BIOREMEDITION OF TEXTILE DYE WASTEWATER USING EXOPOLYSACCHARIDES PRODUCED BY BACTERIA CULTURED FROM COTTON GIN TRASH SOIL

BY

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DECLARATION

DECLARATION

STUDENT'S DECLARATION

I hereby declare that this thesis report is my work and has not been presented for degree in

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DEDICATION

I hereby dedicate this report to my beloved sons, Desmond, Emmanuel and Darian for their prayers and moral support throughout this journey. Your encouragement kept me going, may God bless you abundantly.

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ABSTRACT

Synthetic dyes possess high thermal and photo stability making color removal from textile wastewater difficult hence causing major water pollution problem. Exopolysaccharides (*Eps*) have attracted interest owing to their safety and possible application in bioremediation. The main objective of this research was therefore to extract bacterial *Eps* and assess their efficiency in bioremediation of Reactive Black 5 (RB5) textile wastewater in comparison with chemical coagulants. Specific objectives were: to optimize chemical coagulation of the wastewater; to isolate bacteria with *Eps* production capacity from cotton gin trash soils; to screen for *Eps* with activity on the wastewater; to optimize bioremediation of the wastewater by the *Eps*; to identify *Eps*-producing isolates, *Eps* and *Eps*-treated wastewater. The wastewater was collected from Rivatex while cotton gin trash soils were grab sampled from three ginneries in Meru, Kitui and Baringo. In this work, Polyaluminium Ferric Chloride (PaFC), Polyferrous Sulphate (PFS) and alum were optimized by varying pH (2-10) and doses; alum, 60-140g/L, PFS, 20-100mg/L and PaFC, 140-280mg/L. *Eps*-producers were isolated using nine different media. Nutrient Broth +Sucrose, Nutrient Broth+ Glucose and Glucose, Yeast Extract and Magnesium Sulphate ($GPYMgSO_4$) were used to assess *Eps* activity on the wastewater. The broths were dosed with the wastewater, *Eps* from high (≥90%) producers and incubated at 30 ^oC, pH 7-8 for 72h. *Eps* that supported at least 94% dye removal were optimized in Molasses, Soya, Yeast Extract and Magnesium Sulphate ($MSYMgSO_4$) broth by varying temperature, incubation time and molasses concentration. *Eps* that gave at least 80% dye removal were characterized by FTIR and GC-MS, isolates identified molecularly and the treated wastewater analyzed by UV-Vis and FTIR. Out of the nine media used, YEA, YEPD, NA+G and PDA were the most efficient in isolation of *Eps*-producing bacteria. $GPYMgSO_4$ supported growth of 50% of the isolates whose *Eps* had at least 94% dye removal. Three *Eps* gave at least 80% dye removal in $MSYMgSO_4$ at 30 ^oC, 72h and 20g/L molasses optimal conditions which was comparable to that of PFS, 86%. The isolates were identified as *Bacillus sp*. (Baringo and Kitui) and *Alcaligenes Faecalis* (Meru). GC-Ms indicated that *Bacillus sp. Eps* were heteropolysaccharides while *Alcaligenes Faecalis* was homopolysaccharide. FTIR determined that the *Eps* mainly contained hydroxyl, carbonyl and carboxyl active groups that were associated to the dye binding by adsorption, since no new peaks were formed in the treated wastewater. UV-Vis confirmed the dye removal by the *Eps* that gave at least 80% efficiency by absence of the peak at 597nm in the treated wastewater. In conclusion, *Eps* produced by bacteria isolated from gin trash soil were found to possess capacity to remove at least 80% of RB5 from textile wastewater. $MSYMgSO_4$ was found suitable to substitute $GPYMgSO_4$ for production of the *Eps*. The *Eps* had comparable dye removal efficiency to that of PFS. Cotton gin trash soil is therefore recommended as a suitable source of *Eps* with at least 80% RB5 removal capacity using $MSYMgSO_4$ at 30^oC for 72h.The bacterial *Eps* are hence proposed as effective substitutes to the chemical coagulants.

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ACRONYMS

RB5	Reactive Black 5
PaFC	Polyaluminium Ferric Chloride
PFS	Polyferrous Sulphate
Alum	Potassium aluminum sulfate
PaC	Polyaluminium Chloride
PaS	Polyaluminium Sulphate
PFC	Polyferric chloride
YEG	Yeast Extract + Glucose
NA	Nutrient Agar
MEA	Malt Extract Agar
TSA	Tryptic Soy Agar
YEA	Yeast Extract Agar
YEPD	Yeast Extract + Peptone +Dextrose
NA+G	Nutrient Agar +Glucose
PDA	Peptone + Dextrose + Agar
NB+S	Nutrient Broth +Sucrose
NG +G	Nutrient Broth +Glucose
$GPYMgSO_4$	Glucose, Yeast Extract and Magnesium Sulphate
$MSYMgSO_4$	Molasses, Soya, Yeast Extract and Magnesium Sulphate
FT-IR	Fourier Transform Infrared
UV-Vis	Ultraviolet-visible
GC-Ms	Gas Chromatography Mass Spectrometry

HPLC	High-performance liquid chromatography
NMR	Nuclear Magnetic Resonance
SEM	Scanning Electron Microscopy
ATR	Attenuated total reflectance
HPAEC	High-Performance Anion Exchange Chromatography
GPYMgSO_4	Glucose + Peptone+ Yeast extract + Malt extract
Cu	Copper
Zn	zinc
Cr	Chromium
As	Arsenic
Eps	Exopolysaccharides
SS	Suspended Solids
TDS	Total Dissolved Solids
COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
Ec	Electrical Conductivity
NEMA	National Environment Management Authority
GRAS	Generally Recognized as Safe
NSB-G	Novacron Super Black G
TCA	Trichloroacetic acid
NEMA	National Environment Management Authority

CHAPTER ONE

1.0 Introduction

High levels of environmental contamination in the modern world are a result of fast industrial development. The production of textiles uses both wet and dry methods. In the typical textile industry, wastewater is mostly created during the pretreatment, dyeing, printing, and finishing processes. Due to the large water consumption of the wet processes which include sizing, de-sizing, scouring, bleaching, mercerizing, dyeing, printing, and finishing, highly contaminated effluent is released into the environment (Siddique *et al*., 2017). According to Mani and Bharagava (2018), the primary industries that pollute fresh water bodies worldwide are agriculture and textile activities. One of the primary sources of wastewaters containing dyes that seriously contaminate the environment globally is the textile industries (Kasavan *et al*., 2021). Textile dyeing and finishing processes are deemed to be responsible for 17–20% of industrial water pollution, and they also account for roughly 20% of wastewater globally (Baban *et al*., 2010; Kant, 2012; Hasanbeigi and Price, 2015; Fleischmann, 2019). According to Chen and Burns (2006), textile dyeing ranks as the second biggest polluter, accounting for 15% to 20% of the wastewater collected. According to estimates, the dyeing process accounts for up to 47% of the textile industry's overall organic load (EWA, 2005). More than 8,000 chemicals, including dyes, are used in the textile industry; most of which pose health risk to human, animals, and the environment if they are dumped into water bodies untreated (Reddy and Osborne, 2020). Global output of 10,000 distinct textile dyes is estimated to be 7.105 metric tons annually (Baban *et al*., 2010; Chandanshive *et al*., 2020; Srivastava *et al*., 2022). Ten percent of the dyes produced are used in the textile and related industries, and about two percent are released into

effluents (Allen and Koumanova, 2005; Prasad and Aikat, 2014; Uma, Tarun, and Biswanath, 2016). Every year, wastewater contains more than 280,000 tons of textile dyes (Fouda *et al*., 2021; Selim *et al*., 2021). According to studies by Trovaslet *et al*. (2007) and Asha *et al*. (2015), dye production industries release between 30,000 and 150,000 tons of dyes into water bodies each year, which results into serious pollution. Two thirds of the market for dyestuffs is accounted for by the textile sector (Elisangela *et al*., 2009). More than shipping and international travel combined, the textile industry's 1.2 billion tons of carbon dioxide (CO_2) equivalent gas emissions in 2015 came from its production processes (Ellen MacArthur Foundation, 2017). The processes involved in dyeing and finishing textiles account for around 3% of global CO_2 emissions; by 2050, it is expected that this percentage will have risen to over 10% (Hudd, 2022). This is why, at the United Nations Climate Change Conference (COP26) in November 2021, nearly 200 countries decided to attain net zero emissions by 2050 and to urgently cut glasshouse gas emissions by 1.5 degrees Celsius. Consequently, the industries have been named as some of the worst environmental polluters in the world, with colour being deemed the most harmful pollutant (Wafaa, 2006; Daneshvar *et al*., 2007; Baban *et al*., 2010).

In contrast to natural textile dyes, synthetic dyes are more frequently employed in textile dyeing due to their greater absorption, fastness, and ease of solubility in water (Jamee and Siddique, 2019). According to Mani and Bharagava (2018), synthetic dyes are made of both organic and inorganic molecules, which are typically made from earth minerals or petroleum byproducts.

Natural textile dyes on the other hand, contain anti-microbial, anti-bacterial, anti-inflammatory, UV protection, and deodorizing qualities despite having low absorbance and fastness (Elsahida *et al*., 2019).

Additionally, a greater volume of water is used throughout the dye bath, printing paste, and rinse phases of the operations. It was estimated by Kdasi *et al*. (2004) and Kant (2012) that after processing 12–20 tons of textiles per day, 1,000–3,000 m^3 of water are released. Large amounts of effluents containing unfixed dyes in varying concentrations of 10-15% are produced during the washing of textile goods that have been dyed or printed (Rajamohan & Rajasimman, 2013). Depending on the method, it is estimated that 200 L of water are needed for the chemical processing and rinsing of 1 kg of cotton textile material (Ghaly *et al*. 2014; Hussain and Wahab, 2018). About 45% of the water used in textile mills is accounted for by pretreatment wastewater, with the remaining 50–55% coming from dyeing and processing. (Wang and others, 2011) Depending on the type of dye and fibre used, water consumption during the dyeing process might range from 30 to 50 L/kg of cloth (EWA, 2005).

Table 1.1 gives a summary of the proportionate water consumption and organic load from the different textile process.

Table 1.1. Water consumption and organic load in different textile processing steps (EWA, 2005)

Process	**Water consumption (% of total consumption of the textile plant)**			**Organic load (% from total organic load of the textile plant)**		
	Minimum	**Medium**	**Maximum**	**Minimum**	**Medium**	**Maximum**
Preparation	16	36	54	45	61	77
Dyeing	4	29	53	4	23	47
Printing	42	55	38	42	59	75
Wetting	0.3	0.4	0.6	0	0.1	0.1
Fabrics washing	3	28	52	1	13	25
Finishing	0.3	2	4	0.1	3	7

1.1 Characteristics of Textile Dye Wastewater

The composition of real textile wastewater varies according to substrate raw materials, dye types, equipment, chemicals, and auxiliaries employed, as well as the kind of cloth being processed (Brik *et al*., 2006; Ramos *et al*., 2021; Yaseen and Scholz, 2019). The kind dye used determines how polluted the wastewater is. Contaminants such as dye ions and heavy metals are dependent on exposure duration, absorbed dose, and concentrations (Mani and Kumar, 2014). Pollutant contents from printing wastewater have been reported to be greater than those from dyeing wastewater (Madhav *et al*., 2018). The chemical compounds and auxiliaries found in dye wastewater have an immediate impact on aquatic life, plants, and human health (Fazal *et al*., 2018; Rahman *et al*., 2016). According to Chandra *et al*. (2006), Soares *et al*. (2006), Ntuli *et al*. (2009), Reddy and Osborne (2020), wastewater from printing and dyeing units is frequently rich in organic compounds, colour residues, Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and Total Suspended Solids (TSS). Additionally, the wastewater may contain alkaline and acidic pH, salinity, electrical conductivity (EC), temperature, and

heavy metal ions, all of which are hazardous to terrestrial, aquatic, and human life. Textile effluents have different concentrations of metals due to the presence of dyes and dyeing additives like sodium hydroxide, sodium carbonate, and salts (Yaseen and Scholz 2016; Velusamy *et al*., 2021). In the process of making dyes and pigments, heavy metals such mercury (Hg), cadmium (cd), lead (Pb), and arsenic (As) are frequently utilized (Türksoy *et al*., 2021). The features of a typical textile mill dye effluent are as shown in Table 1.2.

Table 1.2. Characteristics of typical raw textile dye effluent (Eswaramoorthi, Dhanapal and Chauhan, 2014).

Parameter	**Values**
pH	6-10
Temperature (^oC)	35-45
Biological Oxygen Demand (BOD) (mgL^{-1})	80-6,000
Chemical Oxygen Demand (COD) (mgL^{-1})	150-12,000
Total Suspended Solids (TSS) (mgL^{-1})	15-8,000
Total Dissolved Solids (TDS) (mgL^{-1})	2,900-3,100
Colour (Pt-Co)	50-2,500
Trace elements (Zn, Fe, Cu, Hg, Mn, Pb, Cr, Ni (mgL^{-1})	<10
Sulphate (mgL^{-1})	600-1,00
Oil and grease (mgL^{-1})	10-30

In textile wastewater, colour is the easily noticeable impurity to be identified. The aquatic life is affected by the aesthetic and transparent qualities of water bodies when little levels of certain dyes (<1 mg/L) are present, as noted by Gomes *et al*. (2000) and Gita *et al*. (2017). The effluent affects physical characteristics including odor in addition to corrosive characteristics. Colour obstructs light penetration, which in turn thwarts the photosynthesis process. Dye structure with the (C = C) double bonds swings to absorb light and create visible colours when dye molecules are exposed to light (Akbari, Remigy, and Aptel, 2002). According to Trovaslet *et al*. (2007) and Mani and Bharagava (2018), this could

result in dissolved oxygen (DO) depletion, deterioration of water quality, and serious harmful consequences on aquatic life. If some azo dyes are allowed to persistently contaminate water bodies, they can have harmful effects not only on aquatic and human life, but also on agricultural productivity. These compounds contain mutagenic, poisonous, and carcinogenic substances (Kalme Ghodake and Govindwar, 2007; Kalme *et al*., 2007; Govindwar and Kagalkar,2010; Wu *et al*., 2021). Dyes have a high degree of thermal and photo stability, which means that if they are not handled, they can linger in the aquatic environment for a long time (Yaseen and Scholz 2016; Dos Santos *et al*. 2007; Hubadillah *et al*., 2020; Shah *et al*. 2013). For example, reactive Blue-19 in hydrolyzed form, with a half-life of approximately 46 years at 25 ^{o}C and pH 7.0, might linger in contaminated waterways for years, according to Hao *et al*. (2000) and Carmen and Daniela (2012). It has been demonstrated that certain disperse dyes can bioaccumulate, and large amounts of heavy metal ions from textile effluents have been found in exposed algae and higher plants (Khan and Husain, 2007). In addition to polluting the environment, they also change the toxicity along the whole food chain, which results in biomagnification (Palanivelan *et al*., 2013). According to Madhav et al. (2020), increase in blood pressure, irregular temperature, kidney damage, and cramping are among the physiological diseases caused by these hazardous substances that enter the human body through the food chain and food web. Ayed *et al*. (2011) and Phugare *et al*. (2011) have also reported on the phytotoxic effects of azo dyes on plant growth. Low productivity is caused by textile effluents blocking soil pores and hardening the soil's texture, which inhibits root penetration. According to Chandra *et al*. (2009), wastewater from textile dye effluent discharged into drainage systems corrodes sewage pipes and lowers the quality of drinking water in pumps,

rendering it unsafe for human use. Nese, Sivri, and Toroz (2007) claimed that benzidine and other aromatic chemicals, which are known carcinogens, are used to make numerous dyes. These compounds may undergo changes due to microbial metabolism. Certain trace metals found in these effluents, such as Cr, As, Cu, and Zn, can lead to a number of health issues, such as dermatitis, severe skin irritation, nausea, bleeding, and ulceration of the skin (Mudhoo *et al*., 2020; Wu *et al*., 2021; Al-Tohamy *et al*., 2022). Haemoglobin adducts and disruptions in blood formation are caused by the absorption of azo dyes and their byproducts (toxic amines) through the gastrointestinal tract, skin, and lungs, posing a risk to human and animal health (Chung, 2016). Oral consumption and dust exposure are the two ways that textile dyes can be toxic to humans (Clark, 2011). There are still more than 100 dyes on the market out of the 4000 that have been tested for toxicity and determined to have the potential to generate carcinogenic amines (Lacasse and Baumann, 2012).

According to Carmen and Daniela (2012), dyes are not biodegradable in aerobic wastewater treatment methods, and some of them end up adsorbed in the sludge. Decolorization and degradation of dye wastewater are therefore crucial before disposing it into the environment. In order to preserve the environment and facilitate the recycling of treated wastewater for use as irrigation or further processing in textile factories, textile effluents must be treated. The eradication of toxicity is not always ensured by colour, as some wastewater components may degrade or reduce and produce hazardous intermediary compounds (Dellai *et al*., 2013; Gaviria-Arroyave *et al*., 2018; Al-Tohamy *et al*., 2022). To guarantee the environmental safety and efficacy of the treatment process, toxicological and physicochemical evaluations of the treated wastewater are therefore required (Seralathan *et al*., 2017; Bulacio Gil *et al*., 2018).

1.2 Dye Wastewater Treatment

Since most dyes are fully soluble in aqueous solutions, eliminating them from wastewater is a difficult task (Sanayei *et al*., 2010). Because of their complex aromatic structures and resilience to oxidizing chemicals, sunshine, and bacteria, dyes are difficult to degrade (Asha *et al*., 2015). The primary source of synthetic dyes is their intricate aromatic molecular structure, which is frequently created to withstand fading when exposed to oxidizing chemicals, sweat, soap, water, or light. During the dyeing process, chemical auxiliaries are employed to obtain uniform colour depth and fastness attributes appropriate for the desired end use of the cloth. These include alkali, acids, surfactants, levelling, chelating, and softening agents, emulsifying oils, and binders (Lablu, 2022). They cause the dye to become more stable and challenging to biodegrade. The inability of dyes to break down makes it challenging to remove colour from textile wastewaters. It has been discovered that azo dyes, such as Direct Red 81, RB5, and Acid Yellow 19, are extremely stable in both soil and water conditions (Imran *et al*., 2015). Treatment of wastewater containing azo textile dye is particularly challenging due to the high salinity and several heavy metals present. According to Farah, Daniel and Daniella (2011), the presence of sulfonate groups with azo bonds prevents azo dyes from degrading aerobically.

Textile wastewater is first thoroughly mixed to ensure homogeneity before treatment. The effluent is then mixed with chemicals such as strong sulfuric acid (66°Be), boiler flue gas, concentrated hydrochloric acid (20–22°Be), carbon dioxide, Sulphur dioxide, or nitric acid to neutralize the pH to a range of 5 to 9. Additional basic reagents include ammonia, caustic soda, hydrated lime ($Ca(OH)_2$), soda ash (Na_2CO_3), and limestone ($CaCO_3$) (Liu and Tay, 2004; Babu *et al*., 2007). Colloidal particles then settle as a result of mechanical

flocculation and chemical coagulation, which cause sedimentation. In order to alter the colloidal particles' surface characteristics and induce coagulation and sedimentation, chemical coagulants are added to the effluent. Alum, Poly Aluminum Chloride (PAC), ferrous and ferric chloro-sulfates, and ferric chloride are examples of chemical coagulants that are frequently utilized (Hassan, Li, and Noor, 2009). Through vigorous mixing, tiny particles can be grouped and allowed to settle through a physical process called mechanical flocculation (Tripathy and De, 2006). However, coagulation by chemical and mechanical means produces large amounts of sludge, which presents a significant disposal problem.

1.2.1 Dye Wastewater Treatment Methods

The three categories of wastewater treatment technologies include chemical, biological, and physical methods. Colour removal from textile effluents can be accomplished through a variety of techniques, including flocculation, membrane filtration (including reverse osmosis, nanofiltration, microfiltration, and ultrafiltration), photo oxidation, electro coagulation, adsorption, active carbon, and ion exchange (Wang *et al*., 2011; Al-Tohamy *et al*., 2022). However, most of these physicochemical techniques are not only expensive, but they also produce secondary toxic sludge that needs to be settled, dewatered, and pressed into a cake before being dumped in a landfill (Daneshvar, Salary and Khataee *et al*., 2004; Zaharia, Suteu & Muresan, 2011). As per Shih *et al*. (2001) and Salehizadeh & Shojaosadati (2001), flocculating agents are categorized into three groups: inorganic flocculants, which include aluminum sulphate and poly-aluminum chlorides; synthetic flocculants, like polyacrylamide and polyethyleneimine; and natural or bioflocculant, which includes chitosan, alginate, and extracellular polymeric substances.

The removal of organic pollutants from water bodies is done by use of common chemical coagulants, such as $FeCl_3$, $FeSO_4$, $MgCl_2$, alum, Polyaluminium Chloride (PaC), Polyaluminium Ferric Chloride (PaFC), Polyaluminium Sulphate (PaS), and Polyferrous Sulphate (PFS) (Gupta, Carrott and Ribeiro, 2009; Gao, Yue and Miao, 2003). The primary drawbacks of the chemical coagulants are their high cost, difficult process control, and the influence of contaminants such non-ionic detergents on precipitation (Carmen and Daniela, 2012). According to Zaharia, Suteu, and Muresan (2011), Crini and Lighthouse (2019), Syafiuddin and Fulazzaky (2021), the ensuing sludge also poses an environmental threat. Various techniques such dewatering, pyrolysis/carbonization, oxidation, gasification, landfills, and incineration are employed to mitigate the environmental issues (Wei *et al*., 2018; Wang *et al*., 2019).

Therefore, focus has switched to biological treatment methods in order to minimize the detrimental effects of the inorganic coagulants and flocculants (Sarkar *et al*., 2017). According to Senelisile, Bukisile, and Pinkie (2022), there have been almost 9,000 research papers published on biological treatment approaches in the last five years, as illustrated in Figure 1.1, indicating the interest it has sparked among researchers.

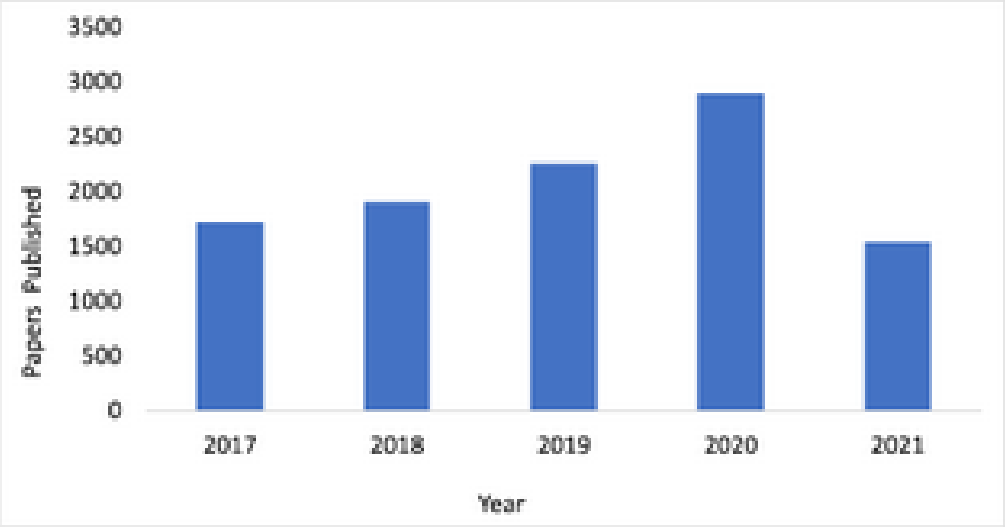

Fig. 1.1: Distribution of research papers on bacterial degradation of dyes published between 2017-2021 on the Science Direct platform (Senelisile, Bukisile and Pinkie (2022).

Compared to other treatment methods, biological wastewater treatment technologies have comparatively cheaper investment and maintenance costs. Additionally, they produce little to no harmful secondary sludge, which reduces their negative environmental consequences (Mingjun, Yanqiu and Xue, 2009; Yin, 2010). Extracellular biological components generated by microbes, primarily polysaccharides and proteins, have the ability to precipitate or flocculate diluted compounds or suspended particles from solutions (Wagner *et al*., 2021). Adsorption by living or dead microbial biomass, as well as enzymatic bioremediation and dye biodegradation, are the foundations of biological technologies.

Numerous microorganisms from various taxonomic families, including actinomycetes, bacteria, fungi, and algae, have been documented to be capable of decolorizing colours (Palanivelan *et al*., 2013). Biodegradation is the process by which organic molecules are broken down by microorganisms like bacteria, fungi, and microfauna, producing carbon dioxide, water, and/or methane. Three major categories can be used to classify biological

processes: aerobic, anaerobic, and combination of anaerobic/aerobic treatment. Generally, bacterial decolorization is faster than fungal one (Jadhav, Kalme, and Govindwar, 2008).

1.2.1.1 Bioremediation of Dye Wastewater

Bioremediation is recommended as safe, effective, and eco-friendly alternative method for treating harmful substances including heavy metal ions and dyes. Biopolymers like chitosan and alginate, as well as extracellular polymeric materials called exopolysaccharides (*Eps*), are examples of natural bioflocculants (Salehizadeh & Shojaosadati, 2001). The process of biosorption of pollutants by aggregation caused by microbial extracellular polymers generated by microbial living cells is known as "bio flocculation." *Eps* biomaterials have generated interest for various industrial applications, including oil recovery, wastewater treatment, textiles, detergents, adhesives, and downstream processing, because of their unique physical-chemical and rheological properties that include flocculation, emulsification, absorption, adsorption, and film formation (Yıldız, 2011). Because they are harmless and biodegradable, bioflocculant *Eps* have emerged as a viable alternative to chemical flocculants in wastewater treatment (Salehizadeh and Shojaosadati, 2001). Decolorization, metal removal, and sludge dewatering are three applications for bioflocculants (Huang *et al*., 2014; Chen *et al*., 2016; More *et al*., 2012).

Eps have been used in the biosorption of dye ions and heavy metals in industrial wastewater treatment (Gong *et al*., 2008; Mohammad *et al*., 2017). Because of their flocculation, viscosity, and stabilizing qualities, they are employed as a binding agent for coloured wastewater through a combination of adsorption, bridging, and charge neutralization

(Zhao, Liu & Zhou, 2013). Due to their capacity to bind ions from solutions, Eps may have utilized in the removal of heavy metals and dye ions from wastewater (Singh, Paul and Jain, 2006; Pal and Paul, 2008; Wei *et al*., 2017; Lo Giudice *et al*., 2020). Through bio-adsorption, negatively charged *Eps* can bond with dissolved hazardous metal cations from wastewater, including Fe, Zn, Cu, Cd, and Co (Zhang *et al*., 2017; Lo Giudice *et al*., 2020). The ability of various microorganisms to synthesize biopolymers with specific qualities for the intended region of application determines the methods for producing and applying *Eps*.

Because bacteria are easier to culture and grow faster than other microbes, using bacterial bioflocculants for wastewater treatment has several benefits. Thus, there is now a lot of interest in the characterization and synthesis of bacterial bioflocculants. Therefore, in order to remediate textile dye wastewater, this research looked into the production of bacterial *Eps* from bacteria isolated from various gin trash soil samples.

1.3 Problem Statement

Synthetic dyes present in textile wastewater are difficult to remove because of their complex aromatic molecular structure, often synthesized to resist fading on exposure to sweat, soap, water, light and/or oxidizing agents. Chemical auxiliaries such as alkali, acids, surfactants, levelling, chelating and softening agents, emulsifying oils and binders are also applied during the dyeing process to acquire uniform colour depth and fastness properties suitable for the intended end use of the textile which renders the effluent more stable and difficult to biodegrade (Lablu, 2022). Dye effluent is therefore often rich in organic compounds, residues of colors, heavy metal ions, pH, SS, TDS, COD, BOD, temperature, pH and salts which are toxic to terrestrial, aquatic and human life (Abdel-Karim *et al*.,

2021). Due to high thermal and photo stability of dyes, their compounds can persist in water environment for an extended period of time if left untreated. Further, most of the physicochemical methods used for the wastewater treatment are not only costly but also have limited applicability and produce toxic secondary sludge. Hence, textile dye wastewater requires proper treatment with low-cost methods that are environmentally friendly before being released into the environment.

1.4 Justification

Textile processing and dye manufacturing plants discharge about 30,000 to 150,000 tons of dyes into water bodies annually causing severe environmental pollution (Allen and Koumanova, 2005; Asha *et al*., 2015). It is estimated that about 17%–20% of industrial water pollution comes from textile dyeing and finishing treatments which contributes to about 20 % of wastewater worldwide (Chen and Burns, 2006; Fleischmann, 2019). The textile industries have therefore been condemned among the world's most polluters of the environment with colour being considered the most undesirable pollutant (Wafaa, 2006; Daneshvar *et al*., 2007; Baban *et al*., 2010). In Kenya, Dye effluent has been found to exceed the limits as stipulated in the Water Quality Regulations of 2006 set by National Environmental management Authority (NEMA) (Waithaka, 2011). According to schedule 4 of the regulations, the most critical parameters to monitor for textile discharge are pH, BOD, TSS, COD, colour/dye/pigment, phenols, sulphide, total chromium, temperature, fecal coliforms, oil and grease (Water Quality Regulations of 2006). Textile processing factory among other industries in Kenya, have therefore been faced with more stringent effluent treatment regulations and are required to treat their wastewater before discharge into the surface water (NEMA, 2020). Rivatex E.A Ltd, Eldoret, from where the dye

wastewater was sampled, discharges its effluent into river Sosiani which is a tributary of river Nzoia that drains into Lake Victoria. Textile dye wastewater treatment has therefore become a research area of increasing interest. Chemical reagents such as $FeCl_3$, $FeSO_4$, $MgCl_2$, alum, polyaluminium Chloride (PaC), PaFC, Polyaluminium Sulphate (PaS) and PFS commonly used in dye wastewater treatment result into secondary sludge that is difficult to treat. These chemicals also remain in the wastewater after the treatment and may cause health and environmental problems. Bioremediation on the other hand is a cleaner and effective alternative that is relatively lower in costs, generates minimal secondary sludge hence fewer damaging effects on the environment, aquatic and human life (Yin, 2010). Therefore, bioremediation of dye wastewater with bacterial bioflocculant, *Eps*, has recently become an area of strong interest as bacteria have shown potential to produce significantly higher yields of *Eps* with many specialties (Siti *et al*, 2021). However, *Eps* production media and broth can account for at least 70% of the total cost which is not economically attractive (Mohammed and Wan Dagang, 2019; Küçükaşik *et al*. 2011). This study therefore aims at producing *Eps* viable for RB5 bioremediation by reconstituting *Eps* production broth with agro- industrial wastes; sugar cane molasses and soya flour.

1.5 General Objective

Evaluation of textile dye wastewater bioremediation using *Eps* produced by bacteria isolated from sampled cotton gin trash soils.

1.6 Specific Objectives

1 To treat RB5 dye wastewater using Alum, PFS and PaFC coagulants

2 To isolate bacteria with *Eps* production capacity from cotton gin trash soil samples using assorted solid media.

3 To screen for the bacterial *Eps* with activity on RB5 dye wastewater using different conventional broth media.

4 To optimize sugarcane molasses concentration in a reconstituted broth medium and process conditions for bioremediation of RB5 dye wastewater by the selected *Eps*.

5 To characterize the *Eps*-treated wastewater, *Eps* extracts and the bacteria isolates whose *Eps* have bioremediation capacity.

1.7 Scope of Research

This study covered comparative assessment of optimal process parameters for RB5 textile dye wastewater treatment using different chemical coagulants; PaFC, PFS and Alum. The pH was varied from 2-10 for all the coagulants. The coagulants dose was varied from 10-50 mg/L for PFS, 30-70 mg/L for alum and 70-140 mg/L for PaFC. All the experiments were done in triplicates at room temperature, 25 °C. Samples treated at the respective optimal pH and coagulant dose for each of the chemicals were assessed for their physicochemical properties; colour, salinity, EC, TDS, COD and turbidity. The physicochemical properties of the chemically-treated samples were compared with those obtained after treatment by the *Eps* produced by the bacteria isolated from three trash soils samples. The gin trash soils were sampled from three ginneries located in cotton growing regions in Kenya; Meru, Kitui and Baringo. Nine different solid media; 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)

were used to assess the most appropriate one for the *Eps* producing bacteria isolation. Three different liquid media; Nutrient broth mixed with sucrose (NB+S), Nutrient broth mixed with glucose (NB+G) and (Glucose, Peptone, Yeast Extract and Malt Extract ($GPYMgSO_4$) were used to evaluate the most suitable one for application in bioremediation of the dye wastewater. The most appropriate one was then reconstituted with sugarcane molasses and soya flour supplemented with yeast extract and magnesium sulphate salt as an intervention to curtail the high cost of the synthetic media. Evaluation of the dye removal using the *Eps* in the different broth media was done at pH 7-8, 30 °C for 72 h in rotary shaker at 150 rpm. The different *Eps* found to have dye wastewater bioremediation capacity were optimized at varied temperature (30-38 °C), incubation time (24-72 h) and molasses concentrations in the $MSYMgSO_4$ broth (10-20 g/L). Full factorial experiment design for 3 factors at three levels each was designed using Mat Lab 17 software. The pH was maintained at 7-8. This research did not asses the effect of varying the dye and *Eps* concentration on the dye removal efficiency. Effectiveness of using concoctions of the *Eps* for the dye wastewater treatment was also not evaluated within this scope.

The bacteria isolate that produced the *Eps* with optimal bioremediation capacity were biochemically and molecularly characterized to determine their specific epithet. The *Eps* with highest bioremediation capacity for the wastewater were extracted and characterized with FTIR, SEM and GC-MS. The optimal *Eps*-treated wastewater was characterized through analysis of physicochemical properties; colour, salinity, Ec, TDS, COD, BOD and turbidity. FTIR and UV-Vis were also used to characterize the *Eps*-treated dye wastewater. The parameters of the optimal *Eps* and chemically -treated wastewater were compared with those specified in Water Quality Regulations, Fourth and Fifth Schedules as set by NEMA.

1.8 Study Significance

Bioremediation of textile dye wastewater with bacterial *Eps* cultured from cotton gin trash soil waste is significant since it will offer a cleaner and effective alternative to the chemicals coagulants commonly used, which result into secondary sludge that is difficult to treat. These chemicals also remain in wastewater after the treatment and may cause health and environmental problems. This intervention will also provide a profitable exploitation of the massive gin trash waste which poses a disposal challenge to the ginneries thus promoting circular economy in the textile value chain. Reconstitution of a chemical broth substitute using sugarcane molasses and soya flour, effective for production of *Eps* with capacity for the RB5 dye wastewater bioremediation will also offer an alternative for profitable utilization of the agro waste materials within their respective value chains. Ultimately, treatment of the dye wastewater before discharging it into the general effluent mainstream in the industry will lessen the chemical loads and reduce the cost of treatment.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter covers literature review on textile dye effluent. Physicochemical characteristics of the common pollutants. Focus has been given to the effluent that originate from azo dyes. A review on the characteristics of the effluent generated from other pre and post dyeing processes has been discusses. Extent of dye fixation and corresponding loss by various dye types and while dyeing different fibres has also been reviewed, with special attention being paid on the reactive dyes. Literature on the commonly used textile dye effluent physicochemical treatment methods has been discussed. Review on the alternative biological dye treatment options in comparison to the physicochemical methods has also been presented. Production, properties and potential application of microbial *Eps* in textile dye bioremediation have been highlighted. Possible intervention measures for alleviating high production cost for bacterial *Eps* for their economical application in industrial effluent bioremediation has been discussed. Appropriate composition of the *Eps* production media and related environmental factors that affect *Eps* production have also been reviewed. Model RB5 dye effluent used in the study and justification for its selection has also been presented.

