparatively, investigation on the removal of RB5 dye from textile wastewater using ferrous sulfate ($FeSO_4$) and magnesium chloride ($MgCl_2$), 400 g/L each, gave respective color removal efficacy of 85% and 97%, achieved at pH 12 (Firdous *et al.*, 2018).

3.5 Characterization of Eps-treated dye wastewater

3.5.1 Lovibond-Day Light Comparator Analysis. Optimal NA+G$_2^{-1}$, PDA B$_2^{-5}$ and YEPD K$_2^{-2}$ *Eps*-treated wastewater samples analyzed for color removal by Lovibond-Day Light Comparator were found to have respective 80, 85 and 70 Hazen values which were higher than the 40 Hazen limit set by NEMA. The high yellowness level of the *Eps*-treated dye wastewater was attributed to the brown color of the molasses syrup used in the *Eps* production broth medium (MSYM) formulation.

3.5.2 UV–Vis spectrophotometer analysis. Figure 3, shows the spectra for the untreated (control) and *Eps*-treated samples. The peak at 597 nm for the untreated wastewater sample disappeared after the *Eps* treatment, indicating the removal of the dye.

3.5.3 FTIR analysis of the Eps-treated dye wastewater. The untreated and *Eps*-treated wastewater displayed spectra as shown in Figure 4 (a)–(d) characteristic with the carbonyl and hydroxyl groups at 1,500–1,750 cm^{-1} and 3,000–3,500 cm^{-1}.

Because no new peaks were formed or shifted before and after the *Eps* treatment, the decolorization was attributed to an adsorption mechanism (Veena *et al.*, 2019). The adsorption mechanism was confirmed by the formation of huge flocs after the *Eps* treatment as shown in Figure 5. The flocs would require to be removed from the treated wastewater before disposal by decanting, filtration or centrifugation methods.

The RB5 dye adsorption was associated with the dye-binding ability of the hydroxyl and carbonyl, carboxyl and amide functional groups in the *Eps*. Similar findings were reported by Mengelizadeh and Pourzamani (2020), who observed that the adsorption of RB5 dye on carbon nanotubes occurred mostly on hydroxyl (–OH) and carboxyl (–COOH) groups, which were predominant before and after the treatment.

3.6 Molecular characterization

Molecular characterization of the NA+GM$_2^{-1}$, PDAB$_2^{-5}$ and YEPDK$_2^{-2}$ bacteria whose *Eps* gave at least 80% RB5 removal capacity were isolated from gin trash soil collected from

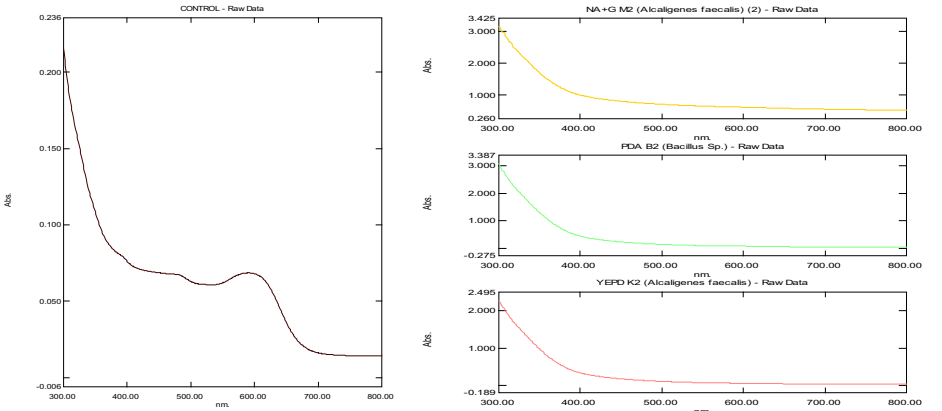


Figure 3.
Spectra for untreated (a) and *Eps*-treated wastewater (b)