2.2 Textile Dye Effluent

Composition of dye effluent from the textile industry varies from mill to mill depending on the process, the equipment used, type of fabric, chemicals added to the dye bath, the weight of the fabric (Ramos *et al*., 2021; Yaseen and Scholz, 2019). Color in dyes and its intensity is a consequence of the presence of chromophoric and auxochromic groups.

Chromophores contain functional groups such as –N═N–, –C═O, $–NO_2$ and -O = (C_6H_4) = O whereas the ionizable functional groups that may be attached to auxochromes are: -NH_2, -COOH, -OH and -SO_3H (Sumathi, 2014; Srinivasan and Viraraghavan, 2010). Dyes are also categorized on the basis of the reactive groups that are in association with -OH and -SH groups through the covalent bond formation which are then used on different fibers. Azo dyes are formed by diazotized amines joined to an amine or phenol, with one or more azo bonds (–N=N–). Azo Dyes account for more than 50% of all the dyes produced globally annually hence the most common colourants released into the environment (Bae & Freeman, 2007; Kusic *et al*., 2011). Majority (60-70%) of the dyestuffs used in the textile industry are azo dyes since they possess good fiber-fixation (up to 85%) properties, as compared with other synthetic dyes (Chequer, Dorta and de Oliveira, 2011). Dyes may also be classified as anionic which includes direct, acid and reactive dyes; cationic which includes basic dyes and non-ionic which includes, pigment, disperse dyes that do not ionize in aqueous phase (Joshi, Bansal and Purwar, 2004; Deive *et al*., 2010). Azo dyes can be anionic (deprotonation at the acidic group), cationic (protonated at the amino group) or non-ionic, depending on the pH value. Figure 2.1 shows dyes classification based on their as anionic, cationic and non-ionic type.

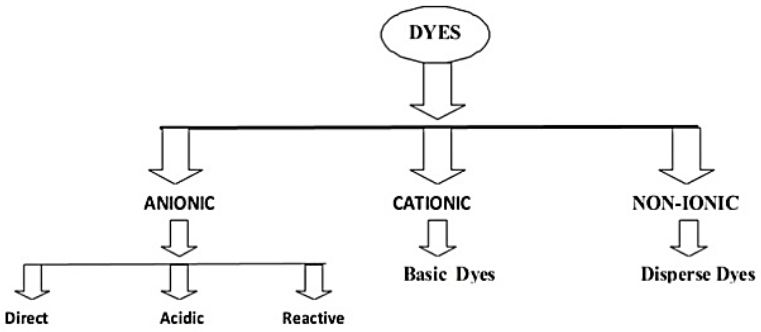

Fig. 2.1: Dye Classification (Fu and Viraraghavan, 2001)

Around 15–20% of the dye is discharged into the environment during the dyeing and rinsing processes (Ouasif, Yousfi & Bouamrani, 2013). Reactive dyes have a greater number of functional groups such as azo (N=N), sulfonic acid (- SO_3-), hydroxyl (-OH) compared to other types of dyes (Ashtekar *et al*., 2014). The reactive dyes colour is due to the presence of the -N=N- azo bonds and the chromophoric groups linked to the –OH or – NH_2 type auxochrome groups. Among the reactive dyes, approximately 66% are azo dyes (Chequer, Dorta and de Oliveira, 2011). The azo dyes are characterized by reactive groups that form covalent bonds with HO-, HN, or HS- groups in cotton, wool and nylon fibres (Devi, 2017; Holkar *et al*, 2016). Anthraquinone dyes form the second largest class of textile dyes, after azo dyes. They have a wide range of colours in almost the whole visible spectrum (Carmen and Daniela, 2012).

Due to their large range of brilliant colour shades, high fastness and affinity to fibres, and ease of application, reactive dyes, which make up the second largest dye class in the colour index (CI), are primarily used for cotton dyeing. In 2011, total production was estimated to be 350,000 tones (Allen and Koumanova, 2005; UNSD, 2013). Nevertheless, these dyes are known to have the lowest rate of fixing and are extremely vulnerable to hydrolysis in alkaline dyeing conditions (Shah *et al*., 2013). Hence, due to their higher alkalinity, higher

concentration of organic compounds, and stronger colour than other dyes, reactive dyes are identified as recalcitrant substances (Barka *et al*. 2010; Sarkar *et al*., 2020). Reactive dye dyeing of cotton produces high pollution because the wastewater has high BOD, COD, colour, pH, and salt load (Zongpin *et al*., 2021).

Reactive dyes have the largest degree of unfixed dye loss (15–50%) among all dye types, however the extent of unfixed dye loss varies. (Wallace 2001; Sumathi, 2014; Mohamed *et al*., 2019; Samsami *et al*., 2020). Reactive dyes form a reactive vinyl sulfone ($-SO_3-CH=CH_2$) group in high pH environments (9–12), at 30-70 ^oC, and with salt concentrations ranging from 40–100 g/L. This group bonds with the fibres. Nevertheless, the vinyl sulfone group does not create a covalent bond because it is readily hydrolyzed in the presence of water, decreasing its affinity with the fibres. Accordingly, the substantial loss of unfixed dye in wastewater (20–50%) is a characteristic of reactive dye components (Dos Santos *et al*., 2004; Almeida Guerra et al., 2012). Upon completion of the reactive dyeing procedure, the bath may include up to 800 mg/L of hydrolyzed dyes. Finally, bioaccumulation results from the unfixed dyes being discharged into the environment (Sreedharan and Bhaskara, 2019; Sarkar *et al*., 2020). According to Almeida *et al*. (2009), reactive dye found in wastewater can be seen by the naked eye at concentrations as low as 0.005 mgL^{-1}. The degree to which various dye classes are fixed onto textile fibres is displayed in Table 2.1.

Table 2.1. Fixation of different dyes classes on textile fibres (EWA, 2005).

Dye class	**Fibre type**	**Fixation degree, %**	**Loss in effluent, %**
Acid	Polyamide	80-95	5-20
Basic	Acrylic	95-100	0-5
Direct	Cellulose	70-95	5-30
Disperse	Polyester	90-100	0-10
Metal complex	Wool	90-98	2-10
Reactive	Cellulose	50-90	10-50
Sulphur	Cellulose	60-90	10-40
Vat	Cellulose	80-95	5-20

It is estimated that 10% of dyes are lost to wastewater for deep colours, 2% for medium shades, and negligible for light shades. Ten milligrams to fifty milligrams of dyes are found in the effluent; of these, one milligram is visible to the unaided eye (Saranraj and Sivasakthivelan, 2014; Saranraj and Manigandan, 2018). If dyes are found in water at even one parts per million (ppm), it is usually not acceptable (Gupta, Ali and Mohan, 2003; Noroozi *et al*., 2008). True, turbidity-free water or visible, untreated water are the two categories for colour in wastewater. Spectrophotometry and visual comparison are the most widely used techniques for determining the colour of effluent, dye solutions or dispersions. By comparing the sample to either known concentrations of coloured standards typically a platinum-cobalt solution or calibrated colour discs, visual comparison quantifies colour; however, this method is not suitable for highly coloured wastewaters. The American Dye Manufacturer Institute (ADMI) and the Tristimulus Filter Method are frequently used in spectrophotometric colour measurement (Carmen and Daniela, 2012).

2.2.1 Physicochemical Characteristics of Dye Wastewater

In addition to dyes, various auxiliary chemicals are used in the pre- and post-dying processes, which also result in wastewater. These chemicals include organic acids, fixing

agents, defoamers, oxidizing/reducing agents, and diluents (Yaseen and Scholz 2016; Sekomo *et al*. 2012; Dos Santos *et al*. 2007; Shah *et al*. 2013). Chemicals used to size components include tallow, carboxymethyl cellulose, polyvinyl alcohol, and starch (Janah *et al*., 2022). Approximately 750 kg of size material are emitted with the effluent from a conventional textile mill (Yaseen and Scholz, 2019). The sizing elements are eliminated by desizing, which can be accomplished through oxidation or hydrolysis. Surfactants, hydrogen peroxide, alkalis, acids, and enzymes can all be used in this procedure. When hydrogen peroxide oxidizes sizing starch, carbon dioxide and water are produced; amylase enzymes convert starch to ethanol (Ul-Haq and Nasir, 2012). The purpose of scouring is to get rid of various chemical and natural impurities that impede the dyeing and finishing processes, like pectins, oils, waxes, minerals, herbicides, and pesticides found in natural fibres like cotton. In comparison to natural fibres like cotton or wool, synthetic fibres require less scouring. Scouring agents include hot alkali and detergents (Tanapongpipat *et al*., 2008; Li *et al*., 2022). To remove the fiber's natural colour, bleaching is mostly done with chemicals like peracetic acid, hydrogen peroxide, and hypochlorite (Wahab, Hussain, and Ashraf, 2022). The purpose of the mercerization procedure, which comes after bleaching, is to improve the material's strength, luster, and ability to absorb dye. During the procedure, a highly concentrated solution of zinc chloride or caustic soda (18–24%) is employed. Following dyeing, printing, and rinsing, a further chemical finishing procedure is carried out to add desired characteristics like softness, fire retardancy, hydrophobicity or hydrophilicity, soil release, UV proof, and so forth (Schindler and Hauser, 2004). Given this, textile effluents from different operating stages typically differ greatly in terms of colour, turbidity, pH, BOD, COD, TDS, and other properties. Colour, pH, SS, TDS, total

organic carbon (TOC), COD, and BOD are all elevated in dye effluents as a result (Mohamed *et al*., 2019; Donkadokula *et al*., 2020; Singh *et al*., 2021). According to studies by Zaharia, Suteu, and Muresan (2011), Desai and Kore (2011), and Chequer *et al*. (2013), dyeing is responsible for the majority of metals, nearly all of the salts, and colour found in the total textile effluent. Table 2.2 displays the possible particular pollutants from textile printing and dyeing.

Table 2.2. Potential pollutants from textile Printing and dyeing processes (Zongping *et al*., 2011).

Process	**Pollutant compounds**
Desizing	Sizes, enzymes, starch, waxes, ammonia.
Scouring	Disinfectants and insecticides residues, NaOH, surfactants, soaps, fats, waxes, pectin, oils, sizes, anti-static agents, spent solvents, enzymes.
Bleaching	H_2O_2, sodium silicate or organic stabilizer, high pH
Mercerizing	High pH, NaOH
Dyeing	Colour, metals, salts, surfactants, COD, BOD, pH, EC, formaldehyde, TDS
Printing	Urea, solvents, colour, metals
Finishing	Resins, waxes, chlorinated compounds, acetate, stearate, spent solvents, softeners.

Figure 2.2 gives a schematic illustration of all the processes involved during textile dyeing and printing, their chemical input components and the respective nature of the resultant wastewater.

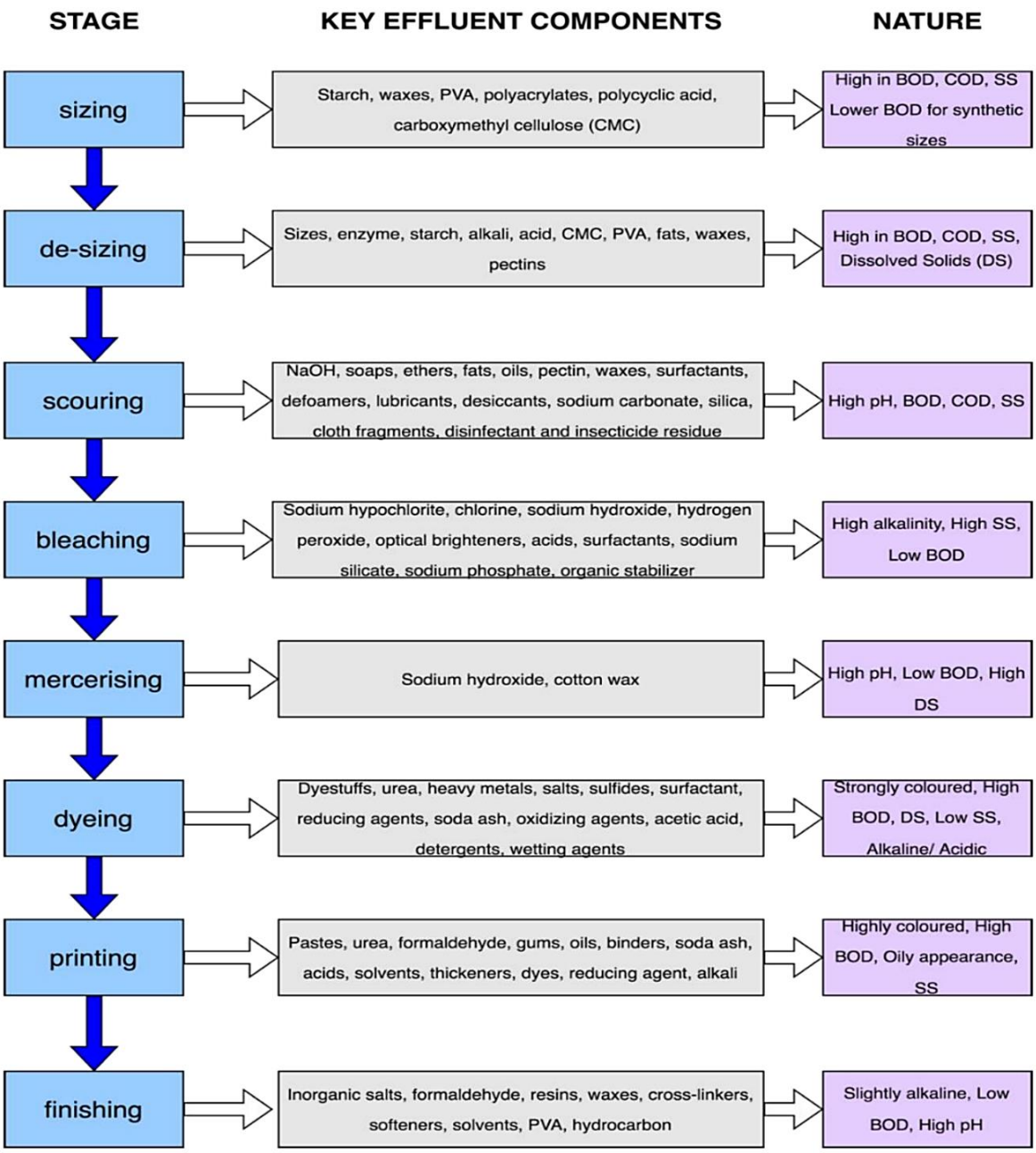

Fig. 2.2: Pre and post dyeing and printing processes with key effluent components and nature of the wastewater discharge (Jahan *et al*., 2022).

The following are the common parameters used to characterize textile dye wastewater;

2.2.1.1 Colour

Residual colour is mainly due to unfixed dyes and pigment chromophore groups as well as other coloured metallic ions such as iron and manganese in form of colloidal or suspended material (Carmen and Daniela, 2012; Vigneshpriya and Shanthi, 2015). Colour increases the turbidity of water therefore hampering light penetration which affects the photosynthesis process (Egli, 2007)

2.2.1.2 Chemical oxygen demand (COD)

This is a measurement of the amount of organic matter that can be oxidized by a potent chemical oxidant in terms of oxygen equivalent. It is used to calculate how much oxygen is present in wastewater that can be chemically oxidized by putting dichromate in an acidic solution. High COD levels indicate hazardous environments and the existence of organic materials that are resistant to biological processes (Groele and Foster, 2019).

2.2.1.3 Biochemical Oxygen Demand (BOD)

This is the quantity of oxygen used for the biochemical breakdown of organic matter and the oxidation of inorganic substance salts over a specific period of time. BOD is a by-product of microorganisms' respiratory functions, and its measurement relates to how well microorganisms break down organic materials (Islam and Mostafa, 2020).

2.2.1.4 Electrical Conductivity (EC)

Water's capacity to conduct an electric current is measured by its electrical conductivity (Golnabi *et al*., 2009). Wastewater becomes more electrically conductive throughout the dyeing process. Changes in dissolved solids, primarily metal ions and mineral salts, can

cause it to change (Tchobanoglous, Burton and Stensel, 2003). Conductivity is affected by the rate at which these salts split into ions, the electrical charge on each ion, the mobility of ions, and the solution's temperature. The concentration of inorganic elements such as alkalis, chlorides, sulphides, and carbonate compounds, as well as soluble ionic salts like sodium carbonate and salt, determines conductivity in wastewater (Morrison, Fatoki and Ekberg, 2001; Uddin, 2021). The primary cause of wastewater's increased conductivity is chloride salts (Bes-Piá *et al*., 2010). Salts are primarily utilized in textile processing to enhance direct and reactive dye bath exhaustion. Salts can also result from other processes, such neutralization, as a byproduct. The unit of conductivity is micro siemens per centimeter, or µS cm-1.

2.2.1.5 Metals

To stabilize the hue of dyes, heavy metals such as cobalt (Co), copper (Cu), and chromium (Cr) are added (Nur *et al*., 2007). In textile wet processes, dyeing, printing, and finishing are the primary causes of heavy metal pollution (Zeiner, Rezic, and Steffan, 2009). According to Hussein (2013) and Velusamy *et al*. (2021) the primary metal ions that pollute the environment include Pb, manganese (Mn), Hg, iron (Fe), Cu, Zn, Cd, and (Cr). According to Adinew (2012), Cu, Cd, Cobalt (Co), and Cr are the metal ions present in the dye chromophores in textile effluents (Hussein, 2013). According to Zongpin *et al*. (2021), Cr has a cumulative effect and a high likelihood of infiltrating the food chain. Even at modest levels of 2.72 µg L^{-1}, cd is regarded as a very hazardous metal due to its toxicity and carcinogenic effects. The neurological, immunological, urinary, and reproductive systems are said to be impacted (Bernhoft, 2013). Additionally, oxidative stress and protein suppression of DNA repair processes have been linked to cadmium exposure (Rani *et al*.,

2014). Heavy metals can change the physiological and metabolic characteristics of microorganisms in wastewaters. According to Cervantes *et al*. (2001), Cr and Cd have the ability to cause oxidative and denaturation damage to microorganisms, hence decreasing their potential for bioremediation. By displacing metals from their natural binding sites or ligand interactions, Cd and Pb cause damage to the DNA structure and disrupt the membranes of microorganisms. Microbes' morphology, metabolism, and growth are all impacted by changes in their nucleic acid structure. These changes disrupt cell membranes and inhibit enzyme activity and oxidative phosphorylation (Olaniran, Balgobind, and Pillay, 2013; Fashola, Ngole-Jeme, and Babalola, 2016). Before releasing heavy metals into the environment, their concentrations must be brought down to manageable levels because majority of them are non-degradable (Deepali, Joshi, and Gangwar, 2009).

2.2.1.6 Salts

The primary source of phosphorus in a textile effluent is dye bath streams that contain phosphate buffers (Hussain and Arif, 2004). Nitrate ions are a functional group found in a variety of colours and their additions (Varsha, Sudesh, and Seema, 2013). Sulphates can come from sodium sulphate, which is a reactive colouring auxiliary. As much as 20–42 g/L of sulphate can be found in the dye bath (Bisschops and Spanjers, 2003). When dyeing with sulphur or vat dyes, sodium hydrosulphite and sodium sulphide are frequently employed as reducing agents. During the dyeing process, sodium lauryl sulphate is employed as a scouring or wetting agent (Verma *et al*., 2012). The use of sulfuric acid, which is used to regulate pH during dyeing, is another source of sulphur compounds. Sodium hydroxide, sometimes known as caustic soda, and sodium carbonate, often known as soda ash, are employed as pH buffer solutions and fixing agents. Worldwide, 200,000–

250,000 tons of salt contaminants are thought to be present in textile wastewater each year (EWA, 2005).

2.2.1.7 Solids

Different values can be measured for solids since they can exist in many forms: total solids, total suspended solids, volatile suspended solids, and dissolved solids. The basis for separation is the application of filters with various pore diameters. TDS is a measurement of the total amount of all organic and inorganic materials present in a liquid in suspended molecular, ionized, or micrograniular form. Remainder or non-filterable material in wastewater is referred to as SS. Occasionally, chemicals are also precipitated as a result of pH changes, which raises the number of suspended particles (Varsha, Sudesh, and Seema, 2013). The osmotic balance of water bodies is disrupted by high or low quantities of dissolved solids, which has an impact on aquatic life.

2.2.1.8 Salinity

Chemical compounds known as salts are often composed of ions such as carbonate, bicarbonate, sulphate, calcium, magnesium, and sodium. These dissolved salts, which are employed in dyeing and printing procedures to improve the fixation between dyestuffs and textile substrates, include sodium chloride, magnesium sulphates, potassium nitrates, and sodium bicarbonate (Aouni *et al*., 2012).

2.2.1.9 pH

pH of the dyeing effluent remains between 10 to 11 after pretreatment with alkali at 90 ^{o}C in the desizing, scouring and mercerization processes. The pH influences bioavailability of

some nutrients, metals and pesticides to plants and animals. Therefore, the dye wastewater pH needs to adjusted before discharge.

2.3 Dye Effluent Treatment Options

Extensive research has been carried out in the recent past on treatment of textile dye wastewater as shown in Figure 2.3.

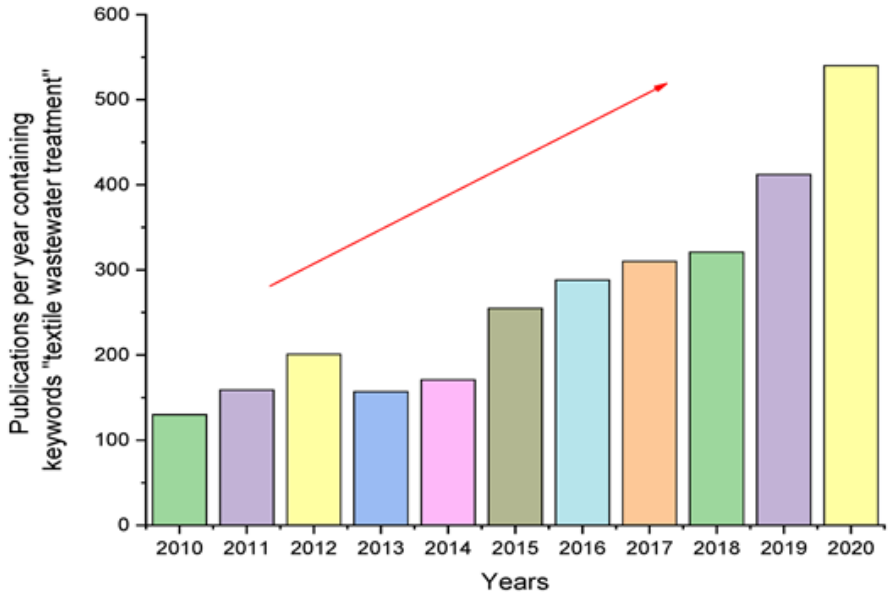

Fig. 2.3: Number of papers reported on textile wastewater treatment per annum; indexed in the core collection of Web of Science from 2010 to 2020 (Wang, Jiang and Gao, 2022).

Industrial effluents have been treated using a variety of techniques and materials, including artificial wetlands, modified bentonite, membrane filtration, activated carbon, ozonation, photooxidation, adsorption, and reverse osmosis (Merzouk, Madani, and Sekki, 2010; Jain *et al.*, 2020; Ismail and Sakai, 2021; Wang, Jiang, and Gao, 2022). When it comes to managing industrial effluents, all of these treatment technologies are important. The wastewater's composition, discharge laws, operating expenses, and the potential for reuse or recycling of the treated effluent all have an impact on the dye wastewater treatment

method that is selected (Jegatheesan *et al*., 2016). The classification of the most popular dye wastewater treatment techniques is shown in Figure 2.4.

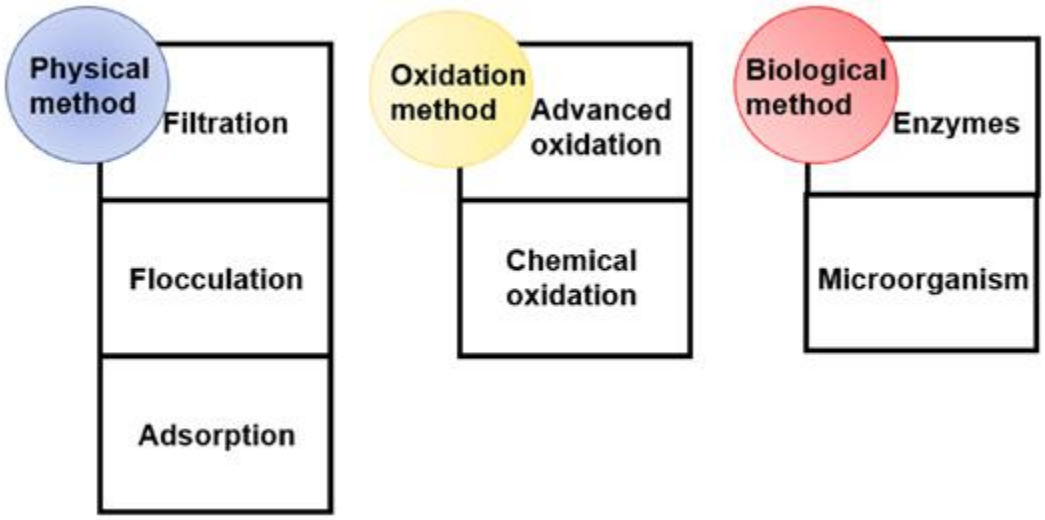

Fig. 2.4: Dye wastewater treatment methods (Wang, Jiang and Gao, 2022)

The physical treatment methods involve removal of pollutants through forces such as electrical attractive, gravitational and/or van der Waals forces) or physical barriers. These methods do not cause change in the chemical structure of the substances present in the wastewater (Mani, Chowdhary and Bharagava, 2019).

2.3.1 Adsorption Method

Adsorption is the physical process by which soluble molecules, called adsorbate, cling to the surface of a solid substrate, called an adsorbent, to be extracted from a solution. Because of its cost, ease of use, and capacity to effectively remove both organic and mineral impurities from wastewater, this approach is widely utilized in wastewater treatment (Jehan *et al*., 2022). Adsorption can occur physically or chemically whereby heavy metal ions or textile dyes are adsorbed onto the surface of an adsorbent. Adsorbents use a mix of ion exchange and adsorption to remove heavy metals and dyes from wastewater. While

physical adsorption results from interactions through forces like hydrogen bonds and van der Waals that depend on the structure and functional properties of the surfaces of both the adsorbent and the adsorbate, chemical adsorption happens when a reaction between the adsorbent and the adsorbate creates a complex (Jehan *et al*., 2022). Unlike during biodegradation, the physical structures of the dye and metal ions are not changed during adsorption. The adsorbent's matrix traps the dye, metal ions, and other contaminants (Ali, 2010).

Adsorption techniques for treating wastewater typically involve the use of adsorbents including activated carbon powder, kaolin clay, silicon polymers, chitosan, resins, zeolite, and activated bentonites (Jehan *et al*., 2022). The wastewater is combined with the porous material powder or granules, or it is passed through a granular material-filled filter bed. Adsorbent surface area, particle size, dye/adsorbent interaction, temperature, pH, contact time, particle diameter, and adsorbate concentration are the primary parameters that affect dye adsorption effectiveness (Carmen and Daniela, 2012; Jehan *et al*., 2022). At a pH range of 2 to 11 and an effluent temperature of 30 to 40 ^{o}C, pollutants can be eliminated. The surface of the porous material or filter is where the contaminants are absorbed and eliminated. The most popular type of carbon, activated carbon, may eliminate up to 92.17% and 91.15% of COD. According to Zongping *et al*. (2011), it is capable of successfully eliminating water-soluble dyes, including acid, reactive, basic, and mordant dyes. Reactive yellow 145, methylene blue, and reactive red 198 were removed by Oke and Mohan (2022) using activated carbon generated from textile sludge as an adsorbent. They discovered that the carbon has the ability to adsorb 75.1 mg/L, 101.8 mg/L, and 76.6 mg/L of the dyes, respectively. Good colour removal capacity has been reported for activated carbon in

powdered (PAC) or granular (GAC) form with specific surface area of 500–1500 m^2/g, pore volume of 0.3–1 cm^3/g, and bulk density of 300–550 g/L (Carmen and Daniela, 2012). However, the substance is unable to adsorb suspended solids or insoluble colours found in wastewater, such as pigment, vat, and disperse dyes. However, the expensive cost of activated carbon, the volume of sludge it generates, the high cost of regeneration, and the low treatment effectiveness it has for some dyes limit its application (Pala and Tokat, 2002). The removal of dyes from textile effluents has also been accomplished by using agricultural waste materials such as peat, coal ashes, red soil, rice husk and hulls, tree barks, wood chips and sawdust, ground nut shell powder, sugarcane bagasse pith, and ground sunflower seed shells (Anjaneyulu *et al*., 2005; Suteu *et al*., 2011 (a, b); Zaharia *et al*., 2011; Igwegbe *et al*., 2020). The major reasons why using these materials is beneficial are their low cost and wide availability. Solid state fermentation (SSF) offers the possibility of protein enrichment, however in certain cases regeneration is not required and the "spent" material is burned traditionally. Although employing "low cost" adsorbents to remove textile dyes is profitable, it necessitates a large quantity of adsorbents and is less effective than activated carbon. Composite materials have also been used to treat industrial wastes, and these efforts have resulted in notable heavy metal ion recoveries of up to 98% (Rauf, Shehadi, and Hassan, 2007). Nevertheless, the process of removing metal ions from the surface of the hybrid materials through desorption of the exhausted composite material is costly and time-consuming (Shahadat *et al*., 2014; Aravind *et al*., 2010).

2.3.2 Membrane Filtration Method

Membrane filtration method has attracted immense attention in dye wastewater treatment since it requires fewer chemicals, low resistance to temperature and adverse chemical

environment, minimal equipment, low energy and capital cost, easy scalability, ease of operation, minimal pollution, high separation efficiency and lesser footprint compared to conventional techniques. Different kinds of dyes and contaminants can be removed from wastewater with the aim of reuse of the water (Othman *et al*., 2021; Jehan *et al*., 2022; Wang, Jiang and Gao, 2022). However, large scale application of this technology is still limited because it requires special equipment that is expensive, low permeability and selectivity coupled by the membrane fouling necessitating frequent cleaning and regular replacement (Ranganathan *et al*., 2007). These limitations can be addressed by preparation of charged membranes through adoption of pre-functionalized polymers as additives, use of different surface modification techniques of commercially available pressure-driven membranes, surface coating of copolymers and blending of fillers/nanofillers with commercially available polymers (Abdel-Karim *et al*., 2021).

Membrane filtration method uses the micropores selective permeability to filter particles lower than 2 μm in size and to separate pollutants in wastewater. Use of membrane filtration in dye wastewater treatment is mainly based on membrane pressure, such as nanofiltration (NF), reverse osmosis (RO), ultrafiltration (UF) and microfiltration (MF). Figure 2.5 shows the types of membrane filtration techniques.

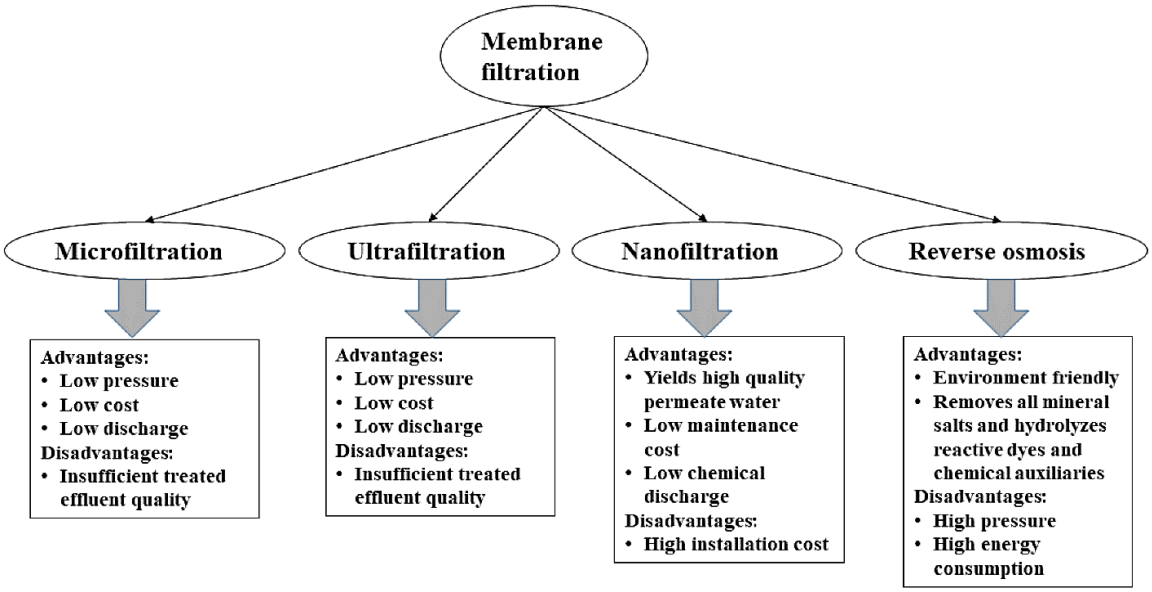

Fig. 2.5: Membrane filtration techniques with respective advantages and disadvantages (Obotey Ezugbe and Rathilal, 2020).

Reverse osmosis (RO) and NF are suggested as additional filtration methods because MF and UF are typically ineffective in treating wastewater (Ezugbe and Rathilal, 2020). MF uses >90% turbidity or silt density index to remove suspended particles, colloids, or macromolecules from effluents having pores between 0.1 and 1 micron. According to Carmen and Daniela (2012), it can be used to cure dye baths that contain pigment colours as well as subsequent rinse baths. UF is used as a pretreatment for RO and separates macromolecules and particles, although its dye removal efficacy ranges from 31 to 76% (Ciardelli & Ranieri, 2001). The pore sizes of UF membranes vary from 0.005 μm to 1 nm, as reported by Nqombolo *et al*. (2018). They work well for removing macromolecules and suspended particles (Barredo-Damas *et al*., 2012). However, because they permit the passage of salts, solvents, and low molecular weight organic solutes, their applications are restricted (Atul, Choudhary, and Verma, 2012). NF technique generates high-quality water that is appropriate for reuse, has a low discharge volume, excellent solvent permeability, is easily scalable, and requires little to no chemical cleaning. According to Alves and Pinho

(2000), NF membranes have a typical pore size of 0.5–5 nm and may hold large monovalent and divalent ions, hydrolyzed reactive dyes, low molecular weight organic compounds, and dyeing auxiliaries with a 70–90% colour removal effectiveness. Tang and Chen (2002) treated textile dye effluent with NF to remove dye with an efficiency of 98%, allowing for reuse. However, because salts can flow through the NF membranes due to their loose surface structure, the clearance rate of sodium chloride was less than 14% (Ye *et al*., 2018).

More so than NF membranes, the RO approach is employed to efficiently extract organic chemicals and mineral salts (Barredo-Dama *et al*., 2012). According to Atul, Choudhary, and Verma (2012), the pores in RO membranes are smaller than those in UF membranes. According to Ramesh Babu *et al*. (2007) and Nqombolo *et al*. (2018), they eliminate ionic chemicals, smaller particles, microorganisms, hydrolyzed reactive dyes, and chemical auxiliaries. According to Kurt *et al*. (2012), the RO method works well for desalting and decolorizing dyebath effluents and can be used to recycle water.

2.3.3 Ozonation Method

According to Ulson *et al*. (2010), ozonation is an oxidative process where ozone (O^3) is the oxidizing agent utilized. Numerous benefits are linked to this technique, including low amounts of organic matter being broken down efficiently, low ozone oxidation potential, adding oxygen to water, and minimal temperature sensitivity. By initiating and accelerating azo link breakage and oxidizing a sizable number of COD, ozone colour removal is often efficient and rather quick. The concentration of dye affects how much colour can be removed from wastewater by ozonation (Soares *et al*., 2006). By oxidizing resistant

contaminants into more readily biodegradable intermediates, this technique can also increase the biodegradability of hazardous and nonbiodegradable components in effluents without increasing the amount of wastewater or sludge (Zongping *et al*., 2011). Nevertheless, the technique produces disappointing results, particularly for some dispersed colours. It is not advised to employ the procedure because it is also linked to the emission of additional hazardous compounds and carcinogenic aromatic amines (Carmen and Daniela, 2012).

2.3.4 Advanced Oxidation Method

In textile dye wastewater treatment, advanced oxidation procedures (AOP) involve the use of oxidants including acids, hydrogen peroxide, and sodium hypochlorite. The colours and hazardous organic chemicals in the wastewater are oxidized into smaller molecules by the free hydroxyl radicals ($OH^{\bullet}$) of the oxidants (Valh *et al*., 2011). Through hydrogen abstraction, electron transfer, and radical addition, the highly reactive oxidizing agent $OH^{\bullet}$ which has a high oxidation potential of 2.15–2.8V can quickly and non-selectively attack stubborn pollutant species in water (Khan *et al*., 2020). It can remove non-biodegradable organic components from water without leaving behind deposits because of its brief nanosecond lifetime in water, which causes it to self-eliminate from the treatment system by converting the pollutants into CO_2, H_2O, and salts (Zhang *et al*., 2016). The usual catalytic combinations for generating HO• are O_3, H_2O_2, TiO_2, UV light, electron beam irradiation, and ultrasound. The compounds with the highest capacity to oxidize textile wastewater are O_3/H_2O_2, O_3/UV, and H_2O_2/UV. In the presence or absence of an irradiation source, hydrogen peroxide is utilized with catalysts like Fe, Mn, and TiO_2 (Forgacs, Cserhati, and Oros, 2004).

2.3.5 Chemical Coagulation and Flocculation Method

Inorganic coagulants, synthetic polymer flocculants, and inorganic–organic dual-coagulants are physical techniques that are frequently employed to destabilize particles or colloids (Chen, Gao, and Yue, 2010; Verma, Dash, and Bhunia, 2012). Due to its ease of use, effectiveness, and affordability, the procedure is frequently used to remove dyes (Huang *et al*., 2014; Choy *et al*., 2001). In the treatment of wastewater containing textile dyes, inorganic coagulants such iron and aluminum salts are frequently utilized (Liang *et al*., 2014). The following categories apply to chemical coagulants: a) Hydrolyzing metallic salt coagulants, such as magnesium chloride ($MgCl_2$), ferric chloride ($FeCl_3$), and aluminum sulphate (alum; Al_2 $(SO_4)_3$). Since they are easily accessible and reasonably priced, the most often used salts are those of alum, lime, magnesium, iron, and aluminum. Sources: Georgiou *et al*. (2003); Sumathi, 2014; Verma, Dash, and Bhunia, 2012. However, coagulation with alum and $MgCl_2$ produces a significant amount of sludge, which becomes a problem for the environment. (Dotto *et al*., 2019; Blancco, Sancho, and Caballero, 2010). Furthermore, it is thought that treating wastewater with dyes requires a lot of alum, which may cause Alzheimer's disease (Lévesque *et al*., 2000). Using synthetic polymer flocculants can help minimize the high levels of sludge (Huang *et al*., 2014).

b) Hydrolyzing metallic salt coagulants (PaC, PaFC, PaS, and PFS) prior to hydrolysis. Because coagulation may be carried out across a larger pH range, forms larger and heavier flocs, and exhibits superior colour removal effectiveness, pre-hydrolyzing metallic salt coagulants are generally preferred (Akshaya *et al*., 2012; Chen, Gao and Yue, 2010; Verma, Dash and Bhunia, 2012). Many intermediate hydrates are generated during the coagulation process, even though the precipitate of aluminum hydroxide (Al $(OH)_3$) is said

to be the end result for aluminum salts coagulants. According to Ashtekar *et al*. (2014), the intermediate hydrates have the ability to polymerize into metal hydrate units, which are capable of binding the colloidal solids and improving their removal through the transfer of particle charges. PFS is a very effective and soluble coagulant that forms quickly settling flocs over a wide pH range (Verma, Dash, and Bhunia, 2012). PFS generates a lot of easily flocculate polynucleic complexions such as $(Fe_2 (OH)_3)^{3+}$, $(Fe_2 (OH)_2)^{2+}$, and $(Fe_8(OH)_2)^{4+}$ and is essentially soluble in water. Adsorption and charge neutralization are two coagulation mechanisms that PaC and PFS share. According to Gao, Yue, and Miao (2001), PaFC is a good composite coagulant that removes colour by combining the benefits of iron and aluminum salts. Additionally, prehydrolyzed coagulants may provide less sludge and are said to provide superior colour removal even at low temperatures (Verma, Dash, and Bhunia, 2012).

2.3.5.1 Chemical Coagulation Optimization

Different mechanisms, such as electrostatic attraction, sorption (associated to protonated amine groups), and bridging (related to high molecular weight polymers), cause flocculation and coagulation (Jing *et al*., 2015). According to Uma, Tarun, and Biswanath (2016), chemical coagulants have the ability to dissociate when in solution because of their positive ions, which lessen the electrokinetic repulsion between the particles. Through electrostatic attraction, the negatively charged dye molecules are neutralized by the coagulants' cationic functional groups, forming particle-polymer-particle complexes (Jeon *et al*., 2009). The colours, organic substances, heavy metals, oil colloidal particles, and small suspended particles in wastewater become unstable due to these compounds. When flocculants are added to the treated water, these pollutants will precipitate as chemical

sludge, increasing the sedimentation (Verma, Dash and Bhunia, 2012; Guibal and Roussy, 2007). Clariflocculation is the process of sequential coagulation, flocculation, and settling; sedimentation or filtration are used to separate the materials after this process. The coagulation process will work better the higher the charges produced by coagulants (Bratby, 2016). Positively charged groups on the coagulants attract the dye ions, which causes subsequent precipitation to occur and produce very tiny particles with little strength that break down readily. This is because many reactive dyes possess a negative charge in their hydrolyzed dissolved form. (2011) Zaharia *et al*. Variations in dye type, coagulant dosage, sample pH, and temperature all affect how well dye is removed (Verma, Dash, and Bhunia, 2012; Perng and Bui, 2014; Mohd-Salleh *et al*., 2019). The type of dye used determines how the coagulant interacts with the amount and kind of functional groups. As a result, significant differences in dye removal efficiency between dye types can be seen using the same coagulant. The ideal pH is essential for efficient coagulation because it strongly influences the development of intermediate reaction products and the duration of their formation (Gao *et al*., 2008). By creating strong, quickly formed, and quickly settling flocs, the addition of coagulant assistance during coagulation enhances the process (Holt *et al*., 2002). Reactive dye wastewater treatment and the dosage of inorganic coagulants have been shown to be strongly correlated (Joo *et al*., 2007). According to Ashtekar *et al*. (2014), PAC was successful in removing reactive blue dye between a pH of 5 and coagulant concentrations of 100–150 mg/L. They also noticed that, up to a dosage of 500 mg/L and pH 5, the same dye removal effectiveness by alum treatment increased with dosage. Conversely, it was shown that ferrous sulphate was mainly ineffective at removing the colour from the solution when the coagulant was added at high dosage concentrations and

the pH range was wide (3–12). According to Sabur, Khan, and Safiullah (2012), treating textile wastewater with 25 mg/L PAC at pH 7, room temperature, and 100 rpm resulted in removals of COD, TDS, and turbidity of 90.17, 74.09, and 93.47%, respectively. According to Yu-li *et al*. (2006), dye wastewater with 30 mg/L polyferric chlorides (PFCs) at pH 7, room temperature, and 100 rpm reduced COD by 55%.

Chemical coagulation techniques do not result in the breakdown of dyes, and as a result, no poisonous or possibly hazardous substances are produced. The chemical coagulation method's main drawbacks are that it is difficult to control the process, that impurities like non-ionic detergent residues can affect the precipitation rate and floc size, that chemical residues like iron and aluminum salts can remain in wastewater, and that the resulting sludge can have negative ecological and health effects. According to Zongping *et al*. (2011), coagulant chemical loading in the effluent additionally raises the concentration of salt during the sludge generation process, which results in insufficient dye removal. However, sludge generation can be reduced by choosing the right coagulant and optimizing process parameters (Aboulhassa *et al*., 2006; Verma, Dash, and Bhunia, 2012).

2.3.6 Biological Methods

Because biological treatment methods create less sludge and are more environmentally friendly, interest has switched towards them as a way to prevent the negative health and environmental impacts of chemical coagulants (Sathiyanarayanan *et al*., 2013; Singh *et al*., 2015). To treat dye effluents, a variety of biological technologies are used, including sequencing batch reactors, fluidized bed reactors, rotating biofilms and sequential bioreactors, aerobic and anaerobic packed beds, and up flow anaerobic sludge blanket

reactors (Zed and Villaverde, 2005; Maas & Chaudhari, 2005; Rathour *et al*., 2021). These techniques have demonstrated the ability to remove colour, BOD, and COD from textile effluents while using less energy and producing less sludge. According to reports, bacteria break down soluble organic waste to remove BOD and COD, with a reported biological clearance rate of 80% to 85% (Periyasamy *et al*., 2018). Albahnasawi *et al*. (2022) examined the efficacy of an aerobic sequential batch reactor in the treatment of actual textile wastewater and discovered that, during a 12-hour retention period, the system could reduce by 86.6% and colour by 62.44%. Numerous factors, including the organic load, microbe concentration, temperature, incubation period, nutrition levels, carbon/nitrogen ratio, oxygen content, dye concentration, and dye structure, all determine how effective biological dye wastewater treatment is (Holkar *et al*., 2016).

Numerous microorganisms, including bacteria, fungi, yeasts, actinomycetes, and algae, are capable of decolorizing a wide range of dyes; these microorganisms are typically used as bioflocculants in bioremediation processes to remove pollutants from effluents and the environment (Humnabadkar, Saratale and Govindwar, 2008; Saratale, 2007; Deng *et al*., 2020). Dye is decolored either through biodegradation by the cells or by biosorption on the microbial biomass. Unlike during biodegradation, the dye's physical structure is preserved during this process. The microbial biomass's matrix traps the dye ions as well as other contaminants. The process of microbiological bioremediation can be either anaerobic or aerobic. The dye undergoes anaerobic conversion into hazardous amino compounds, which are subsequently transformed into intermediate CO_2 and inorganics by an aerobic reaction. According to Khan, Bhawana, and Fulekar (2013), the breakdown of chromophoric group

bonds aids in the degradation and decolorization of dyes, eliminating the dye's colour and toxicity.

Unlike other biological systems, fungi are adaptable and display a range of non-specific methods of decolorization. A variety of lignolytic enzymes, including lignin peroxidases (LiP), manganese dependent peroxidases (MnP), laccases (Hatvani and Mécs, 2001), and reductase (Bhosale, Saratale, and Govindwar, 2006) from white-rot fungi may break down a broad range of textile colours. According to reports, effluent containing colours including Orange II, Tropaelin O, Congo red, and Azure B can be decolored by *Phanerochaete chrysosporium, Hirschioporus larincinus, Inonotus hispidus, Phlebia tremellosa, and Coriolus versicolor* (Jebapriya and Gnanadoss, 2013). However, because of their slow growth rate, mycelial ageing, and sludge generation, filamentous fungi (molds) are not well suited in dye effluents (Parshetti *et al*., 2010). One advantage of bacteria is that they may break down and mineralize dyes, which results in a shorter growth period (Pereira *et al*., 2019). *Pseudomonas, Alcaligenes, Acinetobacter, Paracoccus, Methylobacterium, Bacillus* and *Hyphomicrobium* bacteria genera are have been commonly identified as the microbial flora used in wastewater treatment plants (Wagner et al., 2002)

2.3.6.1 Microbial *Eps*

The ability to synthesize and excrete *Eps*, high molecular weight biopolymers with unique chemical compositions, properties, and structures that have potential applications in various fields, is possessed by a multitude of microorganisms. These biopolymers are produced during the metabolic processes of certain microorganisms, including fungi and bacteria. Compared to *Eps* derived from plants (cellulose, starch, and pectin), animals

(glycogen), and algae (agar, alginic acids, and alginates), microbial *Eps* offer benefits. Microbial *Eps* are inexpensive, simple to extract from cell-free supernatant, and don't require harsh chemicals for cell lysing that harm the environment. The compositions of plants and algae differ, and climate conditions have a significant impact on them (Schmid, Sieber, and Rehm, 2015; Pereira *et al*., 2019). Due to their ease of replication and quick production timeframes, bacteria are the most widely used microorganisms for the manufacture of *Eps* (Pereira *et al*., 2019). During the extraction procedure, the loosely linked mucoid layers of the *Eps* can be readily removed from the cells. According to Elkady *et al*. (2011), Wang, Salem and Sani (2019), Angelin and Kavitha (2020), Abdelhamid, Mohamed and Selim (2020), and Wang, Salem and Sani (2019), bacteriomeric epoxides (BCEs) are nontoxic, biocompatible, biodegradable, and possess good adhesive ability, viscoelasticity, and pseudo-plasticity. When applied in the culinary, pharmaceutical, cosmetic, chemical, textile, oil, and gas sectors, (*Eps*) can be used either alone or in conjunction with other materials as thickeners, emulsifiers and suspension stabilizers, flocculants, and additives (Khalid and Arif, 2022). *Eps* have demonstrated the ability to tolerate a range of environmental stressors, including high salinity concentrations, freeze-thaw cycles, high temperatures, and severe pH levels.

Following the breakdown of the chromophoric group bond, these biopolymers cause synthetic dyes to degrade into relatively less toxic inorganic compounds, removing colour and reducing the issue of secondary pollution (Sutherland, 2001; Vert *et al*., 2012; López, Vlamakis and Kolter, 2010; Babu *et al*., 2015).

Because of full mineralization, the intermediate products produced by biological treatment techniques are nontoxic and use less water than those produced by physicochemical techniques (Saratale, 2011). Nonetheless, it's important to keep an eye on the end compounds' toxicity and regulate a number of process variables, including the kind and concentration of the substrate, the amount of agitation and oxygen present, the temperature, pH, and the textile dye content (Saratale *et al*., 2011). Thus, interest in *Eps* manufacturing has increased due to the growing need for natural polymers that have potential for a variety of industrial uses (Sharma *et al*., 2017).

2.3.7 Combined Methods

Chemical treatment techniques like flocculation and coagulation are efficient in removing dyes, but they are expensive because they produce a lot of toxic residues that need to be treated again (Verma, Dash and Bhunia, 2012; Huang *et al*., 2014; Buscio *et al*., 2015). Therefore, for the efficient treatment of dye wastewater, a mix of several treatment techniques is typically advised. Wastewater containing synthetic dyes has previously been treated using NF and chemical coagulation/flocculation (Liang *et al*., 2014). According to the authors, when the pH of the mixed dye wastewater was 3, the ideal dosage of PAC/polydiallyldimethyl ammonium chloride (PDDA) was 400/200 ppm, and chemical coagulation/flocculation achieved about 90% dye removal efficiency. It was discovered that the coagulation/flocculation and NF techniques worked well together. According to Riera-Torres, Gutiérrez-Bouzán, and Crespi (2010), the colour removal efficiency of RB5 using chemical coagulation/flocculation method was 90%; however, after treatment combining the coagulation with NF method, the efficiency rose to >98%. By using AOPs in conjunction with flocculation or coagulation as a pretreatment technique to lessen

turbidity in textile effluents, the effectiveness of the method was demonstrated. Roughly 99 percent of the turbidity was eliminated. Furthermore, the AOPs' colour removal rate was about 100% (López-López *et al*., 2016). Biological treatment is typically applied before ozonation and sophisticated oxidation techniques to increase biodegradability and remove colour more effectively. According to reports, this combination raised the BOD/COD ratio after ozonation. This is likely because hazardous chemicals are more biodegradable (Sevimli and Sarikaya, 2002).

2.4 Bacterial *EPS* Production

According to Ben Rebah, Mnif, and Siddeeg (2018), a diverse range of microbes isolated from different origins, using different nutrient sources, and growing under varied conditions, can create *Eps* with different features. The initial stage of *Eps* biosynthesis is the absorption of carbon substrates. Subsequently, intracellular polysaccharides are produced, and ultimately, the cells exude polysaccharides (Onkar *et al*., 2020). *Eps* shield cells from UV rays, unfavorable pH values, osmotic stress, phagocytosis, and chemical agents (antibiotics, heavy metals, and oxidants) (Merino *et al*., 2019; Kaur and Dey, 2022). *Eps* also shield cells from extreme temperature (Casillo *et al*., 2018; Wang, Salem, and Sani, 2019), salinity (Isfahani *et al*., 2018), aridity, and desiccation (Knowles and Castenholz, 2008). Additionally, they support the bacterium's ability to adhere to surfaces, colonize new areas, and absorb nutrients (Janczarek *et al*., 2015; Kalpana *et al*., 2018). *Eps*-producing microorganisms can be identified macroscopically in their colonies, which have a mucoid or watery surface and a glistening, slimy look on an agar plate (Ruiz *et al*., 2016; Sutherland, 2001). The physicochemical environment in which the biofilm matrix forms and the genetic makeup of the microbial cells determine the chemical content and

structure of epibiosis. These environmental factors include temperature, agitation, dissolved oxygen, trace minerals, carbon dioxide, pH, carbon/nitrogen (c/n) ratio, phosphorous concentrations, and availability of nutrients and mineral ions (Zhang *et al*., 2002; Öner, 2013; Salehizadeh and Yan, 2014; More *et al*., 2014). Every microbial strain should have its ideal growth conditions for *Eps* determined by optimizing its culture parameters, including pH, temperature, aeration, and inoculum size, as well as media composition (carbon and nitrogen) (Simphiwe, Ademola and Balakrishna, 2012; Mohamed, Raed and Shimaa, 2014; Mohammad *et al*., 2017). The ideal temperature is required for the growth and metabolic processes of bacteria. The generation of *Eps* is impacted by the inactivation or deactivation of cells caused by low or high temperatures. Research has indicated that the ideal temperature range for bacterial cultures and consortiums to degrade dyes is between 30 and 40 0C, beyond which the bacteria's capacity to survive is compromised (Çetin and Donmez, 2006; Garg and Tripathi, 2017). Although it varies depending on the species, most bacteria prefer a pH of between 6 and 8 for the formation of *Eps* (Yuyan *et al*., 2010; Hereher *et al*., 2018; Razack, Velayutham and Thangavelu, 2013).

According to Zhang *et al*. (2009) and Buthelezi, Olaniran, and Pillay (2012), bioflocculants molecules become negatively charged at pH 7 as a result of the ionization of hydroxyl and carboxyl groups, which improves the sorption of the positive dye cations through electrostatic forces.

Bacteria form good biosorbent flocculants due to their ability to grow under controlled conditions and resilience to harsh environmental conditions (Srivastava, Agrawal and

Mondal, 2015). Kaur and Dey (2022), Wünsche and Schmid (2023) and Díaz-Cornejo *et al*. (2022) reported that *Eps*-producing bacteria belong to different phylogenetic groups which include Gram-negative classes; *Alphaproteobacteria* including *Acetobacter, Gluconobacter, Gluconacetobacter, Komagataeibacter, Kozakia, Neoasaia, Agrobacterium, Rhizobium and Zymomonas genera. Betaproteobacteria* class; *Alcaligenes* and *Achromobacter genera.* Gammaproteobacteria class; *Azotobacter, Pseudomonas, Enterobacter, Alteromonas, Pseudoalteromonas, Xanthomonas, Halomonas, Erwinia, Vibrio,* and *Klebsiella* genera. They also include Gram-positive bacteria such as *Bacillus sp., Paenibacillus, Lactobacillus, Leuconostoc,* and *Streptococcus* (Rehm, 2010; Barcelos *et al*., 2020; Netrusov *et al*., 2023). *Bacillus subtilis* is also one of the major producers of *Eps* among Bacillus sp. (Hassan and Ibrahim, 2017)

Eps that are negatively charged bind to trace metals like Fe, Zn, Cu, Cd, and Co. as well as dissolved cations. According to reports, lower pH values are associated with positively charged bioflocculant sites, such as the NH_2 group, which may facilitate removal by attracting the removal of anionic dyes because of their negative charge (Xue and Li, 2008; Dong *et al*., 2022). According to Zhong *et al*. (2016), wastewater containing acid brilliant scarlet GR became more decolorized as pH decreased, reaching a maximum removal of 82% and 95% for COD and colour removal at pH 2. Verinder *et al*. (2013) found that raising the medium's pH from 5 to 7 enhanced the yield of *Eps* produced by *Alcaligenes Faecalis* B14, which was isolated from native soil, from 0.25 mg to 0.85 mg. Changes in the medium's H^+ ion content is blamed for this. A pH increase, according to Deng, Yu, and Ting (2005), lowers electrostatic attraction and deprotonates functional groups, which has an adverse effect on the molecules of the bioflocculant. Stirring agitates the media and adds

additional oxygen, which encourages the synthesis of more *Eps*. The important characteristics of *Eps*, such as porosity, density, water content, charge, sorption and ion exchange capabilities, hydrophobicity, and mechanical stability, are therefore ultimately determined by environmental factors (Sutherland, 2001; Maruzani et al., 2019). The efficiency of flocculants is also affected by varying carbon and nutrient sources as well as extraction techniques (Li *et al*., 2013; Nguyen *et al*., 2016). In order to support cell growth and exopolysaccharide excretion, the manufacturing medium for *Eps* must have a higher carbon concentration than nitrogen content. The *Alcaligenes Faecalis* B14, isolated from native soil, found that glucose in the medium produced the maximum output of *Eps*, followed by sucrose, maltose, and lactose. According to Verinder *et al*. (2013), the best source of nitrogen was determined to be yeast extract, which was followed by peptone, ammonium nitrate, and ammonium sulphate. (Razack *et al*., 2013) also noted that when yeast extract was used as the nitrogen source, *Bacillus subtilis* produced the most *Eps.* Generally, *Eps* synthesis is stimulated by high carbon substrate concentration, whereas nitrogen and phosphorus are needed for cell division (Alves, Melo and Vieira, 2002). *Alcaligenes Faecalis* and *Xanthomonas campestris* DSMZ 19000, respectively, yielded the highest *Eps* production when magnesium sulphate ($MgSO_4$) mineral ions were used, according to research by Verinder *et al*. (2013) and Enshasy *et al*. (2011). The catalytic activity of the enzymes responsible for producing *Eps* are enhanced by mineral ions.

Nwosu, Abu, and Agwa (2019) used nutritional agar supplemented with 2% sucrose as a carbon source to extract five distinct bacterial strains that were capable to producing *Eps* of over 1000 mg/L from oil-polluted soil. The 50 ml nutritional medium that was enriched with 2% sucrose was used to produce *Eps*, and it was incubated for three days at 120 rpm

in a rotating shaker at 30 ^{o}C. The growth media's cell-free supernatant was used to extract *Eps*, which were then combined with two volumes of cold ethanol and centrifuged at 15,000 rpm for 20 minutes. The mixture was then chilled at 4 ^{o}C for an overnight precipitation period. The precipitates were dried at 60 ^{o}C to a constant weight, measured by total dry weight, and tested with phenol sulfuric acid to determine the total amount of carbohydrates present. >100 mg/L of carbohydrates were discovered to be present. *Providencia stuarti, Escherichia coli, Shewanella chilikensis,* and *Bacillus nealsonii* were identified as the isolates. Twenty *Eps*-producing bacteria were found in activated sludge from a wastewater treatment plant, according to Bahar (2015). At 30 to 37 ^{o}C, the maximum *Eps* generation was seen in 2.5 to 3% glucose and 0.5% yeast extract. Using the Bradford and phenol-sulfuric acid techniques, the carbohydrate/protein ratio in all of the extracted *Eps* was assessed and was found to range from 1.42 to 15%. The bacteria were members of the genera Klebsiella, Pseudomonas, and Bacillus. Two *Eps*-producing bacteria were identified from a soil sample by Sudha and Gayatri (2021). The isolates were determined to be *Pseudomonas sps* and *Lactobacillus sps*. produced *Eps* precipitates with dry weights of, correspondingly 0.09 and 0.17 grammes per liter. When compared to the quantities acquired from glucose, lactose, and maltose as carbon sources, it was discovered that the isolates that produced *Eps* gave the maximum development of *Eps* with 2% sucrose. Five morphologically distinct strains of bacteria and *Eps* were identified by Muthusamy, Kanapathi, and Karuppiah (2011) using YMG agar media (glucose 10 g, yeast extract 3 g, malt extract 3 g, peptone 5 g, distilled water, and old sea water) which was shaken at 120 rpm for five days at 25 ^{o}C. The *Eps*' FTIR examination showed the presence

of -OH groups at 1631.48 cm-1 and a -OH band at 3415.31 cm-1. It was discovered that glucose and galactose sugars made up the *Eps*.

2.4.1 Bacterial *Eps* Properties

Bacterial *Eps* are secondary metabolites that are high molecular weight (10 to 30 KD) carbohydrate polymers that come from bacteria. According to Schmidt, Sieber, and Rehm (2015), they can be divided into three categories: slime/mucus exopolysaccharides (*Eps*), such as xanthan, sphingan, alginate, and cellulose; capsular polysaccharides (cps), such as K30 O-Antigen; and internal storage polysaccharides, such as glycogen. *Eps* enter the culture media to produce mucus, while Cps attach to the cell walls of bacteria to form capsules (Rehm, 2010; Shukla *et al*., 2019). Since functional groups such amine, phosphate, hydroxyl, carboxyl, and urinate are present in various levels depending on the composition of the *Eps*, the majority of *Eps* are negatively charged (Durán *et al*., 2011). These functional groups affect the capacity to bind with metal ions, flocculation characteristics, and ion exchange (Ding *et al*., 2018; Hassan *et al*., 2022). (Peña-Méndez, Havel & Patočka, 2005; Chouchane *et al*., 2018; Rana & Upadhyay, 2020) *Eps* are mostly formed of carbohydrates, protein, nucleic acids, lipids, and humic components like fulvic and humic acids that indicate presence of the soluble fragments and the insoluble fraction named humin. *Eps* are primarily made up of organic and inorganic components including uronic acid, methyl esters, sulphates, phosphates, acetyls, succinate, glycerol, and pyruvates, as well as carbohydrates/polysaccharides such as glucose, galactose, and rhamnose (Fleming, 2000; Sutherland, 2001). Carbohydrate and protein are the main constituents of *Eps. Eps* are typically composed of 40–95% polysaccharides, 1–60% proteins, 1–40% lipids, and 1–10% nucleic acids, according to Flemming and Wingender

(2002). Either homopolysaccharides or heteropolysaccharides make up the structure of *Eps* (Flemming and Wingender, 2010). The *Eps* molecules are interlaed and networked with each other to form a defined structure Homopolysaccharides are composed of glucose, fructose, galactose, rhamnose, and mannose linked by glycosidic linkages. They can be either unbranched or branched. Generally neutrally charged, the *Eps* exhibit varying degrees of branching and can be categorized according on their predominant linkage and the type of monomeric units they include, including fructans, polygalactan, α-d-glucans, and β-d-glucans. According to Mohd Nadzir *et al*. (2021), homopolysaccharides are classified into four groups: fructans (levan and inulin), β-D-glucans (bacterial cellulose), and α-D-glucans (dextran, alternan, and reuteran). They have a molecular mass of about 107 Da. According to Czaczyk and Myszka (2007), homopolysaccharides include cellulose, pullulan, dextran, curdlan, and scleroglucan. Heteropolysaccharides incorporate repeating units of fructose, glucose, galactose, Mannose, Rhamnose, D-glucose, D-galactose, L-rhamnose, N-acetylglucosamine, N-acetylgalactosamine, uronic acid, or glucuronic acid. They are made up of at least two distinct monosaccharides connected by glycosidic bonds (Hassan *et al*., 2022). There are instances when substitutes like glycerol, phosphate, and acetyl are present (Sarita *et al*., 2022). They have a molecular mass between 104 and 106 Da. Heteropolysaccharides, such as xanthan, alginate, hyaluronic acid, kefiran, and gellan gum, exhibit unique physical characteristics based on the components and branching of their monosaccharides (More *et al*., 2014; Angelin and Kavitha, 2020). According to Ripari (2019), the majority of homopolysaccharides are formed extracellularly, while heteropolysaccharides are synthesized intracellularly and then exported from the cell.

Polysaccharides with polar functional groups and a high molecular weight are good structural characteristics for *Eps*. According to Nwodo, Green, and Okoh (2012), endogenous bacterial polysaccharides frequently have molecular weights ranging from 10 to 1000 kDa. Mechanical characteristics generally improve with molecular weight. Polysaccharides with polar and hydrogen bonding functional groups, like ethers, hydroxyls, and carboxylates, provide strong interchain contact and high adhesion qualities that contribute to cohesive strength. Because of their ionizable groups, which have a significant impact on their physicochemical properties, *Eps* behave like polyelectrolytes in aqueous solution (Zaheer *et al*., 2019).

2.4.1.1 *Eps* Characterization Techniques

Eps is characterized by different techniques such as High-performance liquid chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), Scanning Electron Microscopy (SEM). *Eps* is characterized by different techniques as shown in Figure 2.6.

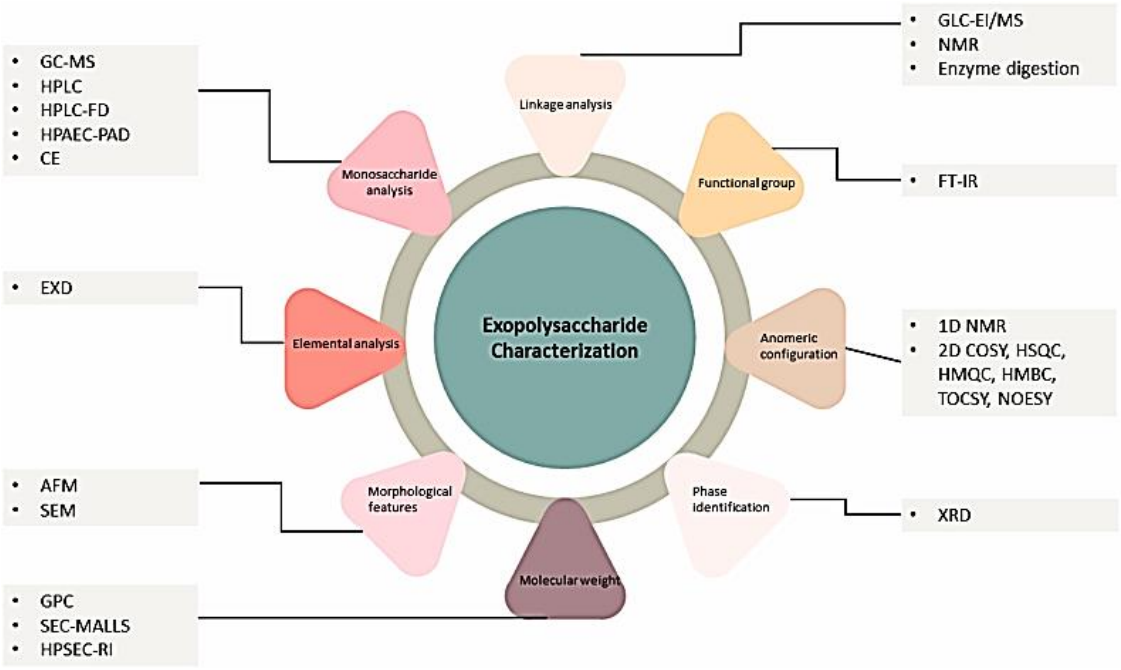

Fig. 2.6: Exopolysaccharide characterization techniques (Krina, Arpit and Meenu, 2021).

High-Performance Anion Exchange Chromatography (HPAEC) and Gas Chromatography-Mass Spectrophotometer (GC-MS) are used to identify and quantify the mono-sugars (Sofia, 2009; Zaki *et al*., 2011). GC-MS is often used to determine the components in organic compounds. All sugars need to be converted into volatilizable and stable derivatives prior to GC-MS analysis due to their high polarity and low volatility. Conventional derivatization methods involve substitution of the polar groups of the sugar. The most popular derivatives for GC-MS analysis of sugars are acetates, trifluoroacetates, methyl and trimethylsilyl ethers (Matute *et al*., 2011; Sohaib, 2015). The resulting alditol acetates are then dissolved and injected onto a GC-MS column.

Through acid hydrolysis, which severs the glycosidic bond separating the monosaccharide in the polysaccharide chain, the composition of *Eps* is determined. The polysaccharides

can be hydrolyzed using hydrochloric acid, trifluoroacetic acid, sulfuric acid, and trichloroacetic acid. In Gas Chromatography (GC) analysis, converting the less volatile sugar monomer into the volatile and stable derivative is a crucial step. Enzymatic degradation, methylation, and NMR spectroscopy are used to analyze glycosidic linkage locations (Madhuri and Prabhakar, 2014). Using NMR, anomeric configuration, linkage type, branching pattern, and non-carbohydrate replacements are investigated. *Eps*'s molecular weight varies according on the species and monomeric composition, and it frequently influences its physicochemical and biological properties. Light scattering, intrinsic viscosity, gel permeation chromatography, Size Exclusion Chromatography (SEC), high-performance size-exclusion chromatography with RI detector, analytical ultracentrifugation, and other methods are employed to investigate the *Eps* molecular weight (Sani and Rathinam, 2018). The analysis of glycosidic bonds and functional groups in *Eps* is done using Fourier Transform Infrared (FT-IR) and attenuated total reflectance (ATR) in conjunction with FTIR spectroscopy (Gómez-Ordóñez and Rupérez, 2011). SEM and X-ray powder Diffraction (XRD) techniques are used to study *Eps* microstructure and identify crystalline and/or amorphous structure (Kavita, Mishra, and Jha, 2011). The thermal stability qualities of *Eps* are measured by thermogravimetric analysis (TGA), Differential Scanning Calorimetry (DSC), and Differential Thermal Analysis (DTA) (Bothara and Singh, 2012).

2.4.2 Bacterial *Eps* Applications

Microbial polysaccharides have garnered interest because of their special qualities and potential for rapid mass manufacturing. Additionally, it has been noted that *Eps* are usually acknowledged as safe (gras) substances, which means that using them won't put the user's

health at risk. *Eps* matrices exhibit hydrophilicity or hydrophobicity, biodegradability, and adsorption capabilities due to their unique components. The capacity to generate hydrogels (depending on structure), the use of renewable resources in synthesis, and minimal toxicity are additional characteristics that set bacterial *Eps* apart (Sutherland, 2001; Onkar, 2020). The features of *Eps*, such as flocculation, emulsification, absorption, and film formation, have sparked interest in using the biomaterial for applications in downstream processing, textiles, detergents, and adhesives (Kanmani and Yuvapriya 2018; Banerjee *et al*., 2021). *Eps* are widely used in many industrial applications, such as food, pharmaceutical, emulsifiers, stabilizers, bio-nanotechnology, metal removal and recovery, and the removal of toxic organic compounds, due to their structural and functional properties (Iyer, Mody, and Jha, 2006; Saravanan *et al*., 2017; Laurienzo, 2010; Kanmani and Lim, 2013). Functional and rheological characteristics of *Eps*, which differ because of their glycosidic linkages, molecular weights, and functional groups, dictate how they are applied in industrial processes (Jiang *et al*., 2021). The absorptive character of the biopolymers towards substances like metal ions is determined by the amount of carbohydrates in *Eps* (Nwodo, Green, and Okoh, 2012). The increased hydrophobicity caused by the protein content of *Eps* increases flocculation activity (Shi *et al*., 2017). The different applications of *Eps* in environmental protection are depicted in Figure 2.7.