Source: Authors' own work

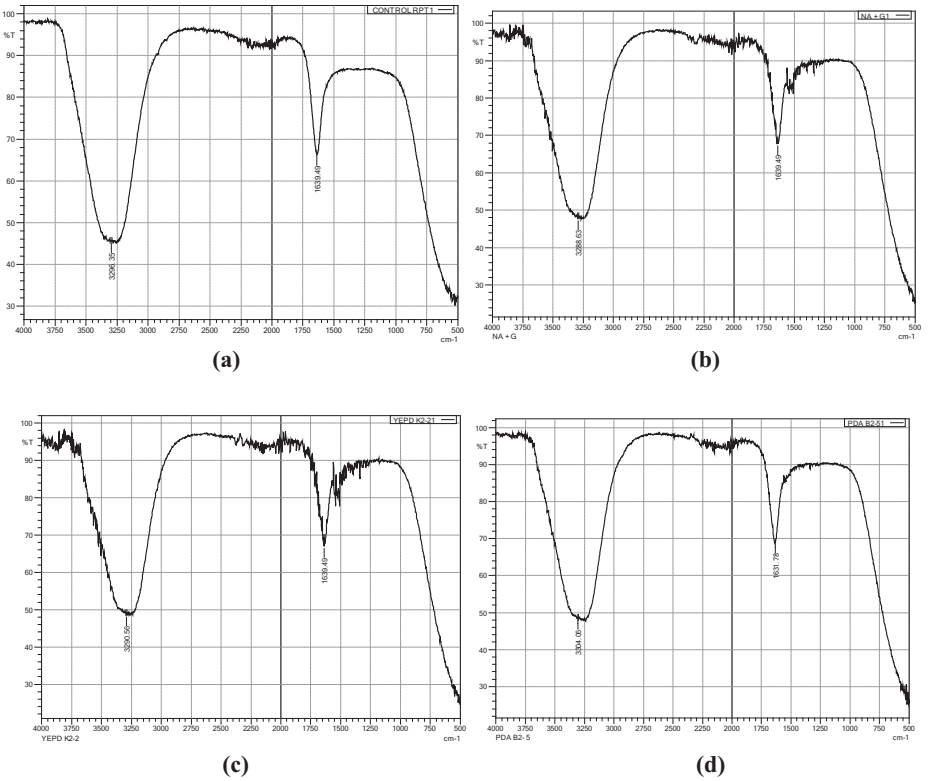
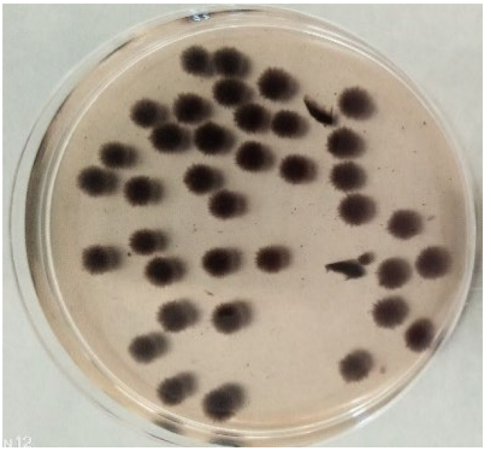


Figure 4.
FTIR spectra for untreated dye wastewater (a), NA+G $M_2{}^{-1}$ *Eps*-treated dye wastewater (b), YEPD $K_2{}^{-2}$ *Eps*-treated dye wastewater (c), PDA $B_2{}^{-5}$ *Eps*-treated dye wastewater (d)

Source: Authors' own work



Source: Authors' own work

Figure 5.
Flocs formed after *Eps*-treatment

Meru, Baringo and Kitui ginneries, respectively. $PDAB_2^{-5}$ and $YEPDK_2^{-2}$ were both predicted to be *Bacillus sp*. with 99.93% and 99.86% identity. The $NA+GM_2^{-1}$ bacteria was predicted to be *Alcaligenes faecalis* with 99.84% identity.

4. Conclusion

The research was done to investigate the production of *Eps* by bacteria cultured from gin trash soil samples collected from ginneries located in different cotton-growing regions (Kitu, Baringo and Meru) in Kenya. The capacity of the *Eps* to remove RB5 dye from textile wastewater was assessed. Out of the nine different solid media used to culture and isolate *Eps*-producing bacteria from the gin trash soils, YEA, PDA, NA+G and YEPD were found to support the growth of the majority of the isolates. MSYM broth was reconstituted using Molasses, 20 g/L, soya, 5 g/L, as substitutes for the conventional medium ingredients. The broth was able to support *Eps* production by the bacteria isolates at 30°C for 72 h incubation time while on an orbital shaker at 150 rpm. Three of the *Eps* produced were able to remove at least 80% of the RB5 dye from the wastewater. The dye removal was established to be through an adsorption mechanism because there was no shift of the dye wastewater spectrum peaks before and after the bioremediation, as determined by FTIR analysis. The dye removal by the three different *Eps* was confirmed by the disappearance of the dye absorbance peaks at 597 nm as determined by the UV–Vis spectrophotometer study. The *Eps*-treated wastewater samples also formed large flocs as a result of the *Eps*-adsorption. The bacteria isolates whose *Eps* were found to have at least 80% dye removal capacity were identified as *Bacillus sp*. and *Alcaligenes faecalis*.

These findings therefore imply that the bacterial *Eps* can offer a cleaner, and more effective alternative to the chemical coagulants that result in secondary sludge and remain in wastewater causing harmful effects to the environmental, aquatic and human health. Treatment of the wastewater by the bacterial *Eps* would also enable textile mills to meet the dye limits allowable by regulatory bodies such as NEMA. Utilization of molasses, soya and gin trash soil for *Eps* production offers alternative uses for the materials which would provide additional revenue generation options for their respective value chain actors.

4.1 Limitations and recommendations for further research

This research did not assess the efficacy of the RB5 dye removal from the wastewater at varied concentrations of the dye and the *Eps*. The rate of aeration through shaking was also held constant at 150 rpm. Further research on the optimization of the dye and the *Eps* concentrations at varied shaker rotations is therefore recommended. A study can also be undertaken on the possible use of the *Eps* concoctions for dye wastewater bioremediation.

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