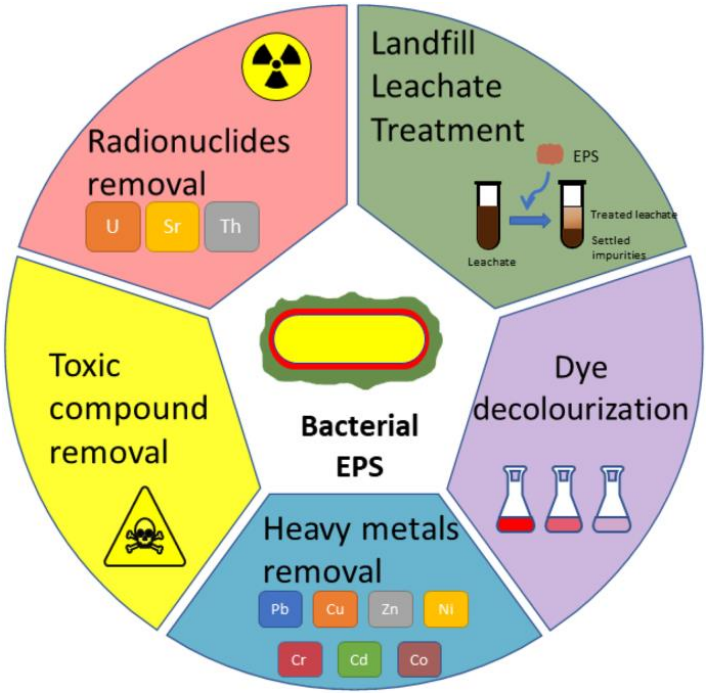

Fig. 2.7: Environmental applications of bacterial *Eps* (Krina, Arpit and Meenu, 2021).

2.5 Application of *Eps* in Dye Effluent Bioremediation

To interact and survive in the presence of inorganic metal ions that might be hazardous, microorganisms use a variety of strategies, including biotransformation, extrusion, the usage of enzymes, and *Eps* (Wu *et al*, 2010; Dixit *et al*. 2015). According to Malik (2004), Ramasamy, Kamaludeen, and Banu (2007), Yang, Chen, and Wang (2015), Wang and Chen (2009), the microorganisms use a variety of metal detoxification mechanisms, including electrostatic interaction, metal-organic complexation, ion exchange, extracellular chemical precipitation, intracellular and extracellular metal sequestration, exclusion by impermeable barrier, and production of metal chelators such as metallothioneins and bio surfactants. There is growing interest in using *Eps* for environmental detoxification and bioremediation because it is thought to be pathogen-free when compared to microorganisms and has features such as emulsification or surfactant (El-Masry, El-

Bestawy and El-Adl, 2004; Díaz *et al*., 2018). The attraction between the dye and the biological components of the biopolymer is attributed to specific functional groups such as amino, carboxyl, hydroxyl, phosphate sulfhydryl, and phenolic, which are responsible for biosorption by *Eps* (Zhang *et al*., 2009; Buthelezi, 2008). Some of the biosorption mechanisms by which these biopolymers remove pollutants include adsorption, charge neutralization, inter-particle bridging, ion exchange, and surface complexation (Aravindhan, Rao, and Nair, 2007; Veena, Rao, and Venkata, 2019). According to Bisht and Lal (2019) and Zhang *et al*. (2007), the hydroxyl groups present in carbohydrate bioflocculants cause the bridging action on dyes, which results in the production of heavy, three-dimensional flocs that eventually settle. According to De Philippis, Colica, and Micheletti (2011), the bioflocculant's uronic acid content contributes hydroxyl groups that are directly linked to the adsorption and flocculation mechanisms. The bioflocculant creates bridges by chemical reactions, van der Waals forces, static electricity, or hydrogen bonds to neutralize and stabilize the charged particles floating in the medium. This process is known as the bridging mechanism (Salehizadeh & Shojaosadati, 2001). Larger flocs are encouraged by high molecular weight bioflocculants because they bind particles, increasing floc density, size, and shear resistance. The bioflocculant's functional groups and pH are important variables that affect how well the adsorption sites bridge (Wu & Ye, 2001; Zhang *et al*., 2010). According to Salehizadeh and Shojaosadati (2001), the charge neutralization mechanism takes place when the bioflocculant's adsorption sites reduce the charge density of the negatively charged particles in a solution. This results in electrostatic repulsion forces that are stronger than van der Waals forces and prevent floc formation and

settling. Low molecular biomolecules are useful for the charge neutralization by bioflocculants, according to Yang *et al*. (2016).

2.5.1 Application of *Eps* in Dye Removal

The type and structure of the dye, the effluent pH, temperature, oxygen, ionic strength, nutrient composition, the concentrations of the dye and *Eps*, and the length of the contact all affect how well *Eps* remove dye (Buthelezi, Olaniran, and Pillay, 2012; Tunc, Tanacı, and Aksu, 2009; Senelisile, Bukisile, and Pinkie, 2022). The amount of dissolved oxygen that is increased by shaking during wastewater treatment promotes the biosorption activity. The pH of effluent has been demonstrated to have an impact on biodegradation, with neutral to slightly alkaline pH being the most effective (Pinheiro *et al*., 2022). It's critical to make sure that *Eps* can operate without being inhibited by dye concentrations in order to enable successful industrial applications in decolorization.

According to Zhang *et al*. (2009), various functional groups such as amino, carboxyl, hydroxyl, phosphoric, sulfhydryl and phenolic cause attractive forces between the dye and the *Eps*, which accounts for the biosorption capacity of *Eps*. Chouchane *et al*. (2018) state that the addition of metallic cations like K^+, Mg^{2+}, and Ca^{2+} can help bridging and charge neutralization procedures by aiding in the neutralization of the negatively charged functional groups. Because of the neutralization and stabilization of the negative charges of their functional groups, the metal cations are conductive and cause an electrostatic repulsion between dyes and flocculants (Buthelezi, Olaniran, and Pillay, 2012). The contribution of the charge neutralization and bridging mechanisms to the floc formation between dyes and bioflocculants is shown in Figure 2.8.

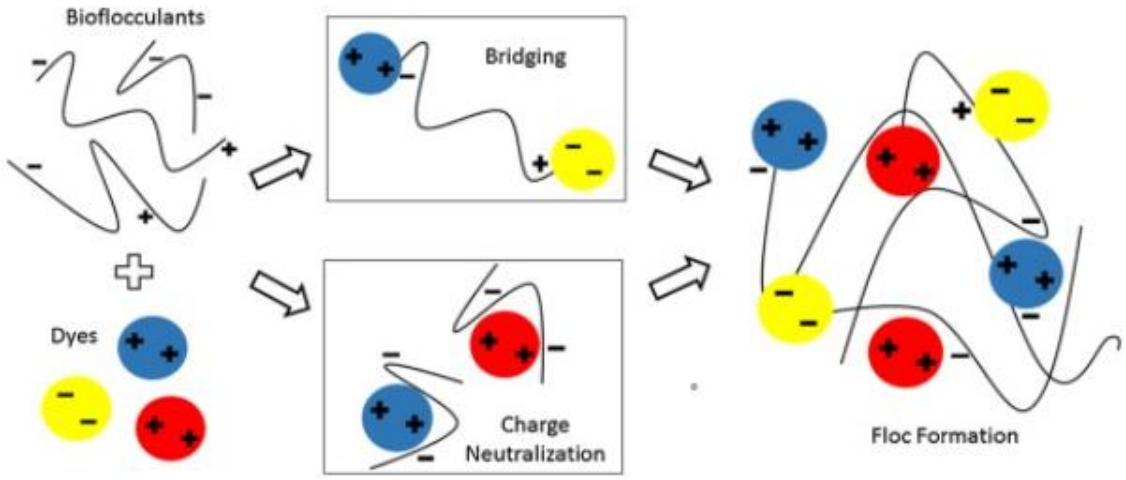

Fig. 2.8: Flocculation mechanisms of charged dyes ions by bioflocculants (Wagner *et al*., 2021).

Eps are utilized in the textile industry as wastewater dye binding agents (Mahmoodi *et al*., 2011; Zhang *et al*., 2013; Mayowa Carolina and Anofi, 2016; Zayed *et al*., 2018). *Bacillus megaterium* KY848339.1 was reported to be able to decolorize 91% of 500 mg/L Acid red 337 at pH 7 in less than 24 hours, according to Ewida *et al*. (2019). Additionally, it has been found that at pH 7, *Bacillus sp.* decolorizes both Navy blue and Methyl red (Ezhilarasu, 2016). *Bacillus sp.* TERI VB2 created the bioflocculant BF-VB2, which was suggested for possible treatment of dye wastewater due to its outstanding flocculation activity throughout a broad pH range without the need for cation addition. According to Bisht and Lal (2019), the flocculation revealed reductions in turbidity, colour, COD, and TSS of 99.6%±1.0%, 82.78%±3.03%, 92.54%±0.24%, and 73.59%±0.71%, respectively. A bioflocculant produced by *Aspergillus parasiticus* after 72 hours of cultivation, had a decolorization efficiency of 92.9% and 92.4% for Acid Yellow 25 and Reactive Blue 4, respectively. This was caused by the negatively charged dye molecules being drawn to the positive charges of the bioflocculant (Deng, Yu, and Ting, 2005). The majority of

positively charged microbial flocculants primarily achieve flocculation via charge neutralization of the negatively charged suspended particles, without the need for metal ions to activate throughout the process (Liu *et al*. 2015; Mohammed and Dagang 2019). Dye removal from wastewater by *Eps* produced by bacterial strains such *Bacillus, Exiguobacterium, Klebsiella, Pseudomonas, and Staphylococcus* (Hassan and Ibrahim, 2017). As seen in Figure 2.9, *Bacillus sp. and Pseudomonas sp*. have been mostly mentioned in the previous 20 years for use in dye effluent treatment, according to Lee and Chang (2018).

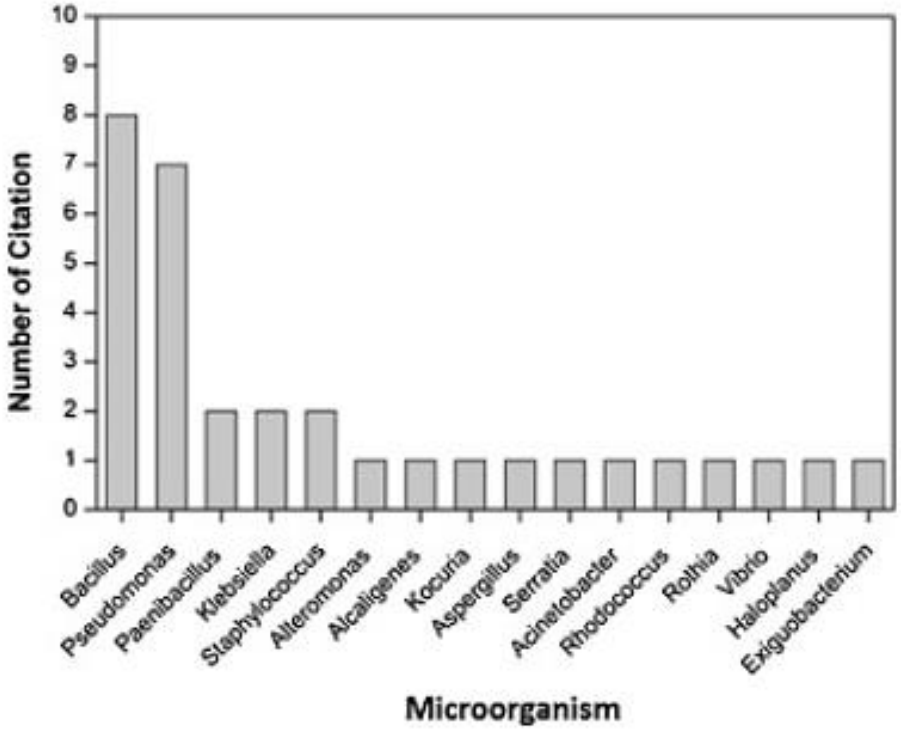

Fig. 2.9: Bacteria bioflocculant strain producers applied for dye removal (Wagner *et al*., 2021).

According to Bisht and Lal (2019), *Bacillus sp.* isolated from soil was able to produce a bioflocculant that could lower the colour of indigo dye, TSS, COD and chloride ions by

83, 92, 74, and 82% respectively from dye wastewater. It has been observed that *Bacillus cereus* B-11 grown on molasses wastewater produces *Eps* that are efficient at eliminating reactive colours from aqueous solutions. 25 mL of *Eps* was found to have 97.5 and 94.7% decolorization efficiency of a solution containing 100 mgL^{-1} reactive Yellow K-4G and Turquoise Blue KN-G respectively (Mao *et al*., 2011).

According to Zobaidul *et al*. (2019), three bacterial isolates, *Alcaligenes faecalis*, AZ26, *Bacillus cereus*, AZ27, and *Bacillus sp*., AZ28, had the ability to decolorize Novacron Super Black G (NSB-G) reactive dye, found in textile industry effluents. About 90% of the 200 mg L^{-1} NSB-G was decolored by the three bacterial isolates after 96 hours of incubation at 37 ^{o}C and pH 7.0 in static conditions. The media's initial dye concentration was adjusted to range between 50, 100, 200, 500, and 1000 mg L^{-1} every 24 hours and the amount of dye decolorized was analyzed. Investigations were conducted at pH 7.0 and temperatures of 22, 30, 37, and 45 ^{o}C to determine the ideal temperature for the dye removal. At specific time interval during the decolorization process, the percentage of the dye supernatant was calculated using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan) set to 600 λmax. Although the greatest decolorization attained by *Bacillus sp.*, AZ28, *Bacillus cereus*, AZ27, *A. faecalis*, AZ26 and *Bacillus sp*., AZ28, was around 91%, 92, and 93%, respectively, with 200 mg L−1 of the dye, decolorization was found to decrease with a rise in NSB-G dye concentration. Although a noticeable decolorization happened at 22–30 ^{o}C, 37 ^{o}C was the ideal temperature for the three isolates to decolorize the dye optimally. Following a 96-hour decolorization period, the maximum absorbance wavelength in the visible spectrum changed by *B. cereus*, AZ27, *A. faecalis*, AZ26, and *Bacillus sp*., AZ28, respectively, to 410 nm, 378 nm, and 373 nm. This shift was attributed

to the formation of new metabolites as a result of the parent compound's biodegradation. However, in adsorption decolorization, there is no shift in the absorbance spectra or new peak, and the strength of all peaks varies nearly proportionately (Veena, Rao, and Venkata, 2019). During FTIR analysis, biodegradation is further indicated by the formation of new peaks or the elimination of old ones. The stretching vibrations of S=O at 1134 cm−1, C–N at 1051 cm−1, C–O at 1004 cm−1, C–N at 1342 cm−1, C=N at 1639 cm−1, and C–Cl at 632 cm−1 were represented in the FTIR spectra of the parent NSB-G. As a result, the parent NSB-G's stretching vibrations vanished from the metabolites' FTIR spectrum after 48 hours, and new peaks appeared in various locations. Asha *et al*. (20115) report that an *Enterobacter asburiae* bacterial strain isolated from Varanasi, Uttar Pradesh, India's textile effluent was able to decompose 98% of the effluent at 35 mg/mL of bacterial biomass in 60 hours at 32ºC and pH 8 under aerobic conditions. The treated effluent's UV-Vis spectrophotometer study revealed that the peaks at about 630 nm and 410 nm were vanished. An analysis using FTIR spectroscopy showed the existence of a new, strong peak at about 1403 cm-1, indicating that the dye effluent had biodegraded.

According to Saha *et al*. (2021), *Pseudomonas putida* was more effective at removing the cationic dye rhodamine-B than *Pseudomonas montelli* in removing the anionic Congo red dye. A maximum decolorization of 82% was reported by Amal *et al*. (2022) using laccase-like active cell-free supernatant from *Coriolopsis gallica*. This was reached at 120 minutes with 0.5 U mL−1 supernatant, dye concentration of 25 mg L^{-1}, HBT of 4.5 mM, pH of 4.2, and temperature of 55 ºC. As a result of the amino and carboxyl groups, the bioflocculant generated by *Alcaligenes Faecalis* isolated from ramie degumming wastewater medium was discovered to include significant levels of proteins and uronic acid, which enhanced

the number of active sites for the selective adsorption of 86% dispersive blue (Chen *et al*., 2020). According to Hossen *et al*. (2019), under 37 °C, pH 8.0, 96 hours, and static circumstances, *Alcaligenes Faecalis* AZ26, Bacillus cereus AZ27, and Bacillus sp. removed 90% of 200 mg/L Novacron Super Black G (NSB-G). According to Nur *et al*. (2007), *Ochrobactrium sp., Salmonella enterica, and Pseudomonas aeruginosa* removed Reactive Black B from 332 to 103.1 mg L^{-1} and Cr (VI) from 199 to 127.6 mg L^{-1} at pH 8. Within 2-4 days, the consortia removed 90% to 99% of Cr (VI) from a medium containing molasses with or without dye. Synergistic effects of combining bioflocculants with conventional commercial coagulants and flocculants have been documented in an attempt to lower the manufacturing cost of *Eps*, despite the fact that most bioflocculants are fundamentally negatively charged (Guo and Chen 2017; Guo *et al*. 2015b). Bioflocculant was applied in conjunction with PAC and aluminium sulphate to achieve a 7% increment in the decolorization of dispersive yellow dye, as reported by Huang *et al*. (2014), Huang *et al*. (2015), and Zhao *et al*. (2012). It was observed that the best bioflocculant dosage for eliminating SS, BOD, COD, nitrate, and turbidity from dairy effluent was 0.5 mg/mL. The flocculation activity, compared with chemical flocculants such PAC, PEA, and alum, was discovered to be higher. Huang *et al*. (2015) found that the microbial flocculant and aluminium salt effectively remedied synthetic dye wastewater by increasing the floc formation speed in alkaline or neutral aqueous solutions and improved the floc size in acidic conditions. As impacts of bioflocculant increases on zeta potential, this was attributed to the adsorption and bridging effects associated to the bioflocculant, which are contradictory to the dominance of charge neutralization. When used with PAC, a bioflocculant called MBFGA1 was shown to decrease the dosage of PAC while

simultaneously improving coagulation efficiency when applied to kaolin suspension. Comparable outcomes were observed when aluminium sulphate and CBF, a bioflocculant, were used for the treatment of humic-kaolin water (Yang *et al*., 2009; Huang *et al*., 2014).

2.5.2 Application *Eps* in Metal Ions Removal

According to Gavrilescu (2004), the adsorption of metal ions occurs in negatively charged sites of *Eps*, including hydroxyl, alcohol, phosphoryl, amine, carboxyl, ester, and sulfonate groups. Thus, the use of microbial enzyme purification (*Eps*) in the remediation of wastewater from the mining and metallurgical sectors is a growing field in bioremediation (Rehm, 2009; Rawlings and Johnson, 2007; Tao *et al*. 2021; Harshitha, Annapoorna, and Louella, 2021). Because of their flocculation characteristics and capacity to bind metal ions from solution, *Eps* may play a part in the removal of heavy metals from wastewater (Gupta *et al*., 2006; Pal and Paul, 2008; Li *et al*., 2013; Prasad *et al*., 2013). To aid in the detoxification process, functional groups of *Eps* (C=O, O–H, CH, C–O, and C–C=O) interact with metal ions (Mathivanan *et al*., 2021). Through electrostatic interactions, the protein and carbohydrate moieties of the *Eps* enhance their ability to bind metal cations (Zhang *et al*., 2017). According to reports, *Eps*'s higher protein content increases its hydrophobicity, which improves flocculation action (Shi *et al*., 2017). Metal ions removal effectiveness is influenced by environmental factors such as pH, temperature, initial metal concentration, *Eps* concentration, stirring rate, and charge density (Salehizadeh and Shojaosadati, 2003).

According to De, Ramaiah, and Vardanyan (2008), Cd and Pb could be eliminated by *Alcaligenes faecalis, Bacillus pumilus, Pseudomonas aeruginosa, and Brevibacterium*

iodinium. It has been found that in roughly 72 hours, *P. aeruginosa and A. faecalis* can remove 75% and 70% of Cd, respectively. Over the course of 96 hours, *Brevibacterium iodinium and Bacillus pumilus* eliminated 87% and 88% of Pb, respectively. According to Chug *et al*. (2016), *Eps* isolated from *Bacillus subtilis and Azotobacter beijreinckii* have been shown to remove 26% and 48% of the chromium from aqueous solution. Heavy metals including Pb, Cu, and Co might be adsorbed by ionic *Eps* produced by the halophilic bacteria, *Halomonas almeriensis* (Llamas *et al*., 2012). Tao *et al*. (2021) report that the maximum adsorption of 99.01 ± 0.61% for Malachite Green was attained by 10 mg of *Eps* at 100 mg/L, at 120 rpm for 75 minutes, and with a maximum adsorption capacity of 247.5 mg/g. The *Eps* were produced by *Lysinibacillus sp*. SS1. Under aerobic conditions, *Bacillus sp*. isolated from tannery activated sludge was shown to decrease 50 mg/L of chromium (vi) in less than 24 hours (Zhu *et al*., 2019). According to reports, metal ions interact with functional groups (C=O, O–H, CH, C–O, and C–C=O) of *Eps* generated by *Bacillus cereus* KMS3-1 to aid in the detoxification process (Mathivanan *et al*., 2021).

Eps produced by *Bacillus sp.* YP03 at pH 7.5 with trivalent aluminium ions showed flocculation, coagulation, and reductions in chemical oxygen demand and total suspended solids from municipal wastewater of 47% and 89%, respectively (Kanmani and Yuvapriya, 2018). It has been observed that earthworm's gut-produced *Eps* from *B. licheniformis* strain KX657843 are effective at remediating heavy metals, as they remove 86% and 81% of Cu (II) and Zn (II), respectively, at pH 8 at a concentration of 25 mg/L. At a concentration of 100 mg/L *Eps*, an overall metal removal effectiveness of 94.8% was noted for both metal ions (Biswas *et al*., 2020). *Eps* secreted from the bacterial consortia of *R. radiobacter* F2 and *B. sphaeicus* F6 showed absorption of 87.12% of Cd, 19.82% of Zn, and 37.64% of

Cu from a hydrocarbon-contaminated water body in 60 minutes at optimal pH 7 (Martins, De Almeida, and Leite, 2008). According to Samarth, Chandekar, and Kaustubh (2012), gram-negative bacterial consortia's epiphytic colonies were found to be effective in eliminating a range of metals from an aqueous solution, including 77.15% Zn, 78.18% Pb, 74.48% Cr, 66.63% Ni, 71% Cu, 72.71% Cd, and 76.12% Co. The metal removal efficiency in the molasses-containing media differed significantly from that of the control group without molasses, with the molasses-containing media showing significantly greater levels of Ni, Cu, and Cr (32%, 75.7%, and 51.1%, respectively) (Kiliç, 2015). *Bacillus subtilis* produced bioflocculant was found to be effective in eliminating heavy metals from samples of lake water and synthetic wastewater. Al, Zn, Fe, and Cu were extracted from the wastewater samples in 92.9%, 94.3%, 86.2%, and 68.1% respectively (Dih, Jamaluddin, and Zulkeflee, 2019). Devi and Natarajan (2015) showed that the use of bioflocculants produced by 10g/L of *Bacillus firmus* and 16.55g/L of *Bacillus licheniformis* resulted in the removal of 85% of Cr (VI) harmful metal ions. According to Agunbiade *et al*. (2019), a biopolymeric flocculant generated by *Terrabacter sp*. was assessed for the bioremediation of heavy metals from wastewater. The removal of Fe (77.7%), Al (74.8%), Mn (61.9%), and Zn (57.6%) was verified through the use of ICP-OES studies. Heavy metals such as As, Zn2+, Pb2+, Cu2+, and Cd2+ have been reported to be removed from industrial wastewater by a unique bioflocculant called QZ-7, which was generated from *Bacillus salmalaya* 139S. For Zn, AS, Pb, Cu, and Cd, the removal rates were 81.3%, 78.6%, 77.9%, 76.1%, and 68.7%, respectively, at pH 7, the ideal dosage for the bioflocculant being 60 mg/L. Additionally, it was verified that the bioflocculant removed

As (89.8%), Zn (II) 77.4%, and Cd (II) 58.4% from actual wastewater (Ragadevan *et al*., 2019).

2.6 *EPS* Production Media

The productivity of *Eps* is said to be influenced by production medium composition parameters as carbon sources, nitrogen sources, carbon nitrogen (C/N) ratio, incubation duration, oxygen, pH, and trace elements (Shukla *et al*., 2019). The limited productivity of bacterial strains and high manufacturing costs have resulted in low commercial production of *Eps* for industrial application. In general, the production of bacterial *Eps* varies between 0.29 and 65.27–100 g/L, contingent upon the type of microorganism and the growing circumstances. The cost of *Eps* productin can be decreased by utilizing inexpensive culture media, isolating new strains with a high capacity for *Eps* formation, and employing genetic engineering to create strains with high productivity (Netrusov *et al*., 2023). Soil-dwelling nonpathogenic gram-positive, rod-shaped, obligate aerobic Bacillus bacteria are of great interest for their production of *Eps*, since they have the ability to yield noticeably higher amounts of *Eps*. Because synthetic media is expensive, use of conventional media for growth is not economically attractive. More than 70% of the entire cost of producing *Eps* can be attributed to the fermentation medium (Salehizadeh and Van Loosdrecht, 2004; Küçükaşik *et al*. 2011; Mohammed and Wan Dagang, 2019). The cost of producing *Eps* can be reduced by switching to a more affordable, natural substrate and by optimizing the conditions under which *Eps* is produced (Freitas, Alves & Reis, 2011; Revin *et al*., 2022). When producing *Eps*, cell development is reliant on the carbon source's bioavailability. According to Netrusov *et al*. (2023), glucose and sucrose are the primary carbon sources that are utilized. Thus, strains with the ability to release enzymes capable of degrading

lignocellulolytic biomasses and concurrently generating microbial flocculants are of both scientific and practical relevance. The many methods that can be used to transform agricultural waste into microbial flocculants are depicted in Figure 2.10.

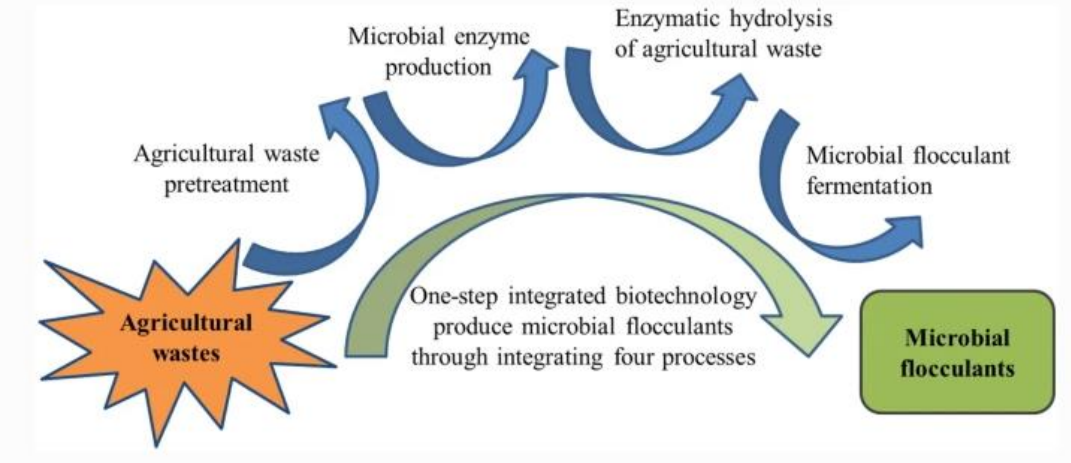

Fig. 2.10: Microbial flocculant production from agricultural wastes (Liu *et al*., 2021)

Therefore, the development of an affordable medium for the manufacturing of *Eps* is chosen to replace the synthetic ones in terms of performance and economy. Hence, search and screening of novel *Eps*-producing microorganisms with good yield at cheap production cost has recently dominated research on *Eps* production (Mohammad *et al*., 2017). For example, *Pseudomonas boreopolis* G22 was found to be able to convert grass lignocelluloses (agave, corn stover, Miscanthus, and wheat bran) into microbial flocculant while secreting xylanase, yielding 3.75 mg flocculant/g dry biomass (Guo *et al*. 2018 a &b)

Thus, research was done on the use of agricultural and industrial wastes as raw materials for the synthesis of *Eps* in reconstituting culture media based on alternative sources of sugars. Agro-waste products that contain carbon are thought to be the most crucial growth medium for the microorganisms' building blocks. Glucose, sucrose, fructose, starch,

maltose, and other forms of organic carbon are among these sources. Agroindustrial residues, including sugarcane and starch molasses, corn-steep liquor, whey, soybean juice, and food wastes (fruit and vegetable peels and pomace), have been shown in this context to be useful as substrates for microbial growth in the production of enzyme-producing peptides (Küçükaşik *et al*., 2011; Ben Rebah, Mnif and Siddeeg, 2018; Saad *et al*., 2018). The earlier research on the synthesis of *Eps* from agro-industrial resources is listed in Table I (Appendices).

In an effort to lower the cost of producing *Eps*, sugarcane molasses, a byproduct of the sugar industry rich in fermentable sugars, has been used in the past. The three sugars found in molasses are sucrose, glucose, and fructose, with sucrose making up the largest proportion. Molasses has a strong growth-stimulating impact on microbes since it also contains vitamins and minerals (Liu, Chu & Chou, 2011). Molasses has been extensively researched as a substrate for the synthesis of commercial polysaccharides such as gellan, xanthan, dextran, curdlan, and scleroglucan because of its high sucrose and other nutritional contents, low cost, easy availability, and ease of storage (Mao, Tian & Zhu, 2011). *Gordonia polyisoprenivorans* CCT7137 was used to produce *Eps* by Fusconi, Mirna, and Nelma (2008) using sugarcane molasses medium at 2%, 6%, and 10%. The results were compared with agar (g/L: 4.0 glucose, 4.0 yeast extract, 10.0 maltose, 15.0 agar) (GYM). The maximum production of *Eps* was found in molasses media at 6% (172.86 g L–1), which was superior and different from that found in GYM media (91.12 g L–1) and 2% SM media (82.86 g L–1). It was also higher than that found in 10% (139.47 g L–1). Up to 23 hours in GYM, 20 hours in 2% molasses, 35 hours at 6% molasses, and

up to 30 hours in 10% molasses, the culture medium demonstrated exponential development.

Eps-producing bacteria are known to exist in habitats with a carbon/nitrogen medium. It has been discovered that Bacillus, an aerobic, gram-positive, rod-shaped and non-pathogenic bacteria that forms endospores and is present in soil and decaying plant material, has a high capacity for producing extracellular polymer synthases (*Eps*) (Arafa *et al*., 2014). These microbes are exposed to abrupt environmental changes, including UV rays, temperature swings, food deprivation, desiccation, osmotic stress, and the impact of antibiotic compounds generated by other microbes. Bacillus has a number of defense mechanisms that allow it to live in these normal soil-related environmental variations. Under conditions of hunger, this organism sporulates, producing spores that are resistant to numerous environmental challenges and are metabolically inactive (Piggot and Hilbert 2004). These bacteria can be isolated and grown from cotton gin waste as reported by ZoBell (2005). They live in the soil of cotton fields and feed on the carbs and proteins found in the cottonseed waste.

As a result, comparative research utilizing bacterial *Eps* and traditional chemical coagulants was conducted to assess the effectiveness of RB5 dye wastewater treatment. The chemical coagulants that were employed were alum, PaFC, and PFS. Because RB5 is one of the most frequently used dyes for dying cotton and other cellulosic fibres and accounts for at least 30% of the world market for textile dyes, it was chosen as a model dye (Sengil and Ozacar, 2009; Zobaidul *et al*., 2019; Hayman *et al*., 2023). According to Iscen Kiran and Ilhan (2007), the dye is an azo dye that is not biodegradable and has a high level

of resistance to light and chemicals. According to Nur *et al*. (2007), dye colour stability has been achieved through the use of heavy metals such as Cr and Co. According to Sarkar *et al*. (2017), the dye has an azo linkage (-N=N-) near the aromatic rings, which results in strong resistance to oxidizing agents and photocatalytic stability. Its chemical characteristics are as follows: With a C.I. index of 20505, a molecular formula of C26H21N5Na4O19S6, a molecular structure as depicted in Figure 2.11, and a λmax (nm) of 597.

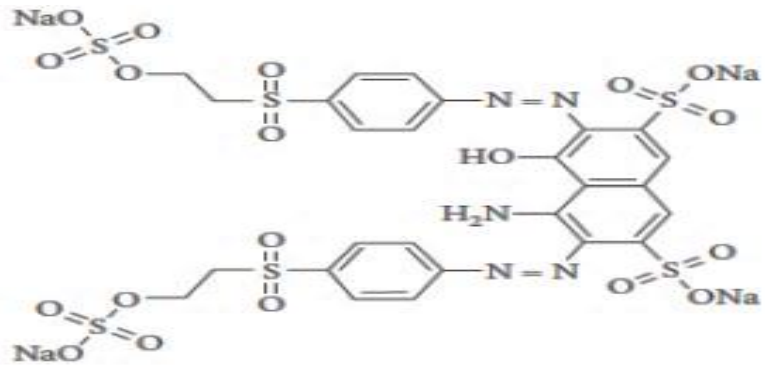

Fig. 2.11: Reactive Black 5 Molecular Structure (Colindres, *et al*., 2010)

Therefore, the purpose of this study was to assess the effectiveness of chemical coagulants versus bacterial *Eps* bioremediation in treating RB5 wastewater. Cotton gin trash soil samples were gathered from specific cotton-growing districts in Kenya, and bacteria that produce ethylene were identified. For comparison, the bacteria were cultured and separated on a variety of solid media. RB5 textile dye wastewater was bioremediated utilizing *Eps* that were optimized for their activity using a media reconstituted with sugarcane molasses and soya, which served as replacements for synthetic carbon and protein sources, respectively. This intervention has the potential to provide a sustainable substitute for

synthetic *Eps* production media and wastewater coagulants, while also generating value from the vast amounts of gin garbage produced along the cotton value chain.

2.7 *EPS* Extraction

The surrounding growing medium is where *Eps* are secreted, and they are readily extracted from cell-free culture supernatant. In order to extract *Eps*, cell-free supernatant must be collected, protein must be denaturized using trichloroacetic acid, followed by ethanol precipitation, dialysis, and drying (Banerjee *et al*., 2022). Polysaccharides can be isolated and purified in a variety of ways while maintaining their inherent characteristics. Thirty to fifty percent of the total cost of producing microbial flocculants is attributed to the extraction and purification of *Eps*. Therefore, investigating effective *Eps* extraction and purification techniques can lower production costs (Liu *et al*., 2021). Various extraction methods are employed for microbial flocculant *Eps*, depending on the required purity. These methods include centrifugation, filtration, dialysis, alkaline extraction, gel adsorption, metals complexation using EDTA or crown ether, ultrasonication, and organic reagent (solvent) precipitation (Sheng, Yu, and Li, 2010; Sivasankar *et al*. 2020; Tang *et al*. 2014). The most common method is organic reagent precipitation (Chen *et al*. 2017; Liu *et al*, 2020). By removing the soluble components of the growth media, solvent precipitation also helps to partially purify the polymer (Razack, Velayutham and Thangavelu, 2013; Tereza *et al*., 2015). Since capsular polysaccharides are tightly bound to the cell membrane, they are primarily recovered from the cells (Casillo *et al*., 2018). Prior to use, the extracted solid microbial flocculant must be dissolved in solution to maximize the likelihood of contact with the suspended solid particles (Liu *et al*. 2015). The process for extracting and purifying *Eps* is shown in Figure 2.12.

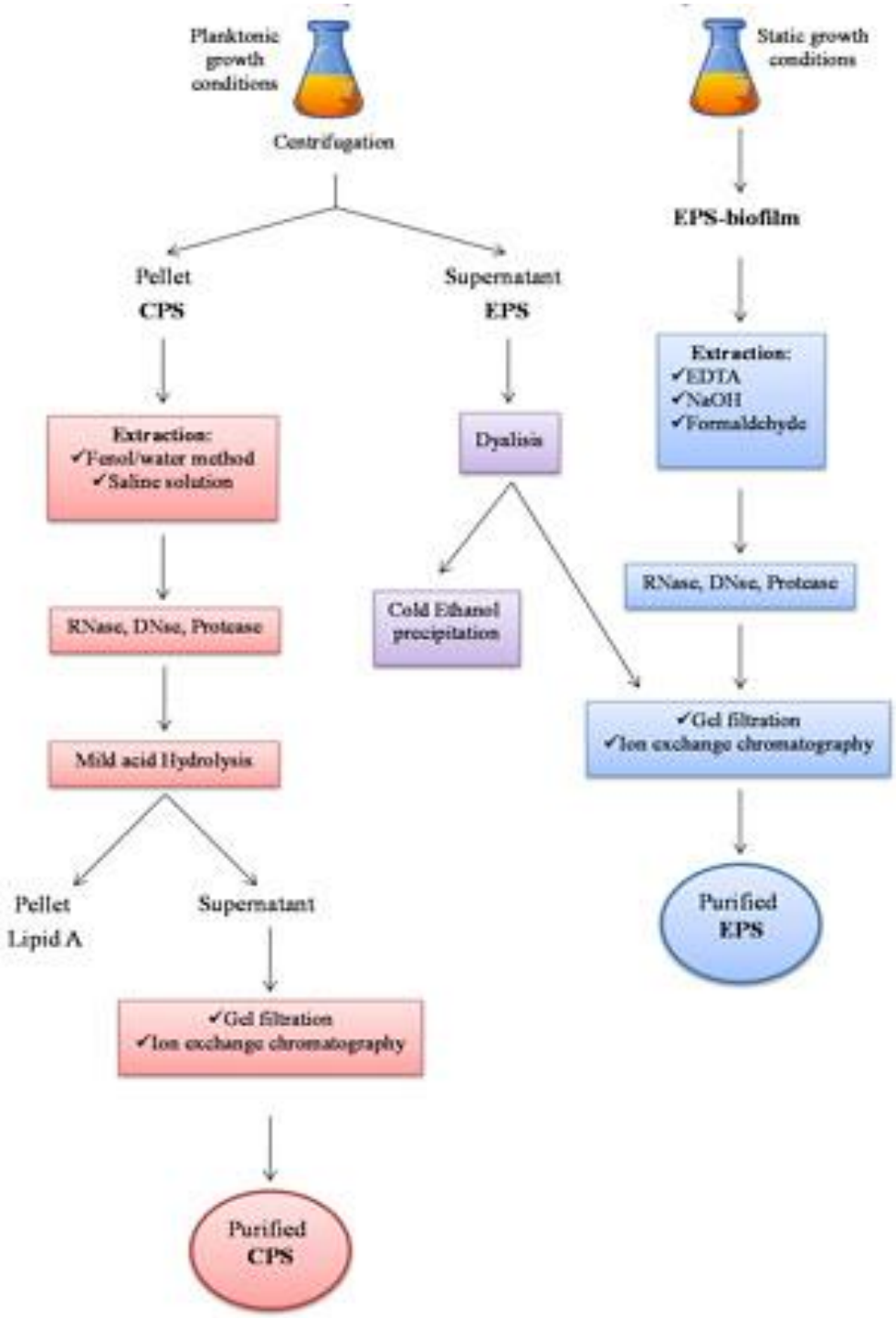

Fig. 2.12: Schematic illustration of extraction and purification of exopolysaccharides from microorganism (Casillo et al., 2018).

CHAPTER THREE

METHODOLOGY

3.1 Introduction

This section presents procedures for sampling, collection and physicochemical characterization of the materials used in this research. Optimization for preliminary treatment of Reactive black dye wastewater using commonly used chemical coagulants method is given. Methodologies for screening of *Eps* producing bacteria on assorted solid and liquid media are presented. Procedures for screening of the bacteria with *Eps* production capacity in the Reactive black dye wastewater presence using different liquid broth media and subsequent reconstitution of the most appropriate media using locally available and affordable materials; molasses and soya have been outlined. Optimization design of bioremediation process conditions; temperature, incubation time and molasses concentration are also elucidated. Physicochemical characterization of the untreated wastewater, chemically and *Eps* treated wastewater procedures are presented. Procedures for extraction and characterization of *Eps* with optimal bioremediation capacity for the dye wastewater is outlined. Characterization methods for the candidate bacteria have also been presented.

3.2 Materials

3.2.1 Collection and Characterization of Gin Trash Soil

Gin trash soil samples were collected from Kitui, Baringo (Salawa) and Meru ginneries in Kenya. Soil from the first seed cotton cleaner machine was mixed well and collected into sterile plastic jars using sterile hand gloves and labelled appropriately. Coning and quarter sampling method was used in accordance with Campos and Campos (2017). All the

samples were transported to the Industrial Microbiology and Biotechnology Research Centre laboratories, KIRDI head office, Nairobi, in a sterile cool box and immediately refrigerated at 4^{o}C to await analysis. Microbial and physicochemical characterization of the gin trash soil samples then commenced immediately.

To extract fine debris, the soil samples were individually sieved using a 25-mesh screen. After being separated from the fine trash through sieving, the organic matter content—which included broken leaves, stalks, burrs, and tangled cotton fibers—was pulverized in a Wiley mill and subsequently sieved into the fine gin trash through the same sieve. The majority of soil biological activity's substrate, carbon content, and nutrient availability are all reflected in organic matter (Bunning and Jimenez, 2003). pH and moisture were measured using the procedures outlined in ISO 10390:2005 and ASTM D 2974:2000, respectively. The amounts of proteins, lipids, carbs (sugars), carbon, and nitrogen were measured in accordance with the Soil Testing Procedure Manual (2008). The tenfold dilution plate method was used to analyse the microbial burden (Inés, Maite, and Barcina, 2013).

3.2.2 Collection and Characterization of Textile Dye Wastewater

A sample of textile dye wastewater was obtained from Rivatex East Africa Limited at the drainage site of a jig dying machine. The American Public Health Association's standard sampling criteria and principles were followed during the sampling process (APHA, 1998). Prior to sampling, the sterile collecting containers were cleaned and rinsed twice using distilled water and the wastewater that was going to be collected. Reactive Super Black R, 4%, sodium sulphate, 80 g/L, sodium carbonate, 30 g/L, and acetic acid, 1 g/L were the

ingredients used to make the dye bath. The sample was promptly shipped in a cooler box to the Industrial Microbiology and Biotechnology Research Centre laboratories at the KIRDI head office in Nairobi, where it was kept cold at 4 ^{O}C until additional analysis could be performed. Using a BIOBASE precision pH/Ion meter, the temperature, pH, EC, salinity, and TDS of the wastewater were measured. The Turbidimeter (ums-UK-Water Quality Analyzer) was used to measure turbidity. The COD-571 Analyzer was used to analyze COD. Using OxiTop-IDS bottles, the sample was incubated at 21°C for five days in order to measure BOD. The UV-vis spectrophotometer (UV-1900 Shimadzu) at 597 nm and the Lovibond-Day Light Comparator 2000 Unit Equipment were used to analyze the colour concentrations. According to ISO 8288:1986, elemental analysis for metal ions (Cu, Pb, Cd, and Cr) was performed using an atomic absorption spectrometer (Analytik Jena, model Nov800).

3.3 Methods

3.3.1 Treatment of Textile Dye Wastewater using Different Chemical Coagulants

The dye wastewater was chemically coagulated using alum, PFS, PaFC, and other inorganic coagulants so that the results could be compared to those expected from bioremediation employing bacterial *Eps* research. According to the guidelines provided by Yildiz *et al*. (2014), Prakash, Vimala, and Jayakaran (2014), and ASTM D2035 (2013) standard, coagulation-flocculation tests were carried out utilizing the jar test protocol. Utilized were commercial-grade coagulants (Alum, PaFC, and PFS) having the following technical specifications. Alum ($Al_2 (SO_4)_3.4 H_2O$); brown liquid PaFC (141g/L, as Fe, pH is around 0.5), Fe_2O_3 content 1.80-2.20 wt%, Al_2O_3 content 4.20-5.30 wt%, density (25°C) 1.20-1.30. As received, the coagulants were employed.

Three sets of 500 mL of dye wastewater per coagulant, each containing nine beakers, were treated to sixty (60) mg/L of alum and twenty mg/L of PaFC and PFS individually. The pH of each coagulant set was adjusted using a solution of 1N HCl and 40% NaOH, ranging from 2 to 10. Each experiment was carried out in triplicate. The identical procedure was used to prepare control samples, but no pH modification was done. The contents of each beaker were rapidly mixed for two minutes at 200 rpm, then slowly mixed for twenty minutes at 30 rpm, and finally allowed to settle for an hour (Chen, Gao, and Yue, 2010). After that, samples of the supernatant were taken out for study of color removal and physicochemical properties to find the optimal pH for each coagulant.

Percentage of color removal was calculated using equation (1) as adopted from Satheesh, Kumar and Jayakumar (2017).

$$\%\ \text{color removal} = \frac{A_{O-}A_f}{A_o} \text{x}100 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (1)$$

Where A_o and A_f are the initial and final absorbance of the wastewater, respectively (Rafiqul and Mostafa, 2020)

Each coagulant treatment's sludge was dried in the beakers for an entire night at 105°C in the oven, resulting in constant weights and the corresponding % weight differences being computed. The process was repeated while varying the dosages of the coagulants at the corresponding pH that was found to be the most effective based on the physicochemical parameters and the findings of the color removal analysis. Supernatant samples were taken out for study of color removal and physicochemical properties to find the best dosage for each coagulant. Additionally, the most efficient pH and coagulant dosage were found for

the clarified samples in order to calculate COD and turbidity. The outcomes of every treatment set were contrasted with the results of the untreated group.

3.3.2 Isolation of Bacteria with *Eps* Production Capacity

After carefully combining freshly sieved soil from all three sources, one gram of each sample was weighed, put into nine milliliters of sterile normal saline, and vortexed for a minute (Sheetal and Arpita, 2016). The soil suspension was then vortexed once more after being serially diluted up to 10-6 by progressively transferring 1 milliliter of each soil suspension into 9 milliliters of sterile normal saline. Within a sterile biosafety cabinet, the samples were incubated for 48 hours at room temperature.

Nine culture mediums were utilized: Malt Extract Agar (MEA), Yeast Extract + Peptone + Dextrose (YEPD), Peptone + Dextrose Agar (PDA), Tryptic Soy Agar (TSA), Yeast Extract Agar (YEA), Yeast Extract + Glucose (YEG), Nutrient Agar (NA), Nutrient Agar + Glucose (NA+G), and Sabaroud Dextrose Agar (SDA). Working beneath the laminar airflow chamber, each medium was prepared according to the instructions on its container, let to cool, and then dispensed into 90-mm-diameter polystyrene sterile plastic petri dishes.

A sterile glass spreading rod was used to evenly distribute 100 microliters (100 μL) of aliquots from each of the dilutions in triplicate on petri dishes that contained the nine distinct media. The plates were then incubated at 37°C. To obtain pure isolates, the cultures were further sub cultured using the appropriate media and ice-cleaned at the same temperatures. After a visual inspection, colonies were chosen for *Eps* production screening based on their glistening, opaque, mucoid, slimy appearance (Pawar *et al*., 2013; Hereher *et al*., 2018).

3.3.3 Screening for *Eps*-Producers Broth

Three different broth media were used to further cultivate two loopfuls of the corresponding pure, glistening, opaque, mucoid, watery, or slimy bacterial colonies that had been screened. Twenty grams of sucrose (NB+S) and twenty grams of glucose (NB+G) were combined with nutrient broth (13 g/L) and the following: five grams of glucose, 2.5 grams of peptone, 4.5 grams of yeast extract, and 4.5 grams of malt extract ($GPYMgSO_4$). The methods used for *Eps* growth and screening were taken from Tereza *et al*. (2015) and Hereher *et al*. (2018). After being inoculated into 20 milliliters of each broth, the pure cultures were cultured for 72 hours at 30 ^oC using an orbital shaker (INCU-Line ILS6) running at 150 rpm. Since 7-8 pH has been shown to be the most suitable for producing *Eps*, it was kept there (Hereher *et al*., 2018; Razack, Velayutham and Thangavelu, 2013). Following incubation, the samples were centrifuged for 30 minutes at 6,000 rpm using a benchtop centrifuge (Biobase-TGL16), and the corresponding cell-free supernatants were examined using a UV-Vis spectrophotometer set at 600 nm for the detection of *EPS* synthesis. In the same way, control samples of the corresponding broths that had not been infected were made. The isolates with the highest *Eps* production were chosen. Utilizing equation (2), the percentage increase in *Eps* was computed.

$$\% \; eps \text{ production} = \frac{A_{i-A_c}}{A_i} \text{x}100 \; \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where A_i and A_c represent the Optical Density (OD) of the inoculated and control samples, respectively. Samples that gave *Eps* production of at least 90% were selected for further screening in the presence of Reactive Black 5 dye wastewater.

3.3.4 Screening for *Eps* Activity on RB5 Dye Wastewater

It was done to screen for bacteria isolates whose *Eps* could extract color from the wastewater. The isolates that were determined to be high *Eps* yielders were each inoculated into 13 g/L of Nutrient Broth (NB), which had been enriched with 20 g/L of sucrose, 13 g/L of glucose, and 5 g/L of glucose, 2.5 g/L of peptone, 4.5 g/L of yeast extract, and 4.5 g/L of malt extract ($GPYMgSO_4$). The mixture was then incubated at 30 ^oC for 72 hours while being shaken orbitally at 150 rpm. As adapted from Vivek *et al*. (2016), Lei (2016), and Ibarra-Molero and Sanchez-Ruiz (2018), each sample was incubated for 24 hours at 4 ^oC, after which it was mixed with 5 mL of 10% (v/v) trichloroacetic acid (TCA) and allowed to stand for 2 hours at 4 ^oC for protein purification (enzyme denaturing). Centrifugation was then used to acquire the appropriate cell and protein-free supernatants for 30 minutes at 6,000 rpm (Mu'minah and Hazarin, 2015; Indira *et al*., 2016).

Each of the three distinct broths' eight milliliters (8 mL) of cell and protein-free supernatant was dosed in duplicate, each but separately, with one milliliter of sterile dye (machine and four percent of the dye—synthetic) wastewater diluted in a 1:500 ratio. After that, all of the samples were incubated for 72 hours at 30 0C with an orbital shaker running at 150 rpm to keep the pH at 7-8. The samples' respective optical densities were then measured at 597 nm using a UV-Vis spectrophotometer to evaluate the dye removal. In the same way, control samples of the corresponding broths that had not been inoculated were dosed using the machine and synthetic wastewater. A reconstituted medium was used to do additional research on bacterial isolates whose *Eps* demonstrated a dye removing capacity more than 94%. Growth in *Eps* was calculated as a percentage using equation (2).

$$\% \text{ color removal} = \frac{A_{c-}A_i}{A_c} x100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

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

3.3.5 Screening for *Eps*-Producers in RB5 Dye Wastewater Using Reconstituted Broth

Using readily available raw materials, molasses and soy flour from the area, along with yeast extract and magnesium sulfate salt, $GPYMgSO_4$ was discovered to promote high production of *Eps* with the ability to remove the color (Arafa *et al*., 2014). A Mettler Toledo Digital Refractometer, model number NR-151, was used to analyze the total sugar content of the molasses.

Molasses (10 g/L), Soya (5 g/L), Yeast Extract (3 g/L), and Magnesium Sulphate (0.2 g/L) were used to screen each of the chosen isolates further for their ability to create *Eps* with the capacity to remove the dye ($MSYMgSO_4$). The broth was used to individually inoculate each isolate in duplicate, and the samples were then cultured for 72 hours at 30 ^oC with an orbital shaker running at 150 rpm. Following the incubation period, each sample was chilled for 24 hours at 4 ^oC. It was then combined with 5 milliliters of 10% (v/v) trichloroacetic acid (TCA) and allowed to stand at 4 ^oC for two hours in order to separate the proteins (enzyme denaturing) and salts. After that, the corresponding cell and protein-free supernatants were recovered by centrifuging for 30 minutes at 6,000 rpm. Each isolate's 8 mL of cell and protein-free supernatant was thereafter dosed separately with 1 mL of sterilized, diluted (500 times) machine wastewater. After that, all of the samples were incubated at 30 ^oC for 72 hours while being kept at a pH of 7 to 8 using an orbital shaker at 150 rpm. The samples' respective optical densities were measured at 597 nm

using a UV-vis spectrophotometer. Equation (2) was used in order to get the percentage dye removal.

For optimization, the isolates with a high dye removal capacity (at least 60%) were chosen.

3.3.6 Optimization of RB5 Dye Removal from Textile Dye Wastewater

Dye removal by the screened bacterial *Eps* was optimized using the reconstituted MSYMgSO$_4$. Full Factorial design of 3 factors (molasses concentrations, temperature and time) at 3 levels ((K^N) each was used to optimize the dye wastewater treatment. The optimization experiments values and their combination levels were designed by MatLab 17 software. Molasses was varied from 10 g/L, 15 g/L and 20 g/L. Amounts for yeast extract and magnesium sulphate were retained as previously used during the media screening for the *Eps* with the dye removal capacity. Nutrient Broth + Glucose and GPYMgSO$_4$ were also simultaneously studied for comparison purposes. The procedure used to screen for *Eps* with dye removal capacity in 3.2.4 and 3.2.5 was adopted. Temperature was set at 30 ^oC, 34 ^oC and 38 ^oC being those likely to be replicated in industrial processes. Incubation period ranged from 24 h, 48 h and 72 h. All the samples were prepared in triplicates and incubated under a shaker at 150 rpm. pH was maintained between optimal 7 and 8 (Simphiwe, Ademola and Balakrishna, 2012; Asha *et al*., 2015; Ben Rebah, Mnif and Siddeeg, 2018; Arafa *et al*., 2014). Bacteria isolates whose *Eps* were found to give at least 80% dye removal efficiency as per Equation (2) were selected for further characterization. Tables 3.1 and 3.2 show the experimental design and corresponding values respectively

Table 3.1: 3^3 Full Factorial Experiment Design

Experiment	Temperature (^oC)	Time (Hours)	Molasses Concentration (g/L) in ($MSYMgSO_4$) broth
1	-1	-1	-1
2	0	-1	-1
3	+1	-1	-1
4	-1	0	-1
5	0	0	-1
6	+1	0	-1
7	-1	+1	-1
8	0	+1	-1
9	+1	+1	-1
10	-1	-1	0
11	0	-1	0
12	+1	-1	0
13	-1	0	0
14	0	0	0
15	+1	0	0
16	-1	+1	0
17	0	+1	0
18	+1	+1	0
19	-1	-1	+1
20	0	-1	+1
21	+1	-1	+1
22	-1	0	+1
23	0	0	+1
24	+1	0	+1
25	-1	+1	+1
26	0	+1	+1
27	+1	+1	+1

Table 3.2: Design experiment values

Experiment	Temperature (^oC)	Time (Hours)	Molasses Concentration (g/L) in ($MSYMgSO_4$) 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

Figure 3.1 gives a summary of the sequential steps for the *Eps*-producing bacteria isolation, *Eps* extraction, screening suitability of different media in *Eps* production and their activity on the dye wastewater.

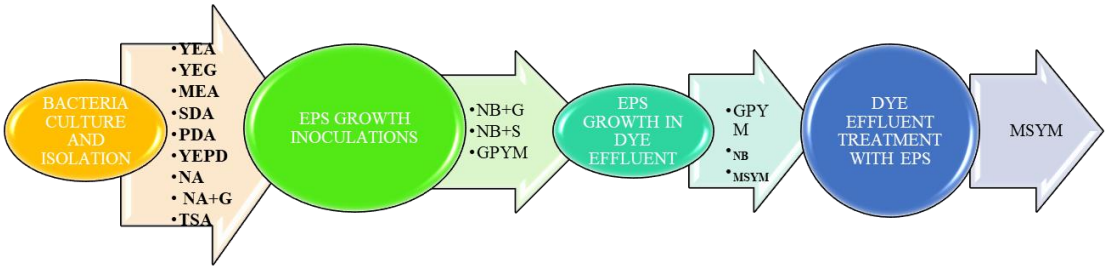

Fig. 3.1: Sequential steps for *Eps* isolation and dye wastewater treatment

3.3.7 Characterization of *Eps* Treated Dye Wastewater

The dye wastewater was further treated by inoculating it with 50 mL of the *Eps* solutions found to have optimal dye removal capacity cultured from broth constituted with 20g/L molasses ($MSYMgSO_4$) and at 30 ^oC for 72 hours while maintaining the pH at 7 to 8. Physicochemical analysis of the optimized *Eps*-treated dye wastewater was done to determine pH, colour, EC, TDS, salinity, COD, Pb, Cd, Cu and Cr using the same procedures used during characterization of the chemically treated wastewater. The results were compared with those of the chemically treated dye wastewater. The optimized *Eps*-treated dye wastewater was also analyzed with FTIR and UV-Vis Spectrometer.

3.3.8 Characterization of *Eps* Producer-Bacteria Isolates

Bacteria isolates whose *Eps* were found to have optimal bio remediation capacity for the RB5 dye wastewater were characterized through bio chemical and molecular techniques.

3.3.8.1 Biochemical characterization

The standard Gram staining procedure was followed for the morphological characterization of the isolates (Bartholomew and Mittwer, 1952). Biochemical tests were done for taxonomic identification of their metabolic activities according to standard protocols (Cappuccino and Sherman, 2005).

3.3.8.2 Molecular Characterization

Molecular characterization of the bacterial isolates was performed at Inqaba Biotechnical Industries (Pretoria, South Africa). The bacteria isolates were subjected to molecular characterization by16S rRNA sequencing. The DNA of the isolates was extracted using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005). The 16S target region was amplified as presented in Table 3.3.

Table 3.3: 16S Primers sequences

Name of Primer	Target	Sequence (5' to 3')
16S-27F	16S rDNA sequence	AGAGTTTGATCMTGGCTCAG
16S-1492R	16S rDNA sequence	CGGTTACCTTGTTACGACTT

Confirmation of DNA was done using agarose gel electrophoresis on 1% agarose gels (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye. The NEB Fast Ladder was used on all gels (N3238) as size standard. Fragment of 16S rRNA gene was amplified by 27F (5-GAGTTTGATCCTGGCTCA-3) and 1492 (R-TACG GYTACCTTGTTACGACTT) universal primers. PCR reaction was performed at 94 ^{o}C for 2 min, 35 amplification cycles at 94 ^{o}C for 30 s, 50 ^{o}C for 30 s, 68 ^{o}C for 60 s, and a final extension at 68 ^{o}C for 10 min. Fragments were enzymatically purified using the ExoSAP procedure (NEB M0293L; NEB M0371). Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. FinchTV (https://finchtv.software.informer.com/1.4/) was used to view the raw chromatogram files (.abi). CLC Bio Main Workbench was used to assemble the forward and reverse

sequencing reads to form a consensus sequence for each sample. BLASTn analysis (with default parameters) was performed on the NCBI website (https://blast.ncbi.nlm.nih.gov/blast.cgi) to determine if a sequence in the database matches the query sequence above a certain threshold (99% query coverage; 99% identity).

3.3.9 Extraction of *Eps*

Bacteria isolates whose *Eps* were found to give optimal dye removal activity, after the optimization experiments, were cultured using the MSYMgSO_4 broth as previously constituted. Soya, Yeast Extract and Magnesium sulphate salt (SYM) was also inoculated and studied as a control for the role of molasses in *Eps* production. Measures of 250mL of the MSYMgSO_4 broth were inoculated with three loopfuls of each of the bacteria isolates and then incubated for 72 hours at 150 rpm at 30 ^{o}C. The samples were then precooled at 4 ^{o}C for 24 hours before being individually mixed with 5 mL of 10% (v/v) trichloroacetic acid (TCA), to avoid *Eps* destruction by TCA. The samples were further refrigerated for 4 h at 4 ^{o}C then centrifuged at 6,000 rpm for 30 min to obtain bacteria cell and protein-free supernatant of *Eps* as adapted from Vivek *et al*. (2016), Lei (2016) and Mu'minah and Hazarin (2015). Three volumes of freeze-cold absolute ethanol were then added to one volume of each of the supernatants, and the mixtures left to stand for at least 12 h at 4 ^{o}C to precipitate the *Eps*. The samples were then centrifuged at 3,000 rpm for 30 min. The respective pellets obtained were then resuspended in distilled water then centrifuged at 3,000 rpm for 30 min. The pellets were then lyophilized to obtain dry *Eps* extracts and respective net weight obtained.

3.3.10 Characterization of Extracted *Eps*

3.3.10.1 Characterization of *Eps* by GC-MS.

GC-MS characterization as done at the College of Pure and Applied Sciences (COPAS) in Jomo Kenyatta University of Agriculture and Technology (JKUAT). The derivatization procedure used was adapted from Sohaib (2015). About 2 mg of purified *Eps* was hydrolyzed with 2 mL of 2 M Trifluoroaceticacid (TFA) at 120^{o}C for 2 h. The hydrolysates were then reduced with potassium borohydride (KBH_4) dissolved in ammonium hydroxide (NH_4OH) and were subjected to N-acetylation using acetic anhydride $(CH_3CO)_2O$. The derivative products were used for determination of the monosaccharide composition by Gas Chromatography. Sample of 0.5 μL was injected into the GC-MS (SHIMADZU) equipped with QP2010SE autosampler and column BPX5(30 m length x 0.25 μm inner diameter x 0.25 mm thickness). The samples were injected in split mode (1:10) at 200 ^{o}C. Helium was used as the carrier gas with 1 mL/min flow rate. The chromatographic conditions used were as follows: the initial column temperature was held at 60 ^{o}C for 1.5 min. A temperature program was used to bring about separation of compounds at that temperature. The temperature was then increased at a rate of 10 ^{o}C/min to 200 ^{o}C and held for 1 min and then subsequently increased at 5 ^{o}C/min to 280 ^{o}C, where it was held at for 8 min. The interface temperature was set at 250 ^{o}C, iron source temperature set at 200 ^{o}C and the solvent cut time set as 4.5 min. The mass spectrometer was run in SCAN mode for masses starting from 35 Hz to 550 Hz. A NIST library was used to predict the name of the scanned compounds based on the mass to charge ratios of the compounds. The chromatographs obtained from the GC were subsequently analyzed and compared with the MS library for identification.

3.3.10.2 Characterization of *Eps* by FTIR

The purified *Eps* samples were further subjected to FTIR analysis to study the functional groups therein. Two milligrams (2 mg) of each sample were blended with 200 mg potassium bromide and compressed into a disc to obtain translucent pellets for FTIR analysis. The background reference compound used was the pelleted form of potassium bromide. Infrared absorption spectra were recorded within wavelengths ranging from 400 to 4000 cm^{-1} with a resolution 4 cm^{-1} and using 32 scans under ambient conditions using Fourier transform infrared (FTIR) spectrophotometer (Bruker Optics GmbH, Germany).

3.3.10.3 Characterization of *Eps* by SEM

SEM characterization of the *Eps* extracts was done at the Food Fortification Lab in Jomo Kenyatta University of Agriculture and Technology (JKUAT). Microstructure and surface morphology of purified dry *Eps* samples were studied using Benchtop Scanning Electron Microscope (JEOL, JCM 7,000) at an accelerating voltage of 15 keV. The *Eps* was mounted on the metal stub (32 mm diameter) and fixed with a carbon tape. Respective *Eps* micrographs were recorded at different magnifications that gave the clear images.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results and related discussion on the gin trash soils and dye wastewater physicochemical characteristics. Comparative results on optimization of the chemical coagulation of the dye wastewater are presented and discussed. Results and discussion on screening of bacteria with *Eps* production capacity using different solid and liquid media are also presented. Findings and discussion on screening of *Eps* production in the dye wastewater using a substitute of the conventional media with one reconstituted with affordable and locally available raw materials (molasses and soya flour) are presented. Results on optimization of the dye wastewater bioremediation at varied temperature, incubation and molasses concentration, subsequent extraction of the optimal *Eps* with the reconstituted broth is as highlighted. Results and discussion on the characterization of the untreated, chemical and *Eps*-treated wastewater have been presented. Findings on characterization of the bacteria that produced the *Eps* with optimal activity on the dye wastewater are also given.

4.2 Gin Trash Soil and Dye Wastewater Characterization

Tables 4.1 shows the respective physicochemical and microbial characteristics of gin trash soil samples collected from Meru, Kitui and Baringo ginneries. No major variations were obtained across the three different gin trash soils collected from the three different regions. The pH of the three samples was within the neutral range (6-7). Baringo sample was contained slightly higher moisture content compared to the others. Kitui sample had the highest carbohydrates and volatile matter compared while Meru sample had the least. Kitui

sample had the highest bacteria population while Meru sample had the least. Figure 4.1 and 4.2 shows comparative illustrations of the physicochemical characteristics and microbial populations for the three gin trash soils.

Table 4.1: Physicochemical and microbial characteristics of the gin trash soil samples.

Test Parameter	**Meru Soil**	**Kitui Soil**	**Baringo Soil**
pH	6.58	6.68	7.02
Temperature (^oC)	25.0	25.0	25.0
Moisture Content (%)	4.570	6.292	7.332
Proteins (%)	7.556	7.103	6.851
Carbohydrates (%)	3.26	8.713	5.426
Fats (%)	2.507	2.332	1.869
Fibre Content (%)	11.25	7.45	4.870
Ash Content (%)	70.86	68.11	56.312
Volatile/organic matter content (%)	23.39	27.23	25.765
Bacteria population (cfu/g of dry soil)	$36.00x10^5$	$60.6x10^5$	$55.2x10^5$
Fungi population (cfu/g) of dry soil	$14.12x10^5$	$15.6x10^5$	$15.7x10^5$
Actinomycetes population (cfu/g of dry soil)	$31.70x10^5$	$16.7x10^5$	$21.9x10^5$

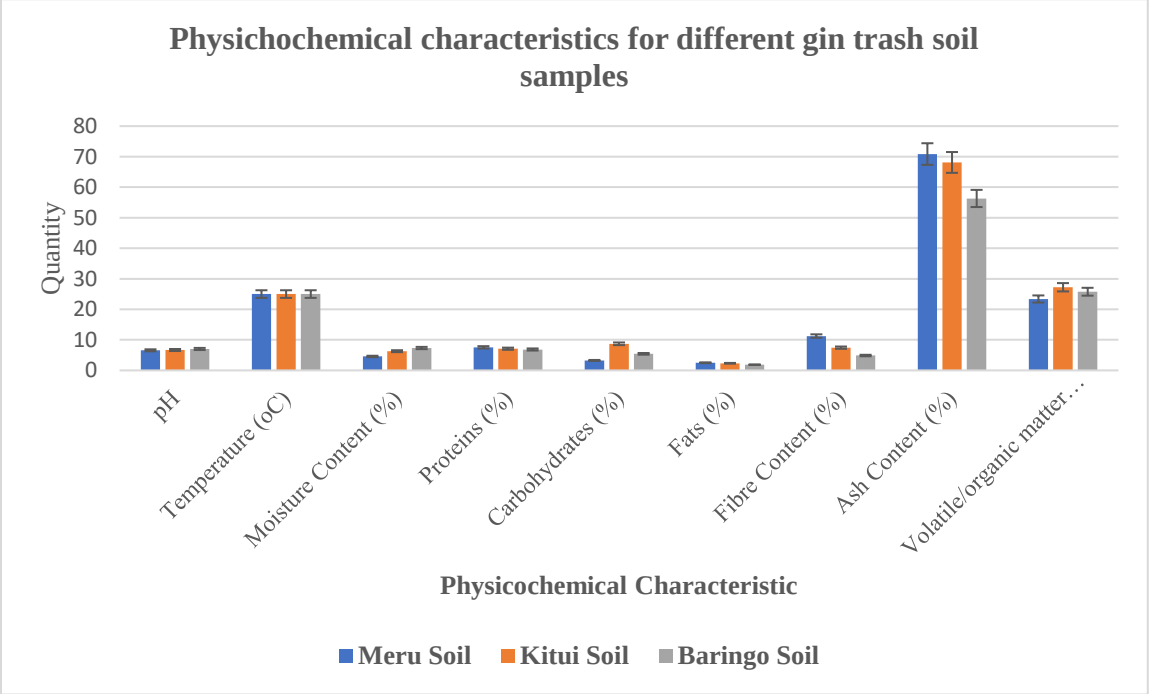

Fig. 4.1: Physicochemical characteristics for different gin trash soil samples

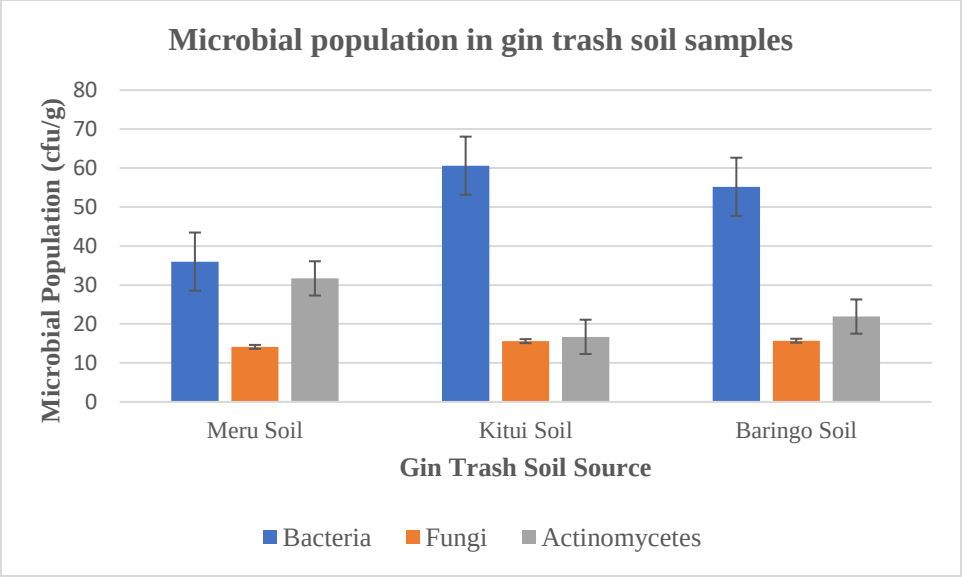

Fig. 4.2: Microbial population in gin trash soil samples collected from different regions in Kenya

Table 4.2 (a) shows physicochemical properties of the textile dye wastewater sample in comparison to the water quality regulations set by National Environment Management Authority (NEMA) (Environmental Management and Co-Ordination (Water Quality) Regulations, 2006).

Table 4.2 (a) Physicochemical properties of the dye wastewater in comparison to NEMA limits

Parameter	**Value**	**NEMA Limits**
pH	10.23	6-9
Colour concentration (Hazen)	>250	≤40
Total dissolved solids (TDS) (mg/L)	81.575	2,000
Total Suspended Solids (mg/L)	8.135	250
COD (mg/L)	1486	1,000
BOD (mg/L)	350	500
Pb(mg/L)	nd	1.0
Cd(mg/L)	nd	0.5
Cu(mg/L)	0.0234	1.0
Cr (Total)(mg/L)	0.1	2.0

ndNot detected

Pb and Cd were not detected in the wastewater. Colour and COD were above the set limits while Cu and Cr were below the limit.

Table II and III (Appendices) show respective monitoring guide for industries, discharging into the environment and the standards for discharge into public sewers as extracted from the Water Quality Regulations.

4.3 Treatment of Textile Dye Wastewater Using Chemical Coagulants

4.3.1 Chemical Coagulation Treatments

4.3.1.1 Effect of pH on Physicochemical Characteristics of Treated Wastewater.

4.3.1.1.1 Total Dissolved Solids

The untreated sample's TDS was 81.58 ppm. While all the coagulants showed minimal changes in TDS with variation of pH, PaFC was found to be the best in reduction of TDS while alum and PFS gave relatively similar results as shown in Figure 4.3.1 (a). This could be attributed to charge neutralization and bridging abilities of PaFC compared with other iron-based inorganic coagulants (Gao *et al*., 2008). The respective coefficient of determination (R^2) obtained from lines of best fit of regression analysis for each of the coagulants gave a moderate linear correlation between the respective coagulants and pH as illustrated in Figure 4.3.1 (b, c & d). Respective R^2 for alum, PFS and PaFC was 55.3%, 53.2% and 50.5%. This implies that TDS removal is moderately affected by pH for all the three coagulants.

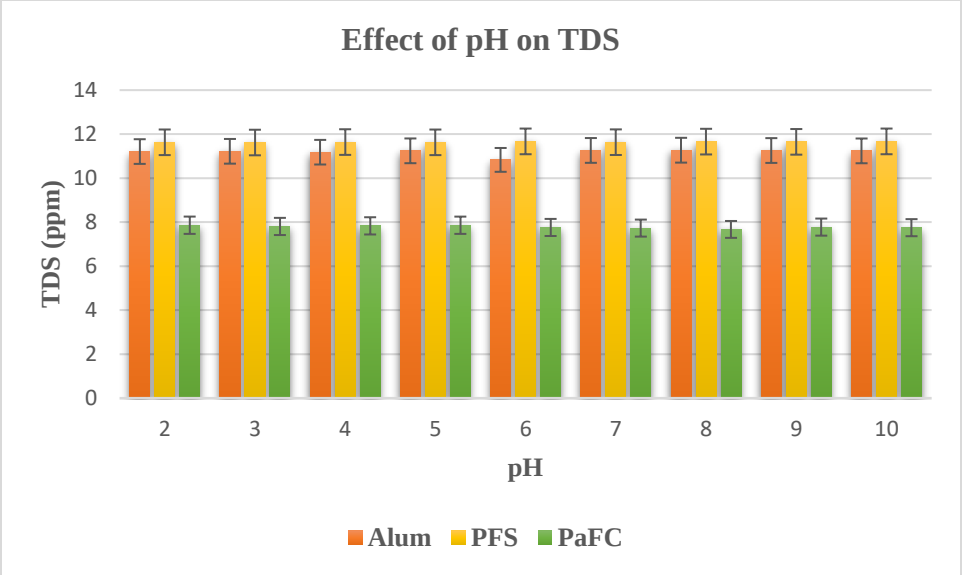

Fig. 4.3.1 (a): Effect of pH on TDS

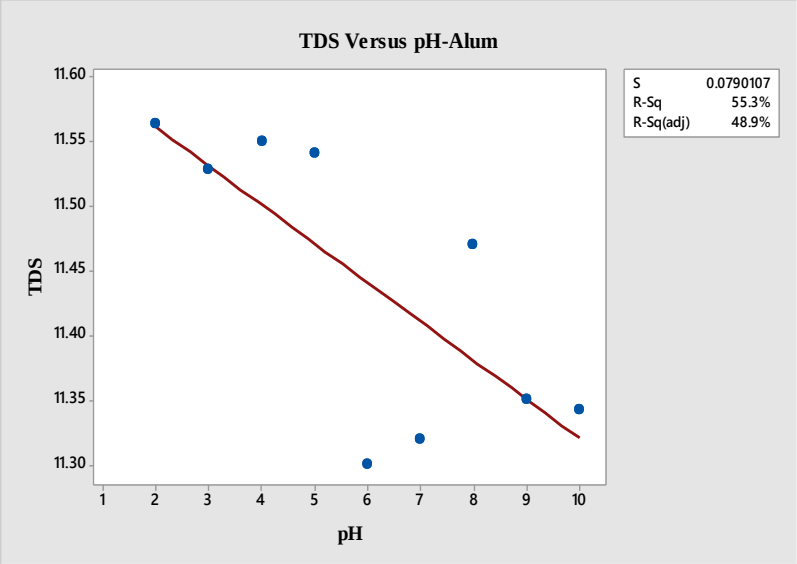

Fig. 4.3.1 (b): Correlation between TDS and pH for Alum

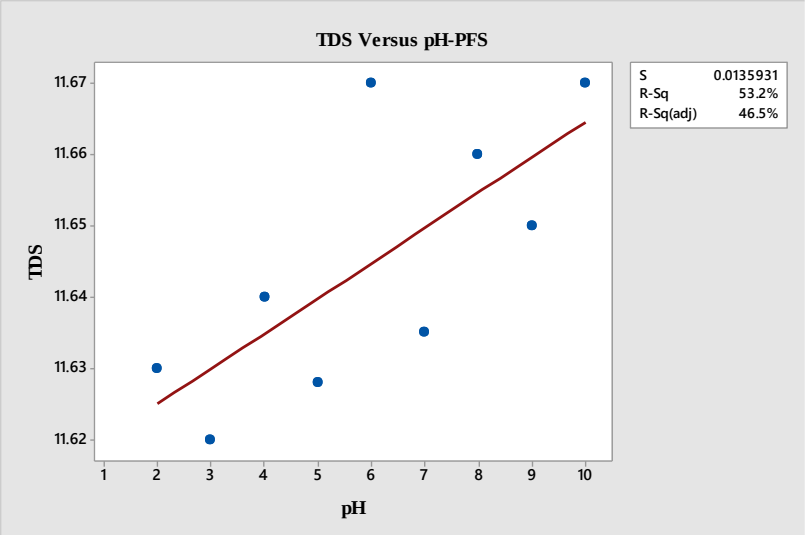

Fig. 4.3.1 (c): Correlation between TDS and pH for PFS

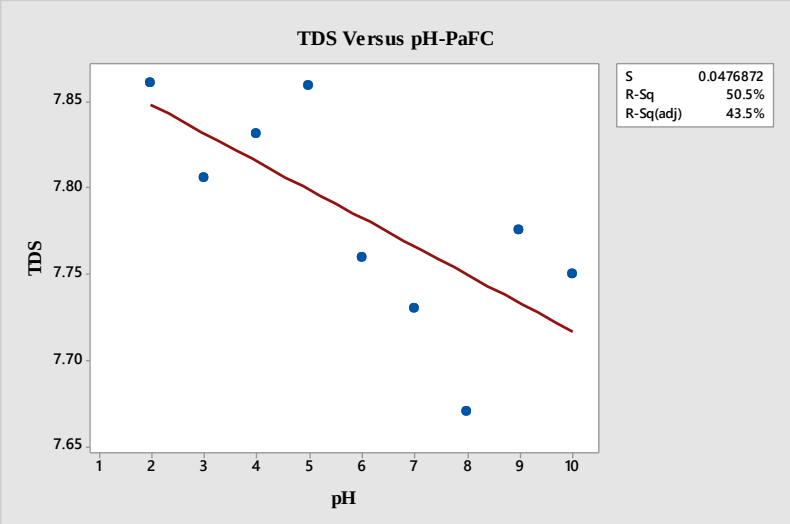

Fig. 4.3.1 (d): Correlation between TDS and pH for PaFC

4.3.1.1.2 Electrical Conductivity (EC)

The EC of the untreated sample was 17.43mS/cm. PaFC gave the lowest values of EC while alum and PFS had similar results across the pH range. PFS and alum gave values that were higher than that of the untreated sample. This can be explained by the fact that EC is directly proportional to TDS (Uwidia and Ukulu, 2013). R^2 for alum, PFS and PaFC obtained from lines of best fit of regression analysis was 85.9%, 60.0% and 48.9% respectively. This shows there is a strong correlation between EC and pH for alum and PFS

compared to PaFC. The high EC values for the alum -treated wastewater could be due to the charges of the free particles formed after the coagulation. Aluminum hydroxide ($Al(OH)_3$) and other intermediate hydrates are formed are reported to be the final by-products for aluminum salts coagulation. The by-products can polymerize into metal hydrate units which carry particle charges capable of conducting electricity (Ashtekar *et al.*, 2014). Figure 4.3.2 (A) shows the effect of pH on EC for the three coagulants. Figure 4.3.2 (b, c & d) gives illustration of the relationship between pH and the respective coagulants obtained from lines of best fit for alum, PFS and PaFC respectively.

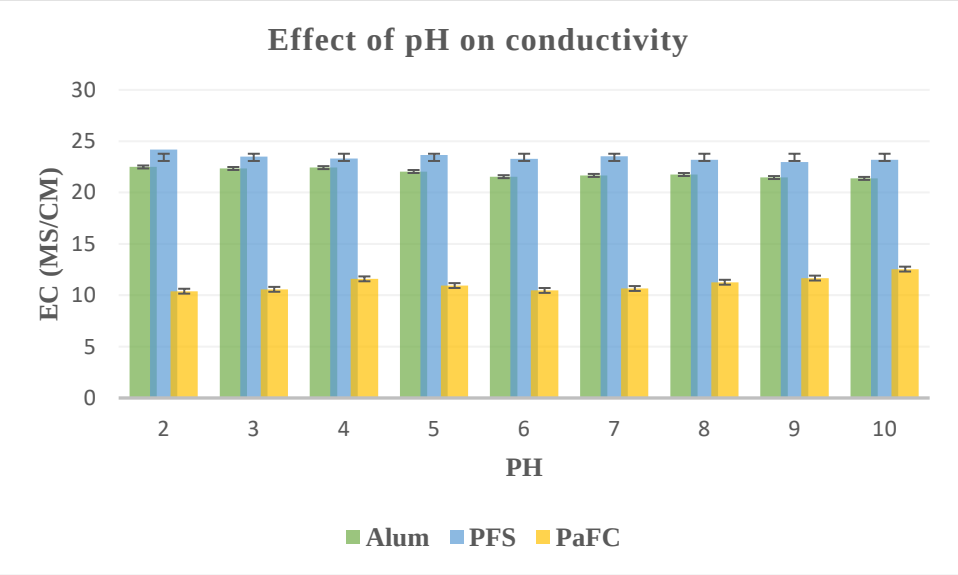

Fig. 4.3.2 (a): Effect of pH on EC

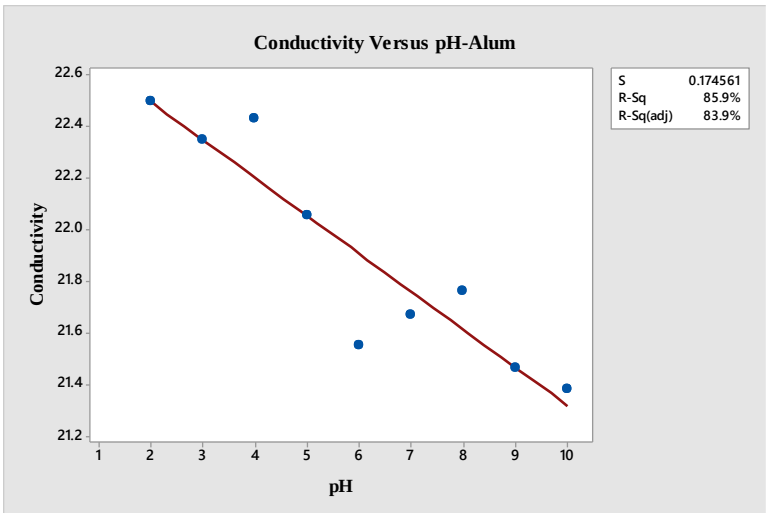

Fig. 4.3.2 (b): Correlation between EC and pH for Alum

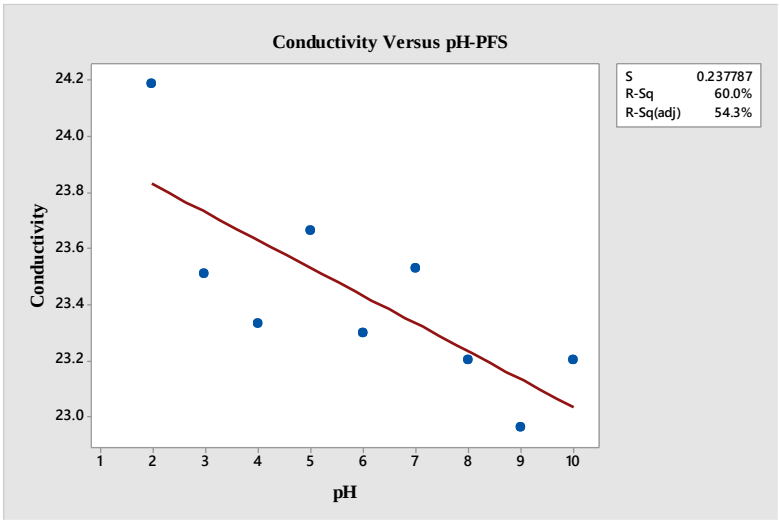

Fig. 4.3.2 (c): Correlation between EC and pH for PFS

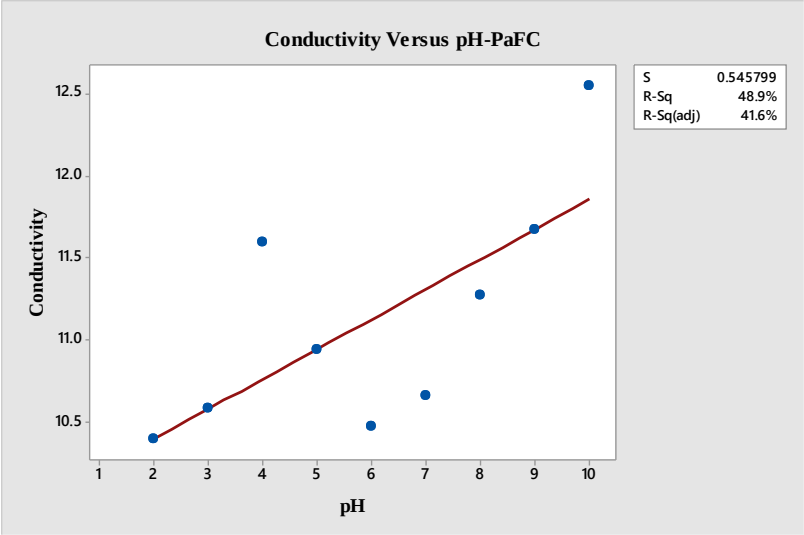

Fig 4.3.2 (d): Correlation between EC and pH for PaFC

4.3.1.1.3 Salinity

Alum and PFS-treated wastewater samples gave relatively similar values of salinity across the pH range that were higher than that of the original sample (10.22 psu). This can be drawn from the respective coagulants TDS and EC results since they give an indication of level of salinity in water (Rusydi, 2018). Figure 4.3.3 (a) shows the effect of pH on salinity for the three coagulants. Similarly, all the coagulants indicated moderate correlations with pH variation as indicated by the respective R^2 for the lines of best fits for alum, PFS and PaFC as shown in Figure 4.3.3 (b, c & d).

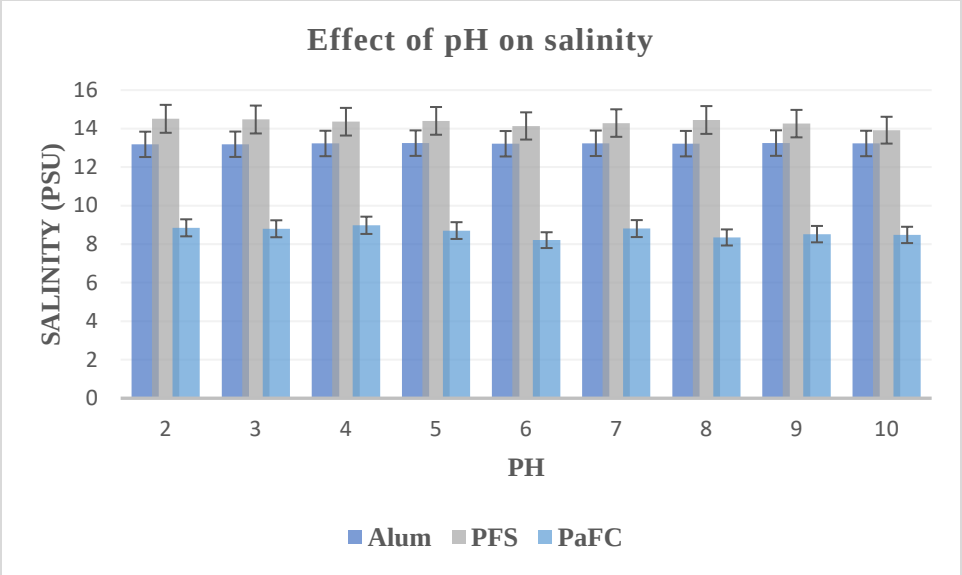

Fig. 4.3.3 (a): Effect of pH on Salinity

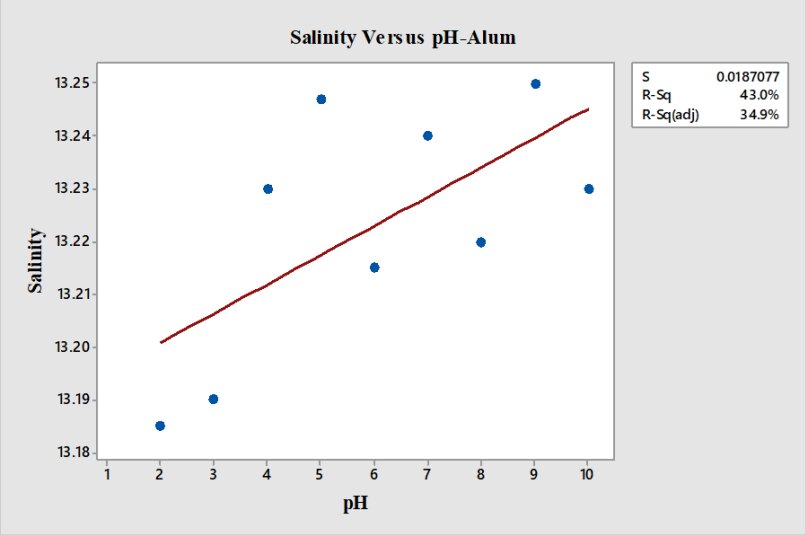

Fig. 4.3.3 (b): Correlation between salinity and pH for Alum

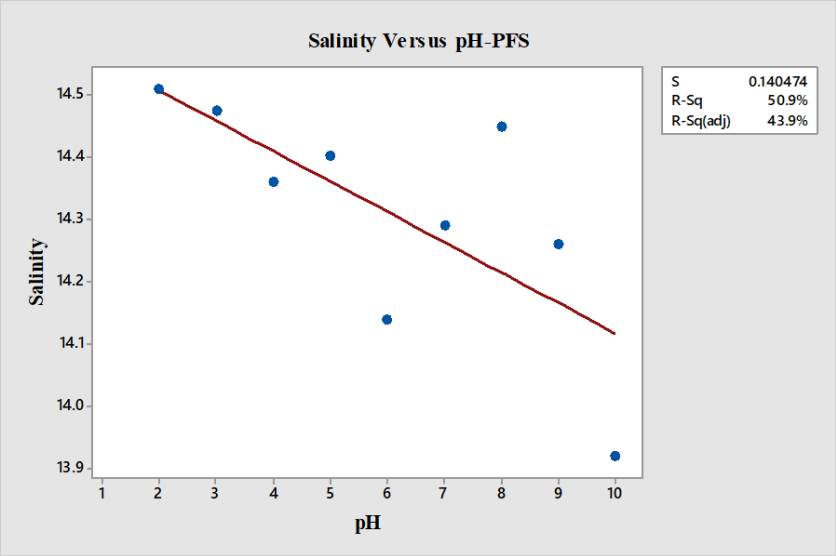

Fig. 4.3.3 (c): Correlation between salinity and pH for PFS

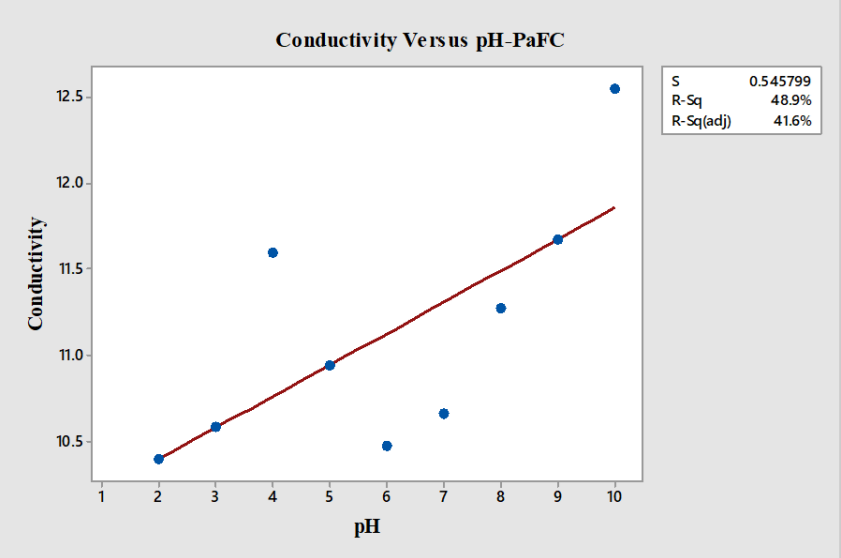

Fig. 4.3.3 (d): Correlation between salinity and pH for PaFC

4.3.1.1.4 Sludge Quantity

Alum generated the highest sludge quantities that increased with increase in pH while PFS generated relatively high amount of sludge that remained moderately constant across the pH range. PaFC gave negligible amounts of sludge across the pH range. When dosed into water, insoluble aluminum hydroxide flocs are formed as a result of the reaction between

the acidic coagulant and increasing alkalinity with rise in pH of the water, hence the high values of sludge generated by alum coagulation (Brandt, 2017). Pre-hydrolyzed coagulants such as PAC, PFS and PaFC have been previously reported to produce lower amounts of sludge hence the low quantities generated by PaFC (Yildiz *et al*., 2014). Figure 4.3.4 (a) gives an illustration of the effect of pH on the amount of sludge generated across the pH range by the respective coagulants.

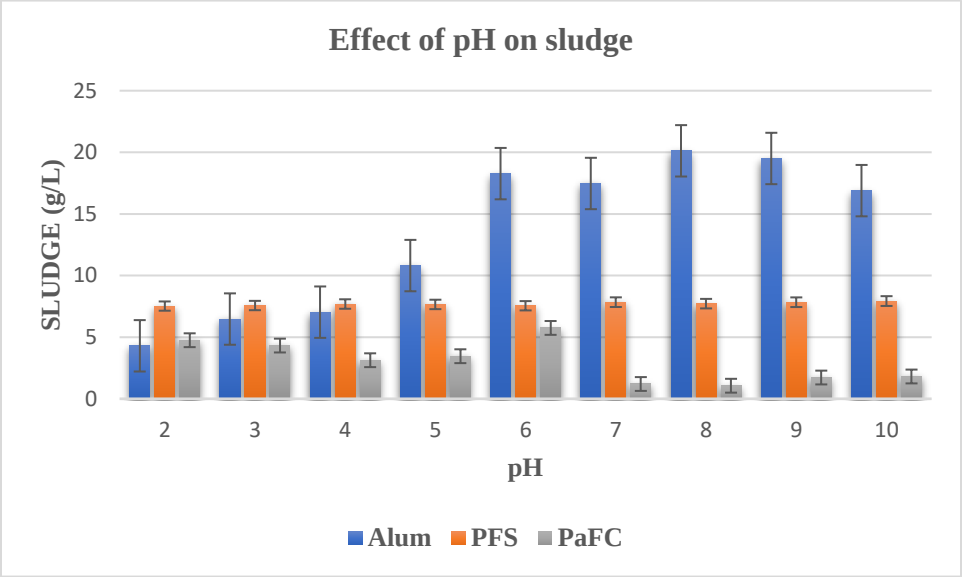

Fig. 4.3.4 (a): Effect of pH on Sludge amount

Consequently, alum and PFS exhibited strong relationship with pH variation compared with PaFC as illustrated by the R^2 of the lines of best fit of the respective data obtained as shown in Figure 4.3.4 (b, c & d). Respective R^2 for alum, PFS and PaFC are 74.8%, 72.4% and 49.6%.

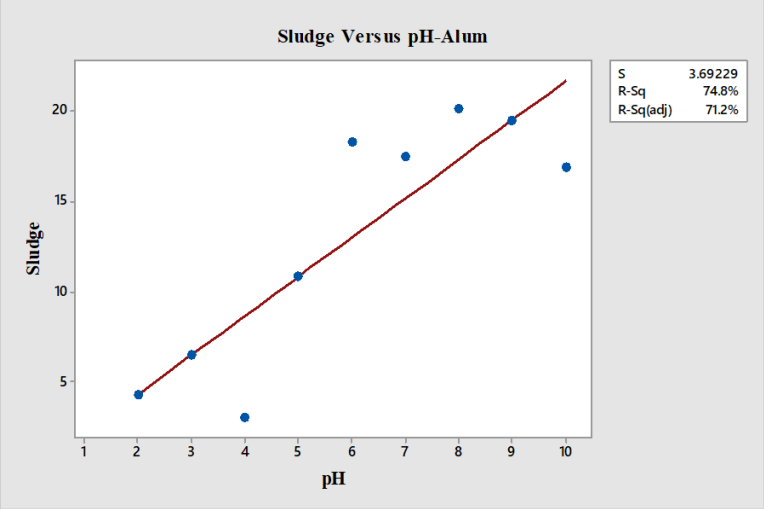

Fig. 4.3.4 (b): Correlation between sludge amount and pH for Alum

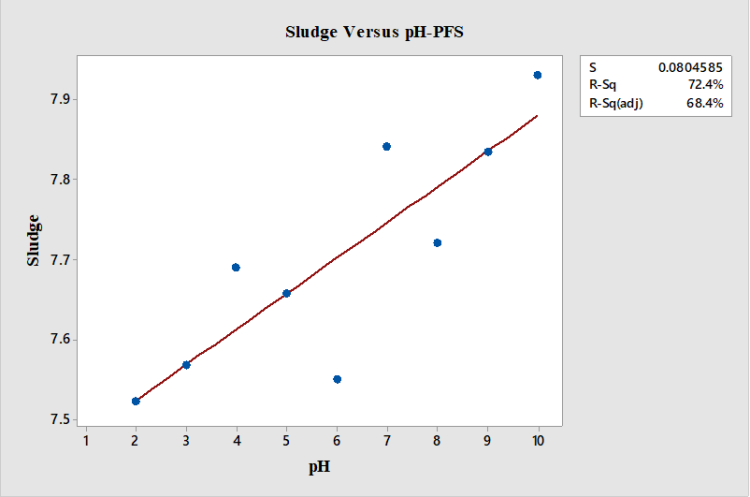

Fig. 4.3.4 (c): Correlation between sludge amount and pH for PFS

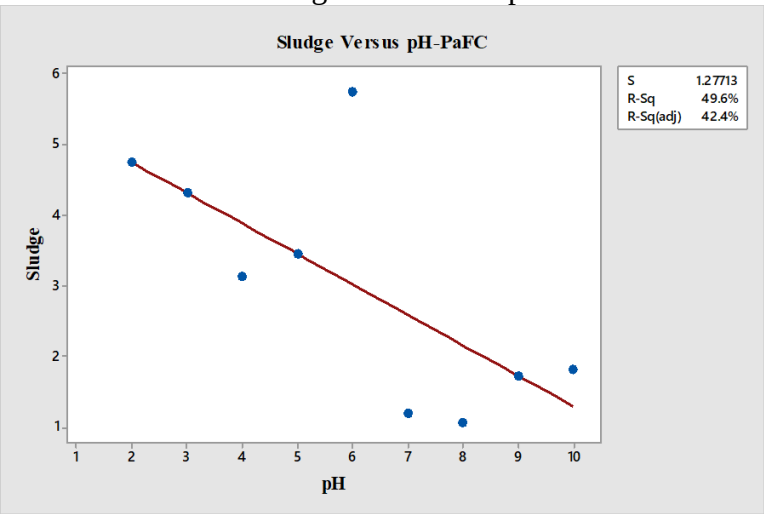

Fig. 4.3.4 (d): Correlation between sludge amount and pH for PaFC

In summary, PaFC was found to be the best in reduction of the TDS, EC and salinity of the dye wastewater while alum and PFS gave relatively higher yet relatively similar values of the same parameters across the entire pH range. This was attributed to the fact that PaFC has charge neutralization and bridging properties compared to alum and PFS hence the simultaneous reduction of the three parameters that have been found to be codependent (Rusydi, 2018). Alum generated relatively higher amounts of sludge compared to PFS while PaFC gave negligible amounts across the pH range. R^2 of respective best lines of fit obtained from regression analysis of the data indicated a moderate correlation between pH and TDS and salinity for all the coagulants. While alum and PFS exhibited strong linear relationship between pH and EC and amount of sludge generated compared with PaFC.

4.3.2 Effect of Coagulant Dose on Physicochemical Properties of Treated Wastewater

4.3.2.1 TDS

Since variation of pH from 2 to 10 during the dye wastewater treatment with the three coagulants gave relatively similar results of salinity, EC and TDS, pH 6-7 was chosen as the optimal. The dye wastewater sample was then treated at the optimized pH at varied coagulant dosages for each of the coagulants. Respective coagulant dosage variation for alum, PFS and PaFC was 60-140 g/L, 20-100 mg/L and 140-280 mg/L. TDS reduction was found to be more effective with lower dosages for alum and PFS while the values remained relatively lower and constant for PaFC across the dosage range. This was attributed to high solubility of the multi-charged complexes formed during coagulation with the aluminum and iron-based salts compared to the stable effect of PaFC (Bratby, 2016; Gao, Qinyan and Miao, 2003). Figure 4.3.5 gives an illustration of the effect of coagulant dose variation on TDS for the three coagulants.

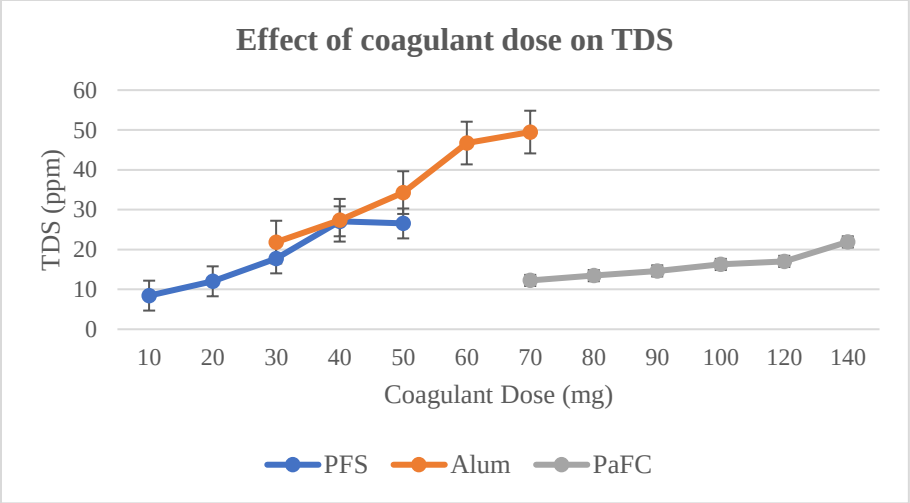

Fig. 4.3.5: Effect of coagulant dose on TDS

4.3.2.2 Electrical Conductivity

Since EC is directly proportional to TDS, reduction in EC was found to decrease with rise in dosage for alum and PFS while the values remained relatively similar for PaFC across the dosage range. Figure 4.3.6 shows the effect of change coagulant dose on the EC.

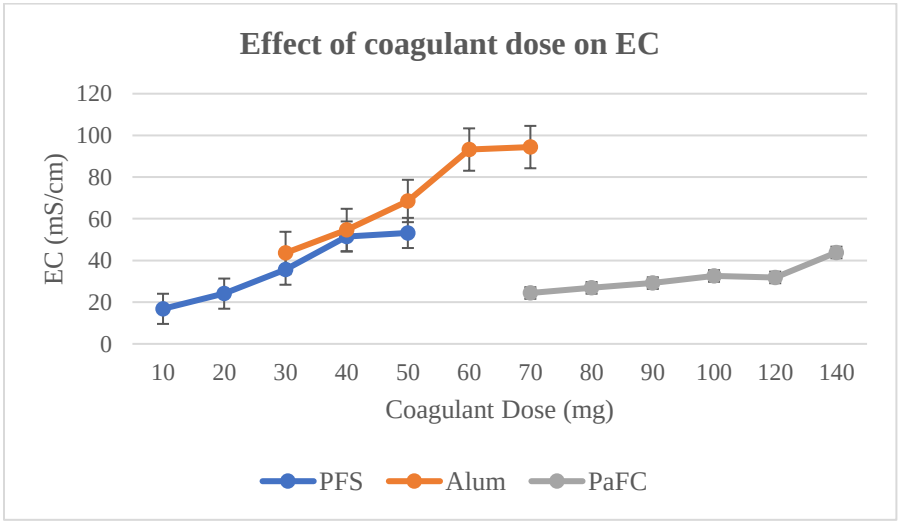

Fig. 4.3.6: Effect of coagulant dose on EC

4.3.2.3 Salinity

Since EC and TDS have been reported to give an indication of the level of salinity in water according to Rusydi (2018), salinity values were consequently found to increase proportionately with all the coagulants dosages as shown in Figure 4.3.7. Salinity amounts obtained from dosage variation were higher compared to those of pH for all the coagulants especially alum, which can be explained by presence of dissolved salts that originated from dissolution of the coagulants during the coagulation process.

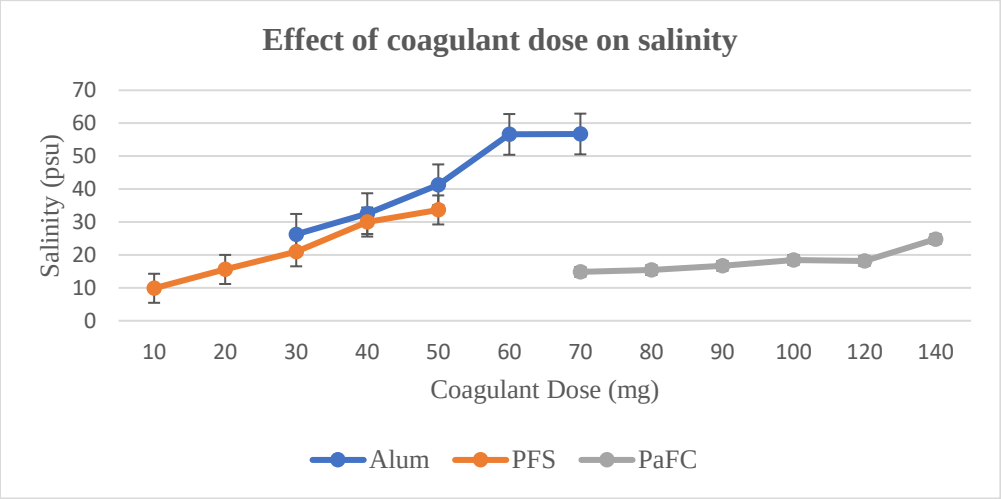

Fig. 4.3.7: Effect of coagulant dose on Salinity

4.3.2.4 Sludge Amount

Alum generated the highest amount of sludge that increased with dosage. While PFS generated relatively high amount of sludge that remained moderately constant across the dosage range, PaFC gave negligible amounts of sludge. This can be explained by the ability of alum to form insoluble aluminum hydroxide flocs with enhanced adsorption characteristics hence the high values of sludge generated after treatment with alum (Brandt, 2017). Pre-hydrolyzed coagulants such PFS have also been reported to produce larger and heavier flocs from disassociation of the dissolved solids (Akshaya, Rajesh and Puspendu,

2012). Figure 4.3.8 shows the effect of coagulant dosage variation on the amount of sludge generated.

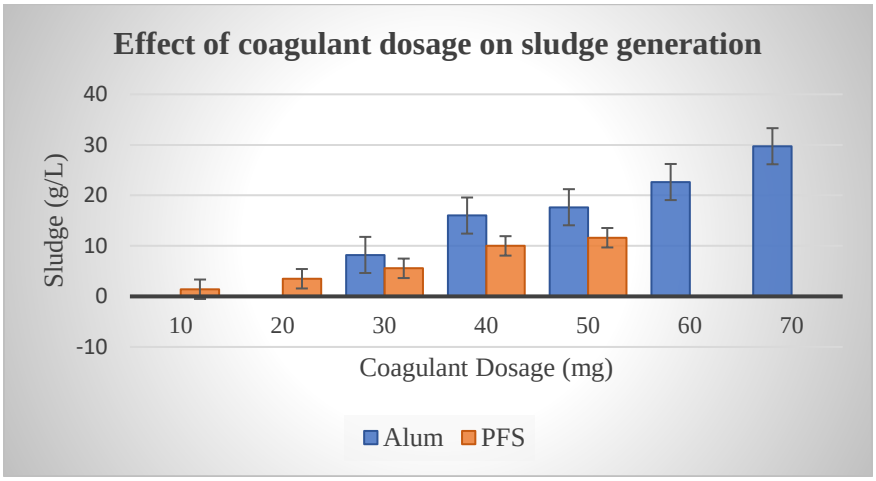

Fig. 4.3.8: Effect of coagulant dose on sludge generation

4.3.3 Effect of Optimized pH and Coagulant Dose on Colour Removal

After coagulation at varied pH (2-10), alum removed the dye optimally at pH 6, 7 and 10 while PFS cleared at pH 6-7. Colour removal reading values (Abs) from UV-Vis Spectrophotometer for alum and PFS across the pH range gave a coefficient of determination (R2) of 84.0% and 72.9% respectively at 95% confidence level. This indicates a strong correlation between the coagulants and pH. PaFC response values on the other hand gave a lowest R^2 of 38% indicating resilience with pH variation.

Colour removal reading values (Abs) for alum, PFS and PaFC after treatment under different dosages gave R^2 of 75.7%, 85% and 35% respectively. This explains that colour removal was directly proportional to coagulant dosage. Aluminum and ferric salt-based coagulants have the ability to attain particulate destabilization through charge neutralization of negatively charged dye ions in the wastewater, hence more appropriate for decolorization (Ghafari *et al*. 2009).

Mohsin (2019) had also found out that PAC and alum were able to clear reactive dyes at pH 3-11 and 10 respectively. PFS has been reported to coagulate by formation of rapidly settling flocs and are efficient over wide pH range (Gao, Yue and Miao, 2001; Sumathi, 2014). According to Gregory and Ross (2001) this is attributed to pre-neutralization of pre-hydrolysed coagulants hence smaller effects on the pH and therefore minimize the need for pH correction. High valence of Iron-based coagulants such as PFS counter ion gives more destabilizing effect and less dose is needed for coagulation (Nawaz *et al*.,2014) 2014. PaFC however did not give significant results across the pH range. This was attributed to the hydrolysis of the dye across the wide pH range that was faster than the coagulation process coupled with the dark colour of the coagulant itself. Perng and Bui (2014) had also found out that PAC could not remove substantial amount of RB5 at alkaline pH.

Figure 4.3.9 shows the comparative analysis of the dye concentrations of treated samples at varied pH. The dye concentration was found to be minimal at pH 7 for PFS while alum gave lowest concentrations at pH 6,7 and 10 and PaFC exhibited relatively similar results across the pH range as shown in Figure 4.3.10 (a).

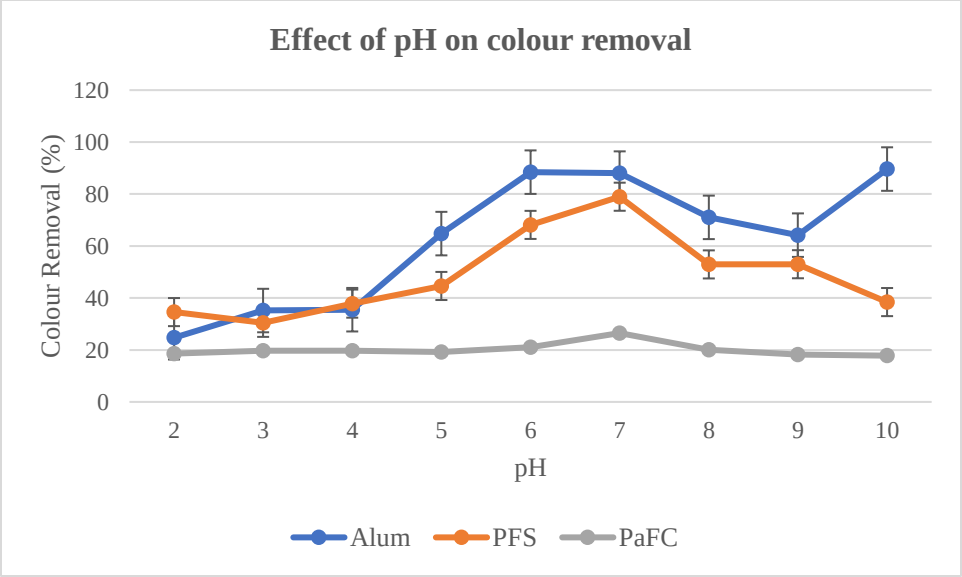

Fig. 4.3.9: Effect of pH on Dye Removal

Colour removal was found to be proportional with coagulant dosages for alum and PFS. This can be explained by the neutralization mechanism between the increasing cationic ions of the coagulants with the destabilized anionic colloidal dye ions (Perng and Bui, 2014). Alum clarified the dye at 140mg/L and PFS at 100mg/L. The lower dose for PFS as compared to that of alum may be attributed to formation of bigger $Fe(OH)_2$ flocs useful for faster adsorption of soluble dye molecules (Perng and Bui, 2014; Joo *et al*., 2007). Gradual addition of PaFC dose from 140mg/L to 280mg/L however did not give significant dye removal results, an observation that agrees with that reported by Mohsin (2019). This can be explained by saturation of the positive charge of the coagulants that were not bonded with dye ions which led to electrostatic repulsion of the particles. As a result, increase of the coagulant dosage did not impact on colour removal efficiency. Colour concentration reduced with increase in alum and PFS dosages as shown in Figure 4.2.10.

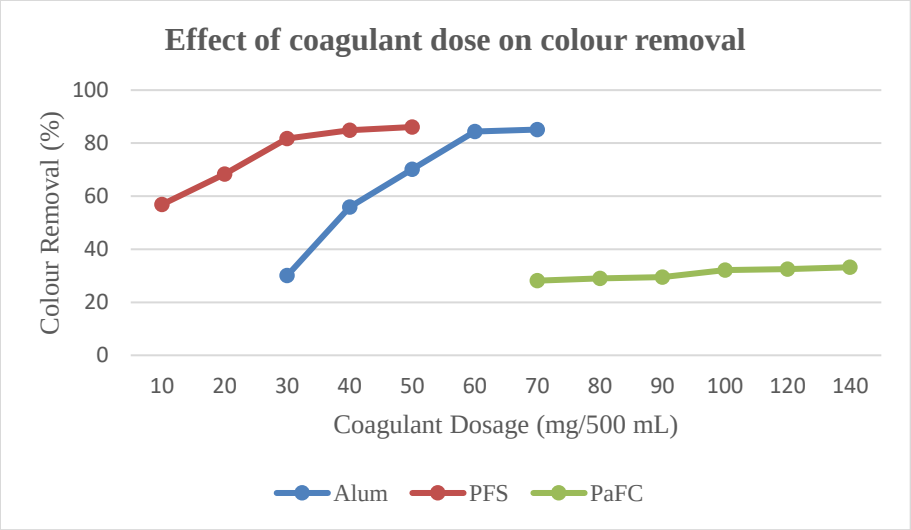

Fig. 4.3.10: Effect of Coagulant Dosage on Colour Removal

4.3.4 Effect of Optimized pH and Coagulant Dose on COD

COD of the clarified samples at optimal pH (6 and 7) and respective coagulants' dose was obtained as shown in Figure 4.3.11 (a & b). PaFC gave the highest COD levels followed by PFS while alum gave the lowest values. This can be explained by the fact that the pre-hydrolyzed nature of PaFC formed relatively stable polynuclear hydroxide flocs through neutralization and bridging whose accumulation caused rise in COD as compared to those formed from charge neutralization and adsorption mechanism associated with alum and ferrous sulphate (Akshaya, Rajesh and Puspendu, 2012).

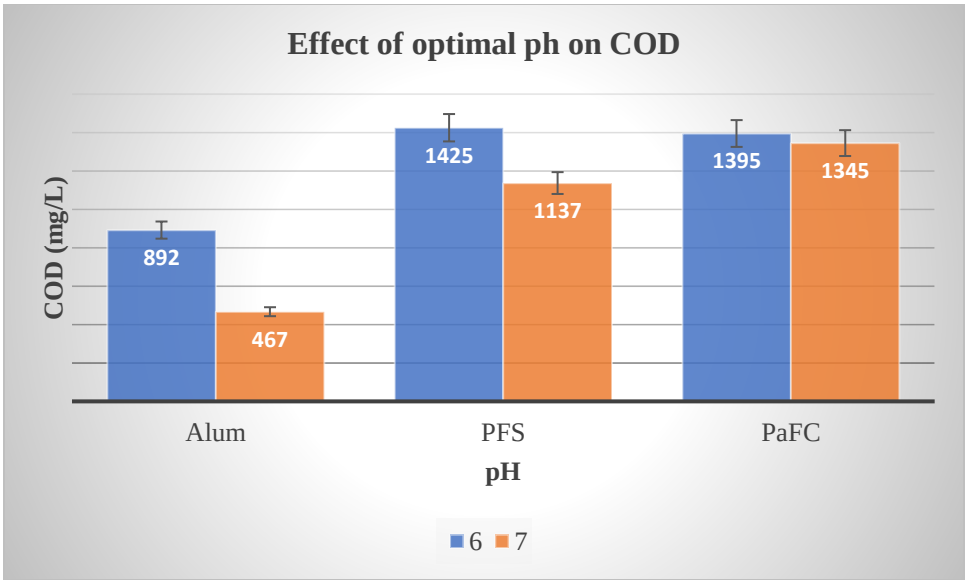

Fig. 4.3.11 (a): Effect of optimal pH on COD

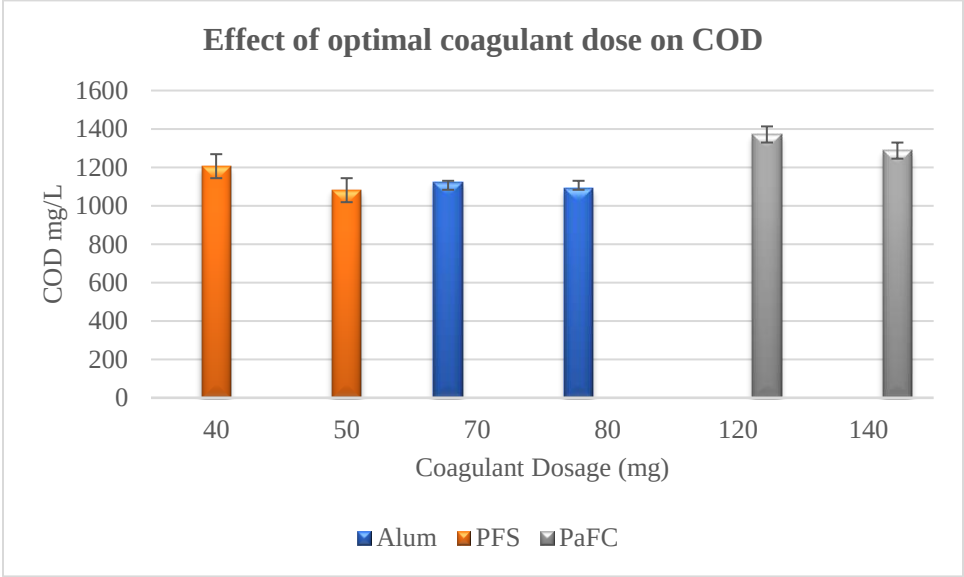

Fig. 4.3.11 (b): Effect Optimal coagulant dose on COD

4.3.5 Effect of Optimal pH and Coagulant Dose on Turbidity

Turbidity for the clarified water samples at pH 6 and 7 and corresponding coagulants optimal dosages were as shown in Figure 4.3.12 (a & b). pH 7 was found to give the lowest turbidity for all the coagulants. This could be attributed to the coagulants ability to form

flocs faster at the optimal pH due to the optimal charge neutralization of the coagulants followed by quick adsorption and/or bridging of the destabilized colloids hence minimal amounts of suspended solids that lead to low values of turbidity. Gao, Yue and Miao (2001) also found out that PaFC removed turbidity better between pH 7.0-8.4.

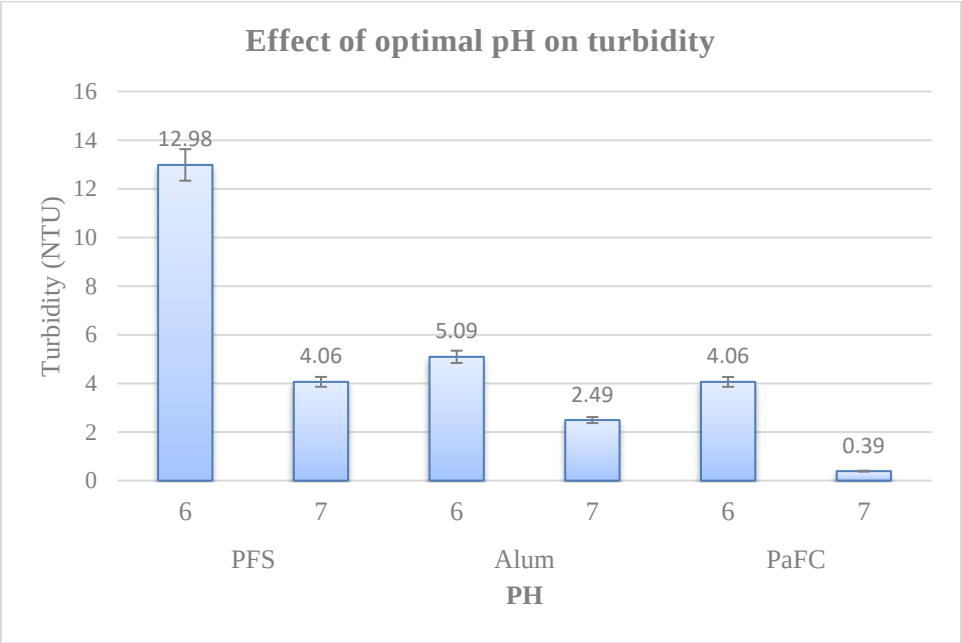

Fig. 4.3.12 (a): Effect of optimal pH on Turbidity

Alum was found to give the highest turbidity values compared to PFS and PaFC. Alum is reported to form flocs that settle slowly hence availability of suspended solids that give rise to high turbidity. Turbidity decreased with the dosage increase of all the coagulants as shown in Figure 4.3.12 (b). This can be explained by the optimal adsorption of the destabilized colour and other suspended solids with rise in coagulant dosage.

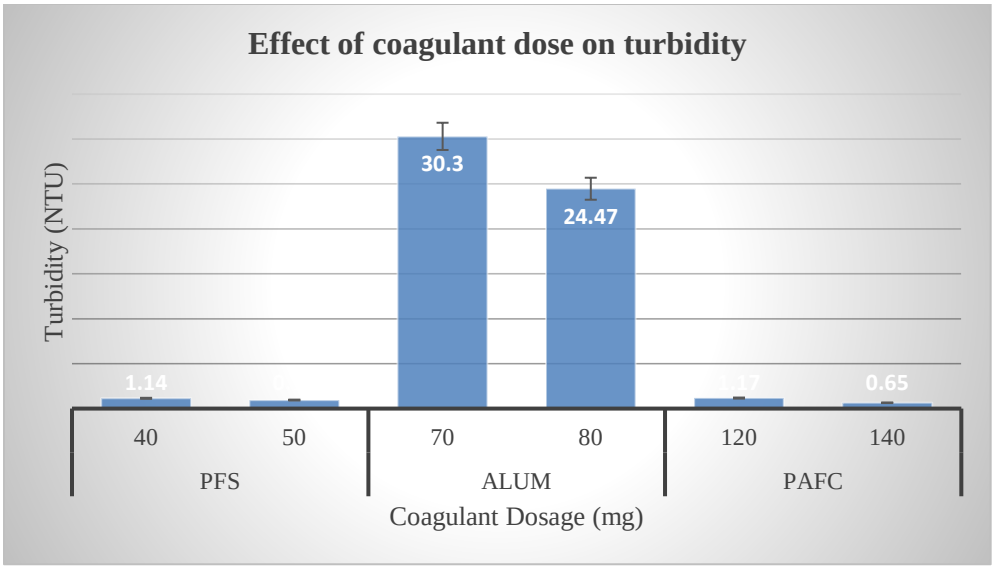

Fig. 4.3.12 (b): Effect of optimal coagulant dose on Turbidity

Table 4.2 (b) gives a summary of physicochemical properties of the dye wastewater sample optimally treated at PH 6-7 and respective doses for PFS, 100 mg/L, PaFC, 280 mg/L and alum, 140 mg/L. The values for each of the parameters were compared with the water quality regulations set by National Environment Management Authority. All the treated samples met the pH and TDS limits. All the samples treated by the three coagulants exceeded the set limit for COD (1,000mg/L). Samples treated by alum met the set limits for BOD (500mg/L) while those of PFS and PaFC exceeded the limit.

Table 4.2 (b) Physicochemical properties of the treated dye wastewater in comparison with NEMA limits.

Parameter	**Untreated wastewater**	**PFS**	**PaFC**	**Alum**	**Limits**
pH	10.23	6-8	6-8	6-8	6-9
Color (Hazen)	>250	10	>250	10	≤40
TDS (mg/L)	81.575	49.48	21.92	26.55	2,000
BOD (mg/L)	350	775	1,000	150	500
COD (mg/L)	1486	1081	1287	1090	1,000

4.3.6 Conclusion

In conclusion, treatment of the dye wastewater at the optimized pH and varied respective coagulants' dosage was found to affect TDS, EC, salinity, sludge generated and amount colour removed from the RB5 textile wastewater. PaFC was found to be the best in reduction of the physicochemical parameters analyzed while alum and PFS were found to have relatively higher yet similar values across the entire pH range. This was attributed to the fact that PaFC combines effects of charge neutralization and bridging which yielded better results (Rusydi, 2018). Alum gave the highest amount of sludge that increased with dosage while PaFC generated negligible amounts accompanied with minimal colour removal across the dosage variation. Dye concentration was found to be minimal at pH 7 for PFS while alum gave lowest concentrations at pH 6,7 and 10 while PaFC exhibited relatively similar results across the pH range. Colour removal was found to be proportional with coagulant dosages for alum and PFS attaining optimal dye removal at 140 mg/L and 100 mg/L respectively. Gradual addition of PaFC dose from 140mg/L to 280mg/L did not give significant dye removal results. PFS was therefore found to be the best in the dye removal at pH 7 and 100 mg/L dose. This may be explained by its ability to form bigger flocs beneficial for quick adsorption and settling of the dye molecules. PaFC gave the highest values of COD at optimized conditions while PFS and alum had high turbidity at their respective optimal pH and dosages. While PaFC was found to be best at reduction of salinity, EC and TDS with minimal sludge generation, it was poorest in colour and COD removal. PFS on the other hand had the best dye removal efficiency followed by alum although they generated high amounts of sludge. Therefore, none of the three coagulants had the comprehensive capability of dye removal and remediation of the physicochemical

parameters studied and a combination of PaFC and either PFS or alum is hence recommended for complementary effects during treatment of the dye wastewater.

4.4 Screening and Isolation of Bacteria with *Eps* Production Capacity from Cotton Gin Trash Soil Samples Using Different Media

4.4.1 Isolation of *Eps* Producers

Figure 4.4.1 shows some of the colonies with *Eps* production capacity characterized by glistening, opaque, mucoid, slimy colonies.

Fig. 4.4.1: Mucoidal and slimy *Eps*- producers isolates

Table 4.3 gives the respective number of the mucoidal and slimy isolates obtained from each of the media per gin trash soil sample.

Table 4.3: Number of mucoidal isolates from respective media

Soil Source	YEA	YEG	YEPD	TSA	SDA	NA +G	NA	MEA	PDA	TOTAL
Meru	17	3	3	9	1	12	10	5	14	74
Kitui	12	8	13	4	0	5	4	3	4	53
Baringo	11	6	6	8	3	7	4	5	14	64

Figure 4.4.2 gives a quantification summary of the isolates with *Eps* production capacity cultured from the three different gin trash soil sources using the nine different media.

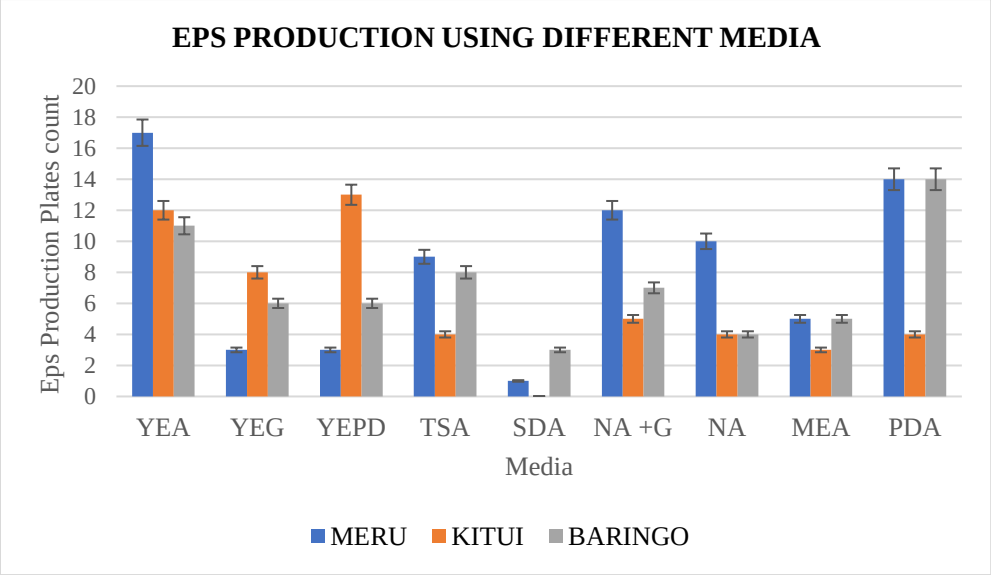

Fig. 4.4.2: *EPS* production using different media

YEA, PDA, NA+G and NA were found to be the best media in supporting the majority of the isolates with the *Eps* production capacity from the soil sample collected from Meru ginnery. Majority of the potential *Eps*-producers cultured from the Kitui and Baringo soil samples were supported by (YEPD and YEA) and (PDA and YEA) respectively. Results from the other media were found to be comparative although SDA supported the least

number of *Eps*-producer isolates across all the three gin trash soil samples as shown in Figure 4.4.2.

4.4.2 Screening for *Eps*-Producers in Broth Media

NB+S, NB+G and GPYMgSO$_4$ broths were used to screen for the bacterial isolates with *Eps* production out of those selected from the three soil sources. 80.0%, 92.3% and 96.3% of the *Eps* producers that gave at least 90% production were supported by NB+ S, NB +G and GPYMgSO$_4$ respectively. Figure 4.4.3 shows respective *Eps* growth as supported by different media broths.

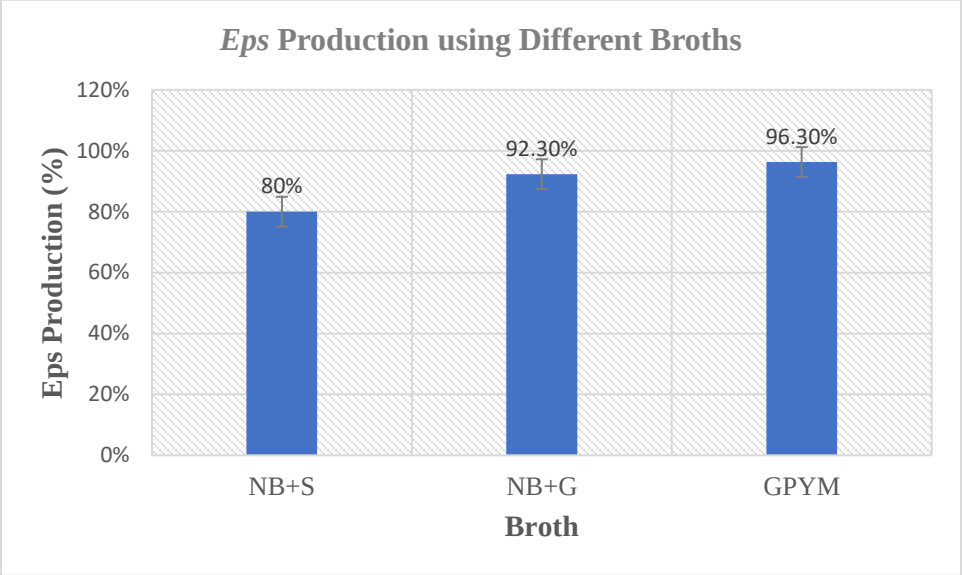

Fig. 4.4.3: Respective *Eps* growth as supported by different media broths

While GPYMgSO$_4$ was found to be relatively better in *Eps* production while basal medium (NB), enriched with glucose also supported the *Eps* growth substantially as shown in Figure 4.4.3. This finding agreed with those reported by Hereher *et al*. (2018), where among eight tested media, the basal medium was found to stimulate the highest yield of *Eps* by different bacteria strains isolated from soil samples. 30%, 42% and 28% of the

isolates found to give at least 90 % *Eps* production capacity originated from Baringo, Meru and Kitui gin trash soil samples respectively as illustrated in Figure 4.4.4.

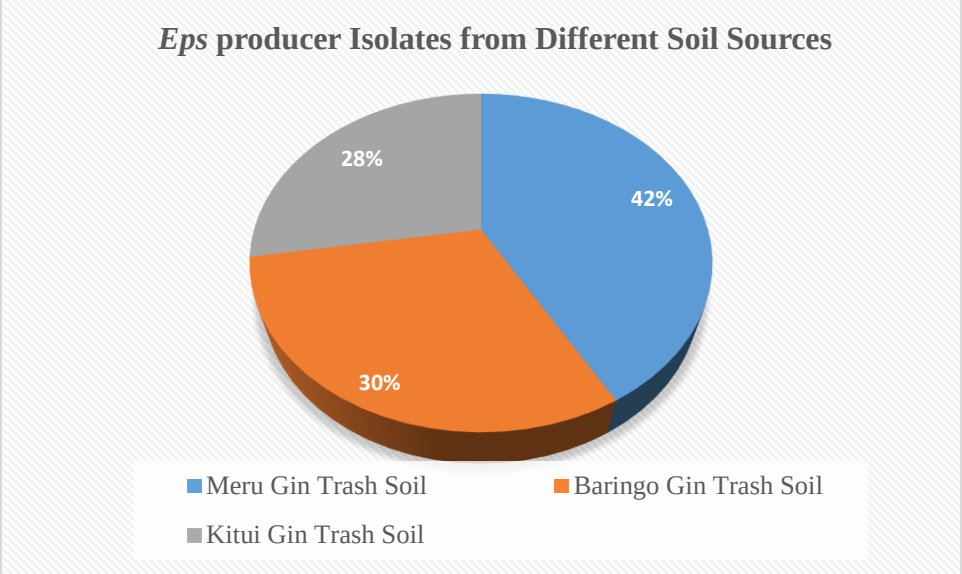

Fig. 4.4.4: Soil sources for high (>90%) *Eps* producer isolates

4.4.3 Conclusion

Isolates screened from bacteria cultured from gin trash soil samples collected from Kitui, Meru and Baringo (Salawa) ginneries demonstrated capacity for *Eps* production that varied with media. Out of the nine different media used to screen for *Eps* producers, YEA, PDA, NA+G and NA were found to support those cultured from Meru gin trash soil sample better. Those cultured from Kitui gin trash soil sample were found to be supported by YEPD and YEA while those from Baringo sample were obtained from PDA and YEA media. SDA medium was however found to be the least in supporting growth of the *Eps* producing bacteria across all the three gin trash soil samples. GPYMgSO_4 broth medium was found to be the best in supporting production of *Eps* compared with NB+G and NB+S. 80.0%, 92.3% and 96.3% of the *Eps* producers that gave at least 90% growth were obtained from NB+S, NG +G and GPYMgSO_4 respectively. Gin trash soil from Meru region

produced the highest number of the *Eps* producing bacteria isolates (42%) compared with 30% and 28% of the isolates that originated from Baringo and Kitui gin trash soil samples respectively.

4.5 Screening for Bacterial *Eps* with Activity in RB5 Dye Wastewater

4.5.1 Screening for *Eps*-Producers in RB5 Dye Wastewater

NB+S, NB+G and GPYMgSO_4 separately dosed with synthetic and machine dye wastewater were used to screen for the bacterial isolates with dye removal capacity using the selected isolates' *Eps*. The broths dosed with the different dye wastewater samples were individually inoculated with the selected isolates' *Eps*.

Respective isolates' *Eps* that exhibited at least 94% dye removal were selected for optimization.

Figure 4.5.1 gives a summary of the isolates' *Eps* that gave at least 94% dye removal efficiency.

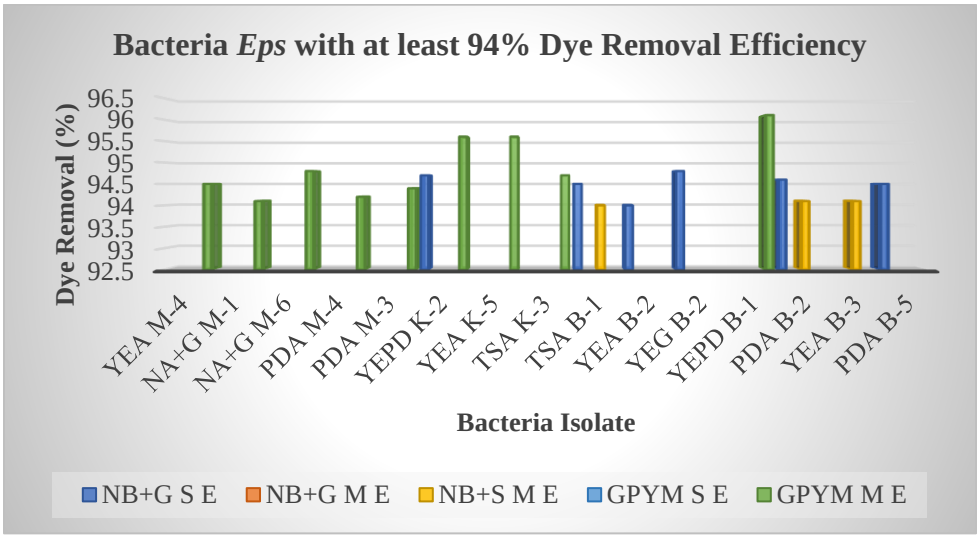

Fig. 4.5.1: Bacteria isolates that gave at least 94 % dye removal efficiency with different broth

50%, 16.7% and 33.3% of the selected *Eps* activity was supported by GPYMgSO$_4$, NB+S and NB+G media broths respectively as shown in Figure 4.5.2. GPYMgSO$_4$ was therefore found to be the most appropriate broth medium for the production of *Eps* in the presence of the dye wastewater. 46.5%, 20% and 33.5% of the selected bacteria with *Eps* producing capacity in the dye wastewater were cultured from Baringo, Kitui and Meru gin trash soil samples respectively as shown in Figure 4.5.3.

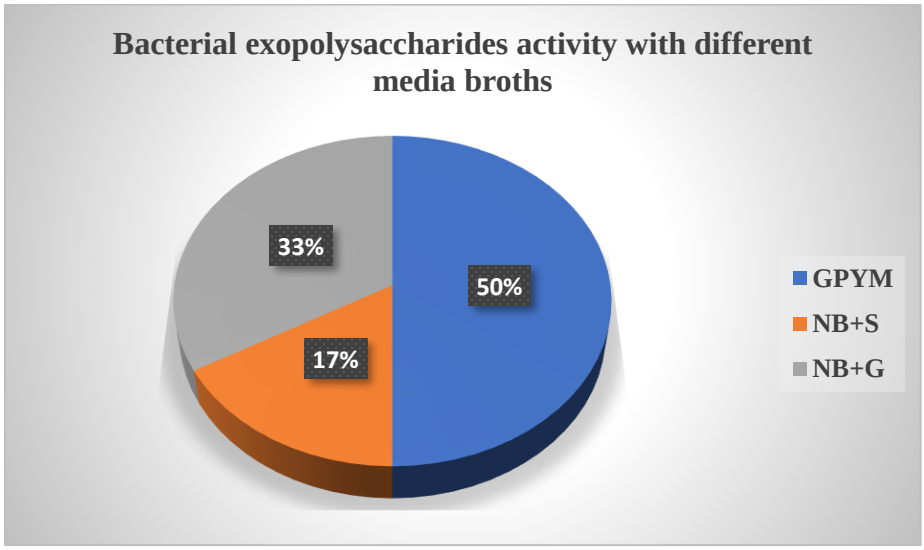

Fig. 4.5.2: Bacterial *Eps* activity in dye wastewater with different media broths

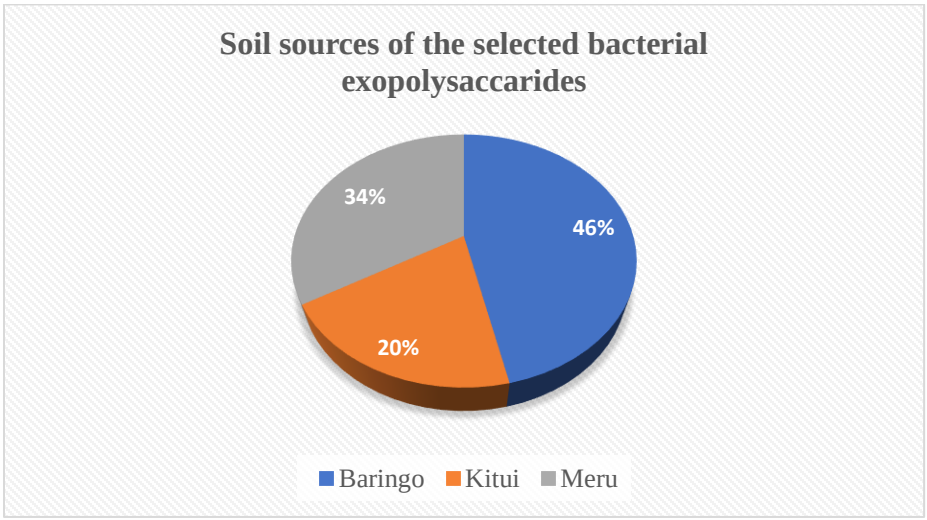

Fig. 4.5.3: Soil source for bacterial isolates with capacity for *Eps* production in dye wastewater

4.5.2 Dye Wastewater Treatment Using Substitute Broth

$MSYMgSO_4$ was screened for its suitability for the dye wastewater treatment as a substitute to $GPYMgS_{O4}$ which had been found to be the best in supporting *Eps* production in the dye wastewater. Total sugars for the molasses used was 81.2% (w/w). Molasses 10g/L, Soya 5g/L, Yeast Extract 3g/L and Magnesium Sulphate 0.2g/L ($MSYMgSO_4$) broth dosed with the dye wastewater was inoculated in duplicates with the *Eps* that had been found to give at least 94% efficiency for the dye removal and incubated at 30 ^oC for 72 h under 150 rpm. Inoculations that gave at least 60% dye removal efficiency were then selected for optimization. Table 4.4 gives a summary of the *Eps* producer bacteria accordingly selected for optimization.

Table 4.4: Summary of the bacteria *Eps* producers selected for optimization

***Eps* Producer Bacteria**	**% Dye removal using $MSYMgSO_4$ broth**
YEA M_2^{-4}	65.89
YEPD K_2^{-2}	62.50
YEA K_2^{-5}	68.28
YEPD B_2^{-1}	60.80
NA+G M_2^{-1}	61.00
PDA B_2^{-5}	65.24

Figure 4.5.4 ((a) & (b)) shows comparative colour removal efficiency for $MSYMgSO_4$ compared to $GPYMgSO_4$ samples.

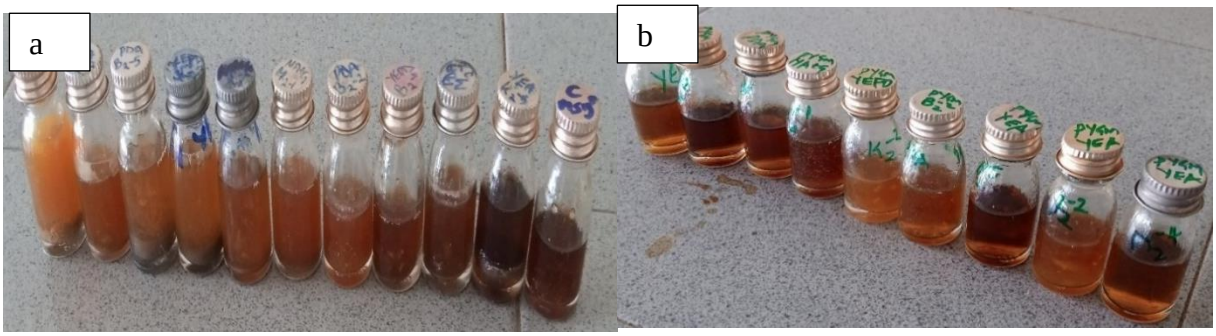

Fig. 4.5.4: Dye removal using $MSYMgSO_4$ (a) and $GPYMgSO_4$ (b) broths

4.6 Optimization of RB5 Dye Wastewater Bioremediation using Bacterial *EPS*.

4.6.1 Optimization of RB5 Dye Wastewater Treatment with Bacterial *Eps*

Eps produced from the six isolates selected were optimized for the dye wastewater treatment using the DOE shown in Table 2. Figure 4.6.1, 4.6.2 and 4.6.3 illustrates the dye removal efficiency (at least 60%) with the bacterial *Eps* for 24, 48 and 72 h respectively.

4.6.1.1 Dye Removal after 24 h Incubation

Incubation of the *Eps* inoculated dye dosed broths for 24 h at 30 ^{o}C did not meet the dye removal threshold of at least 60% with GPYMgSO_4 and MSYMgSO_4 containing 10 g/L and 15 g/L molasses concentration for all the six *Eps*. Only YEPD K_2^{-2} *Eps* gave 69.91% dye removal efficiency with NB + G. YEAM_2^{-4}, YEPD K_2^{-2}, YEPD B_2^{-1} and PDA B_2^{-5} *Eps* had capacity to remove at least 70% of the dye with MSYMgSO_4 that contained 20g/L molasses as shown in Figure 4.6.1 (a).

As shown in Figure 4.6.1 (b), incubation of the *Eps* for 24 h at 34 ^{o}C with MSYMgSO_4 containing 10g/L molasses did not support the dye removal across all the *Eps* while the MSYMgSO_4 containing 15g/L molasses supported dye removal with the YEA K_2^{-5}only. MSYMgSO_4 that contained 20g/L molasses supported the dye removal with NA+GM_2^{-1} and YEA M_2^{-4} *Eps*. NB + G M_2^{-1} and GPYMgSO_4 on the other hand were found to remove the dye at the same conditions across all the *Eps*.

Incubation of the *Eps* for 24 h at 38 ^{o}C supported the dye removal efficiency threshold with MSYMgSO_4 containing 10 g/L and 15g/L molasses for YEA M_2^{-4}, NA+GM_2^{-1} and YEPD K_2^{-2} *Eps* only. While YEA M_2^{-4} and NA+GM_2^{-1} *Eps* were found to remove the dye with MSYMgSO_4 containing 20g/L molasses, GPYMgSO_4 supported the dye removal with

YEPD B_2^{-1} only. $YEAK_2^{-5}$ and PDA B_2^{-5} did not meet the required dye removal target with all the media broths as illustrated in Figure 4.6.1 (c).

4.6.1.2 Dye Removal after 48 h Incubation

Incubation of the *Eps* for 48 h at 30 ^oC supported the dye removal with $GPYMgSO_4$ and $MSYMgSO_4$ containing 20g/L molasses for all the six *Eps* while NB + G supported $NA+GM_2^{-1}$ *Eps* only. $MSYMgSO_4$ containing 15g/L molasses supported the dye removal for $NA+GM_2^{-1}$, YEPD K_2^{-5} and YEPD B_2^{-1} *Eps* only while $MSYMgSO_4$ that contained 10g/L molasses did not support activity with any of the *Eps* at the same conditions as shown in Figure 4.6.2 (A)

Incubation of the *Eps* for 48 h at 34 ^oC supported the dye removal with $GPYMgSO_4$ for all the six *Eps*. NB + G also supported all the *Eps* except for the YEPD K_2^{-2} *Eps*. $MSYMgS_{O4}$ containing 10 g/L and 20g/L supported the dye removal by $YEAK_2^{-5}$ and NA+G M_2^{-1} respectively while $MSYMgSO_4$ that contained 15g/L molasses was not found to meet the dye removal limit with any of the six *Eps* as shown in Figure 4.6.2 (B).

Figure 4.6.3 (c) shows that incubation of the *Eps* for 48 h at 38 ^oC supported the dye removal using $GPYMgSO_4$ for all the *Eps* except YEPD K_2^{-2} and PDA B_2^{-5}. All the other broth media did not support the dye removal at the set level.

4.6.1.3 Dye Removal after 72 h Incubation

Incubation of the *Eps* for 72 h at 30 ^oC supported the dye removal with $GPYMgSO_4$ and $MSYMgSO_4$ containing 20g/L for all the *Eps*. $MSYMgSO_4$ containing 10 g/L and 15 g/L molasses supported the dye removal with $YEAK_2^{-5}$ *Eps* only as illustrated in Figure 4.6.3 (a)

The dye removal after incubation for 72 h at 34 ^{o}C was supported by MSYMgSO$_4$ that contained 15g/L molasses for the NA+G M_2^{-1} and YEPD K_2^{-2} *Eps*. NB+G had activity on the dye removal for all the *Eps*. GPYMgSO$_4$ also supported dye removal with all the *Eps* except that of YEPD B_2^{-1}. MSYMgSO$_4$ that contained 20g/L molasses supported the dye removal for YEA M_2^{-4} and PDA B_2^{-5} *Eps* as shown in Figure 4.6.3 (b).

Incubation of the dye for 72 h at 38 ^{o}C exhibited high variability across all the broths.

NB+G supported the dye removal for all the *Eps* except that of YEPD K_2^{-2} and PDA B_2^{-5}. Dye removal with MSYMgSO$_4$ that contained 10 g/L, 15 g/L and 20 g/L molasses was only supported by (YEA M_2^{-4} and PDA B_2^{-5}), (YEPD B_2^{-1} and PDA B_2^{-5}) and (YEA M_2^{-4} and NA+G M_2^{-1}) *Eps* respectively. GPYMgSO$_4$ only supported the dye removal with YEA M_2^{-4} *Eps* (Figure 4.6.3 (c).

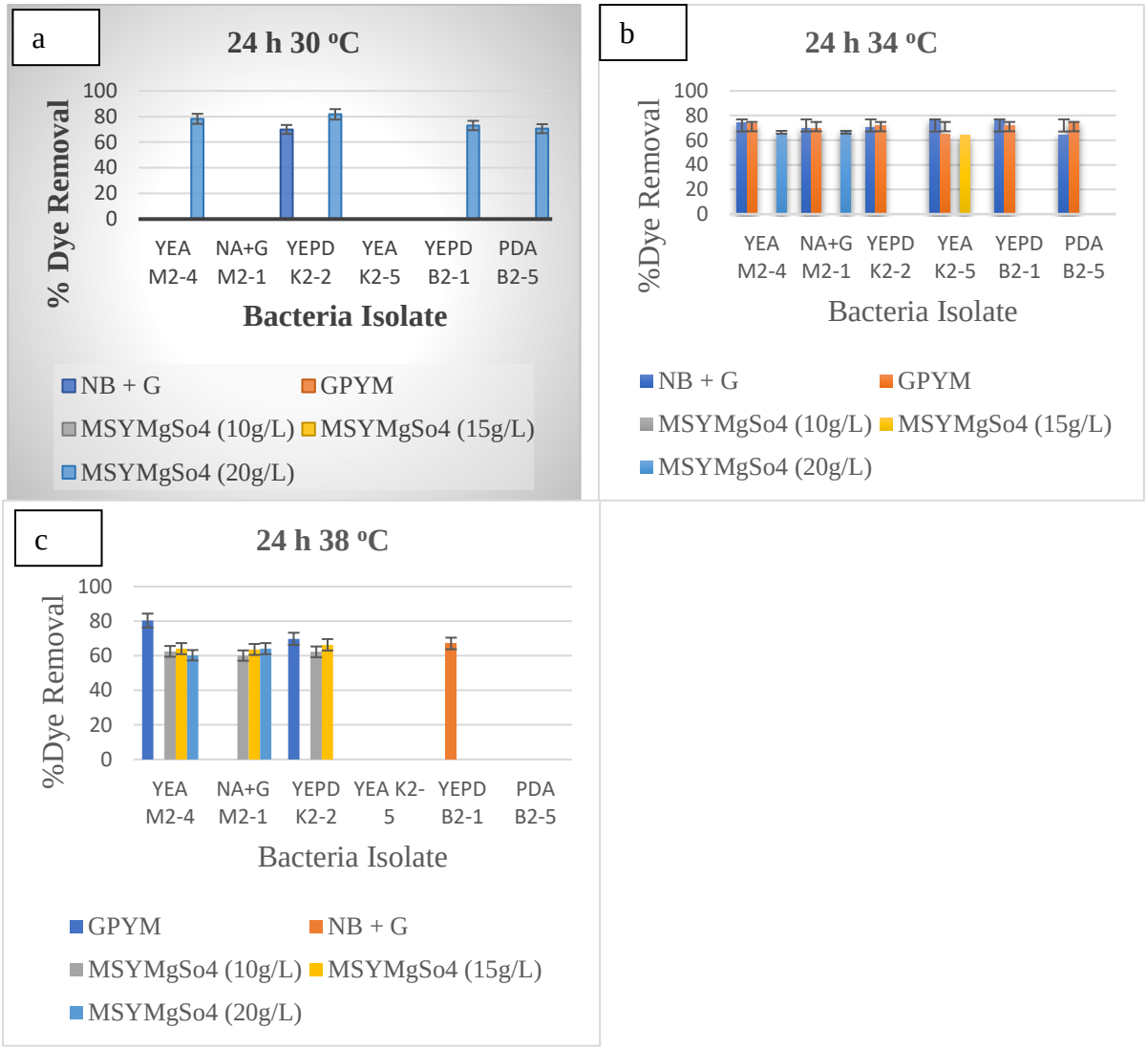

Fig. 4.6.1: Dye removal optimization at 30 ºC (a), 34 ºC (b), and 38 ºC (c) for 24 h

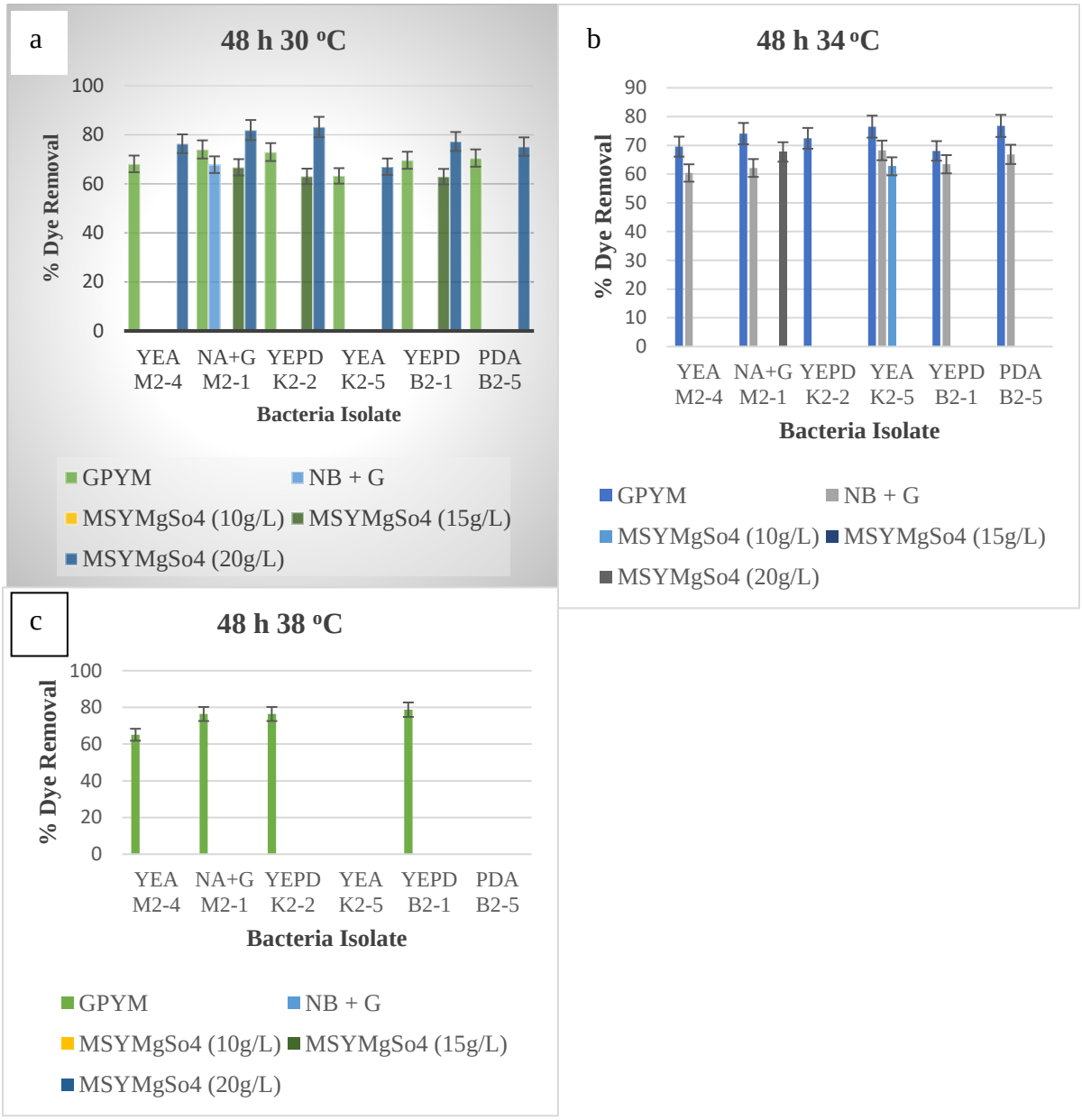

Fig. 4.6.2: Dye removal optimization at 30 ºC (a), 34 ºC (b), and 38 ºC (c) for 48 h

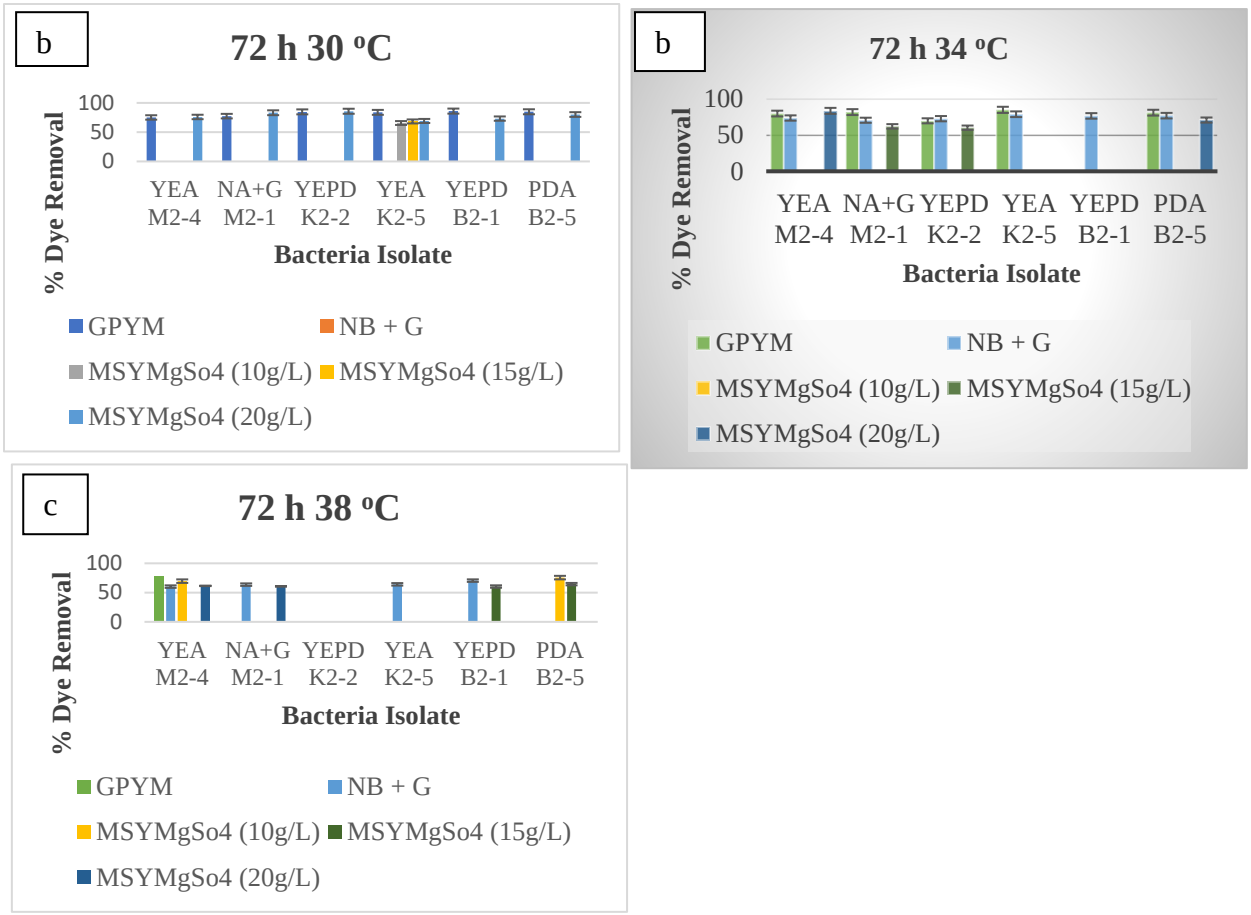

Fig. 4.6.3: Dye removal optimization at 30 ºC (a), 34 ºC (b), and 38 ºC (c) for 72 h

Eps that gave at least 80% of the dye removal efficiency at 30ºC after 24, 48 and 72 h incubation time with the $MSYMgSO_4$ broth were selected for their possible industrial applicability. $MSYMgSO_4$ that contained 10 and 15g/L molasses concentration did not meet the 80% threshold. Figures. 4.6.4 (a (i and ii)) show photos of the wastewater before and after treatment by the *Eps* from different bacteria isolates using $MSYMgSO_4$ containing 20g/L molasses broth. Figure 4.6.4 (b) shows flocs that resulted from the bio flocculation process.

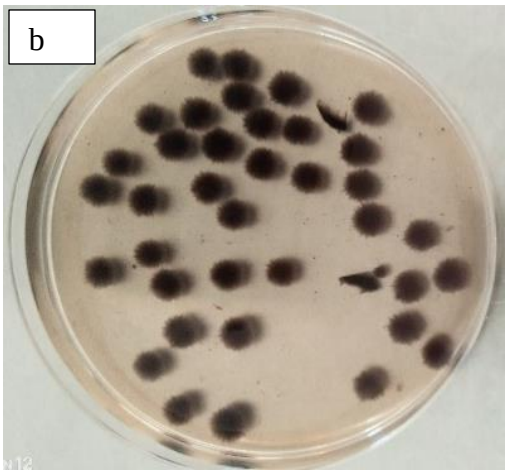

Fig. 4.6.4: (a (i)), control sample, (a (ii)), dye samples after *Eps*-treatment, (b) bio flocculation flocs after *Eps*-treatment

Table 4.5 shows the *Eps* that gave at least 80% dye removal efficiency with $MSYMgSO_4$ containing 20g/L molasses and corresponding $GPYMgSO_4$ values.

Table 4.5 *Eps* with at least 80% dye removal efficiency.

Isolate	**YEPD K_2^{-2}**			**NA+GM_2^{-1}**			**PDA B_2-5**		
Time (h)	24	48	72	24	48	72	24	48	72
$GPYMgSO_4$	69.91	72.961	84.686	-	74.038	77.38	-	-	84.853
$MSYMgSO_4$ (20g/L)	81.686	83.18	85.73	-	81.941	83.177	-	-	80.126

Figure 4.6.5 gives an illustration of the *Eps* that gave at least 80% dye removal efficiency with $MSYMgSO_4$ that contained 20g/L molasses and corresponding $GPYMgSO_4$ comparative broths.

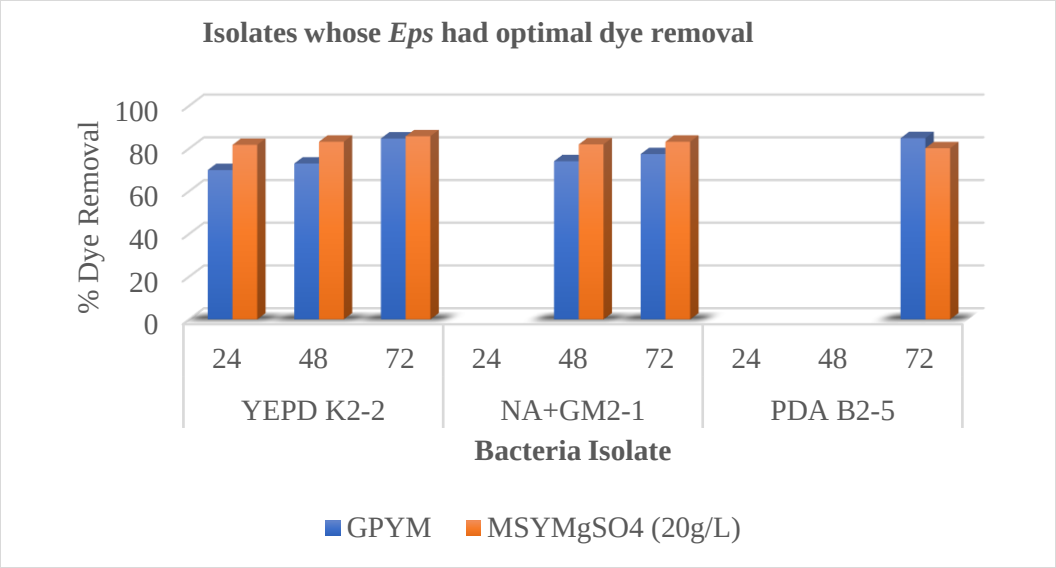

Fig. 4.6.5: *Eps* with at least 80% dye removal efficiency with MSYMgSO4 (20g/L molasses) and GPYMgSO4 broths.

Each of the *Eps* was further analyzed for the variance between the incubation time and the dye removal efficiency at 95% confidence level as shown in Figures. 4.6.6 (a &b). YEPD K_2^{-2} and NA+G M_2^{-1} *Eps* exhibited strong linear relationships between the dye removal efficiency and incubation time as shown by the high R^2 for both the $MSYMgSO_4$ (20g/L molasses) and $GPYMgSO_4$ broths. PDA B_2^{-5} *Eps* achieved the 80% dye removal minimum at 72 h incubation time only as shown in Table 4.6. The strong linear relationship between the dye removal and incubation time can be explained by the biosynthesis of *Eps* which has previously been found to increase with incubation time up to a maximum point where the synthesis starts to drop (Benhadria *et al*., 2017). Dye removal efficiency with *Eps* inoculations at 38^{o}C did not attain the 80% threshold by all the *Eps* while only the YEA M_2^{-4} *Eps* at 34 ^{o}C met the target indicating that *Eps* biosynthesis is hindered by high temperature, a finding that agrees with that reported by Hereher *et al*. (2018). High amounts of molasses (sucrose) in $MSYMgSO_4$ (20g/L molasses) were found to support the *Eps*

production better than the lower concentrations in MSYMgSO$_4$ (10g/L and 15g/L molasses) due to increased carbon content required by the bacteria for *Eps* production. This finding corresponds to that reported by Razack, Velayutham and Thangavelu (2013) who reported that 2% molasses sucrose yielded 2.66 g *Eps*/L compared to maltose and fructose which yielded 1.42 and 0.96 g *Eps*/L, respectively from *Bacillus subtilis* after incubation at 30 ^{o}C for 48 h. They also reported that different concentrations of cane molasses and rice bran in the media showed that 2% cane molasses and 5% rice bran produced the highest yields at 4.86 and 2.14 g/L. Hereher *et al*. (2018) reported that, among nine carbon sources they screened for *Eps* production, 4.5% sucrose was found to be the most efficient carbon source that gave highest yield of 45 g/L at 25 ^{o}C after 96 h incubation of *Micrococcus roseus*.

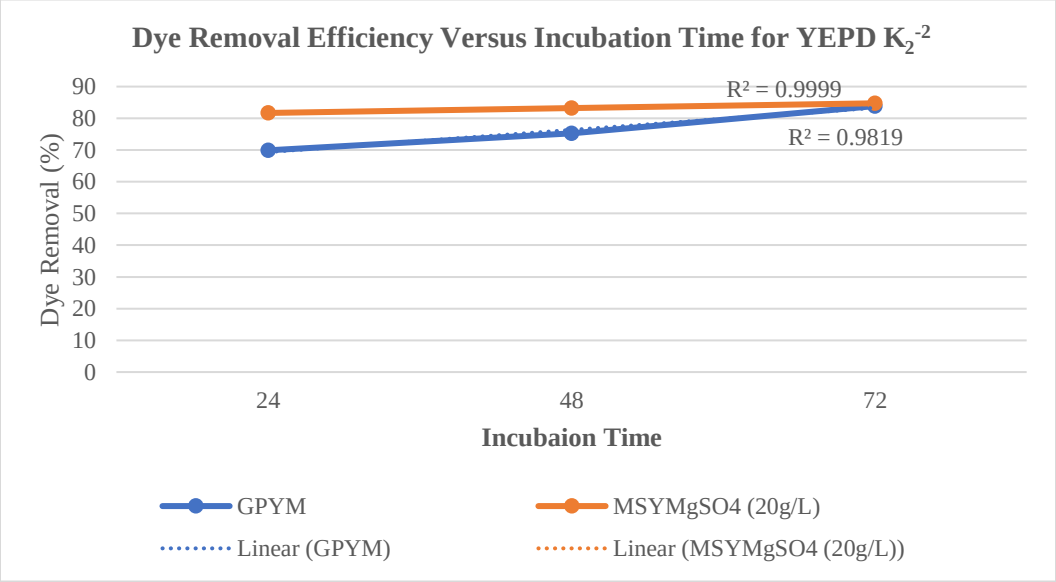

Fig. 4.6.6 (a): Dye Removal versus Incubation Time for YEPD K_2^{-2} *Eps*

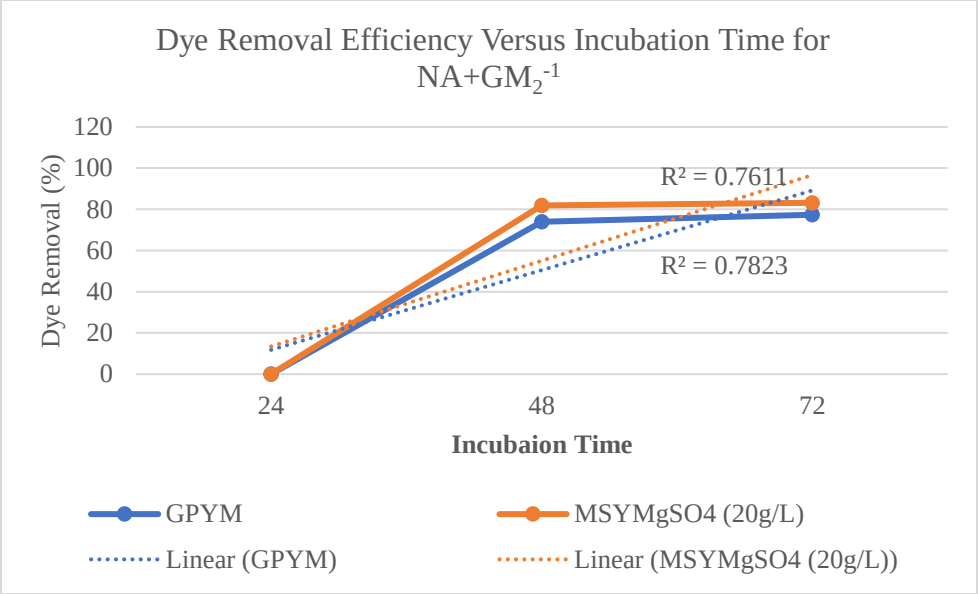

Fig. 4.6.6 (b): Dye Removal versus Incubation Time for NA+GM_2^{-1} *Eps*

4.7 Characterization of *Eps*-Treated Wastewater, *Eps* Extracts and *Eps*-Producing Bacteria Isolates

4.7.1 Physicochemical Characterization of the *Eps*-Treated Dye Wastewater

Figure 4.7.1 shows physicochemical properties of the *Eps* treated dye wastewater using the $MSYMgSO_4$ broth. Colour, salinity, turbidity, COD, BOD, Cu and Cr were found to be reduced while EC and TDS increased after the treatment at the optimized conditions of 72 h, 30 ^oC and 20g/L concentration of molasses in the $MSYMgSO_4$ broth by *Eps* from NA+G M_2^{-1}, YEPD K_2^{-2} and PDA B_2^{-5} as shown in the Figure. This finding indicates that molasses, as carbon source, enhanced production of *Eps* which in turn facilitated bioremediation of the wastewater. Degradation of organics in water has been reported to decrease levels of organic pollution in water such as turbidity (Ibrahim *et al*., 2020). pH was found to remain within the range of 7-8 as adjusted before the treatments.

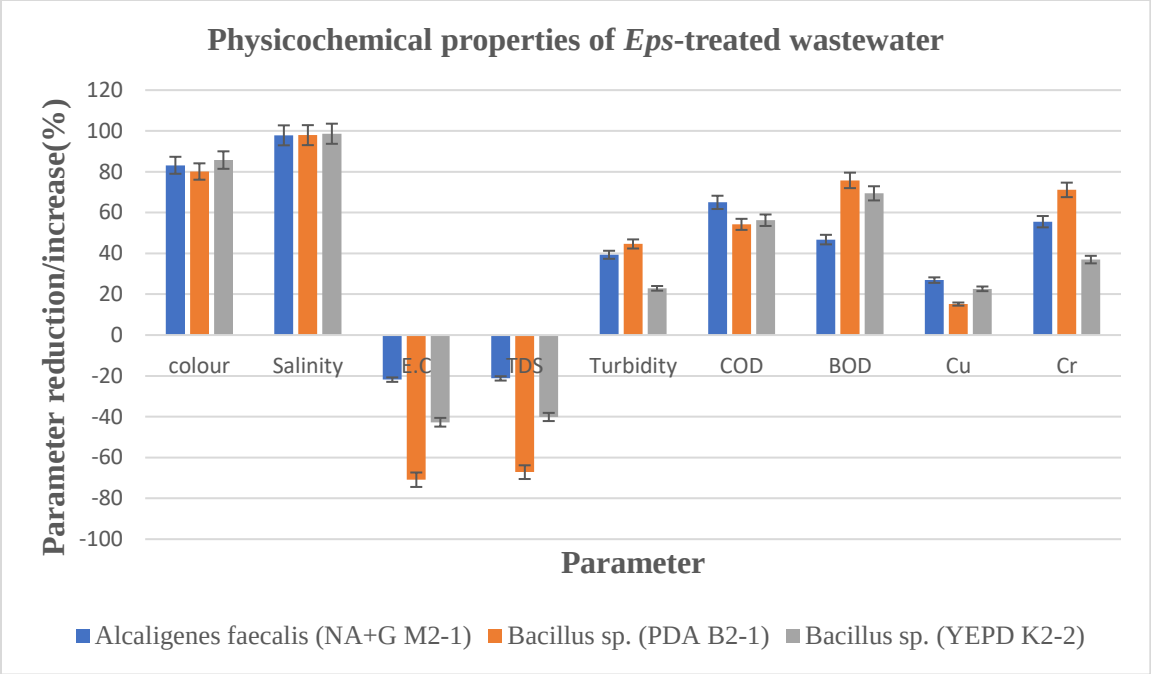

Fig. 4.7.1: Physicochemical properties of dye wastewater treated with *Eps*

Figure 4.7.2 shows the comparative results for the physicochemical properties of the optimized treatment of the dye wastewater with *Eps* and chemical coagulants.

Colour removal efficiency by the *Eps* from the three isolates was found to be comparable with that of the best coagulant, PFS. While PFS had the highest colour removal capacity of 86.088%, *Bacillus sp.* (YEPD K_2^{-2}) gave 85.73%, the highest compared with that of *Alcaligenes Faecalis* (NA+G_2^{-1}-83.17%) and *Bacillus sp.* (PDA B_2^{-5}-80.13%) as shown in Figure 4.7.2 (a). The three *Eps* were found to reduce the salinity of the wastewater by at least 90% compared with that of the optimized PaFC (19.667%) and PFS (3.424%) as shown Figure 4.7.2 (b). This can be attributed the sodium sulphate and sodium carbonate contained in the wastewater and the magnesium sulphate in the broth that may have enhanced the *Eps* production during the wastewater bioremediation process. *Eps* is reported to bind with Na^+ salt ions, minimizing the salinity levels reducing salt stress. Rise

in salt concentration also drives an increase in bacterial *Eps* synthesis, which enhances Na+ chelation (Arora *et al*., 2010). Nigam *et al*. (2001) utilized a microbial consortium consisting of *Alcaligenes Faecalis* and *Commamonas acidovorans* and reported 67-89% decolorization of azo-diazo and reactive dyes. While chemical coagulants reduced EC and TDS of the treated wastewater, the three *Eps* on the contrary, were found to cause rise in the two properties with *Bacillus sp.* giving the highest values as shown in Figure 4.7.2 (c & d). This can be supported by the fact that EC is directly proportional to TDS (Uwidia and Ukulu, 2013). PaFC at optimized conditions (pH 7 and 280g/L) was found to reduce turbidity by higher percentages (>90%) compared to the three *Eps*. *Bacillus sp.* (PDA B_2^{-5}) *Eps* gave the highest reduction in turbidity compared to *Bacillus sp.* (YEPD K_2^{-2}) and *Alcaligenes Faecalis* (NA+G_2^{-1}) as shown in Figure 4.7.2 (E). Figure 4.7.2 (f) shows that COD reduction by alum (68.573%) and *Alcaligenes Faecalis* (NA+G_2^{-1}) (65.007%), the best in chemical coagulants and *Eps*, was comparable.

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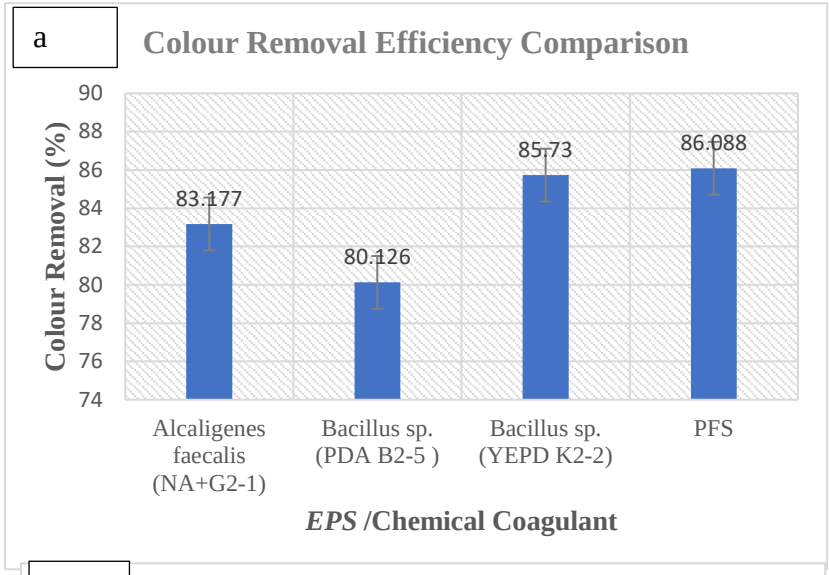
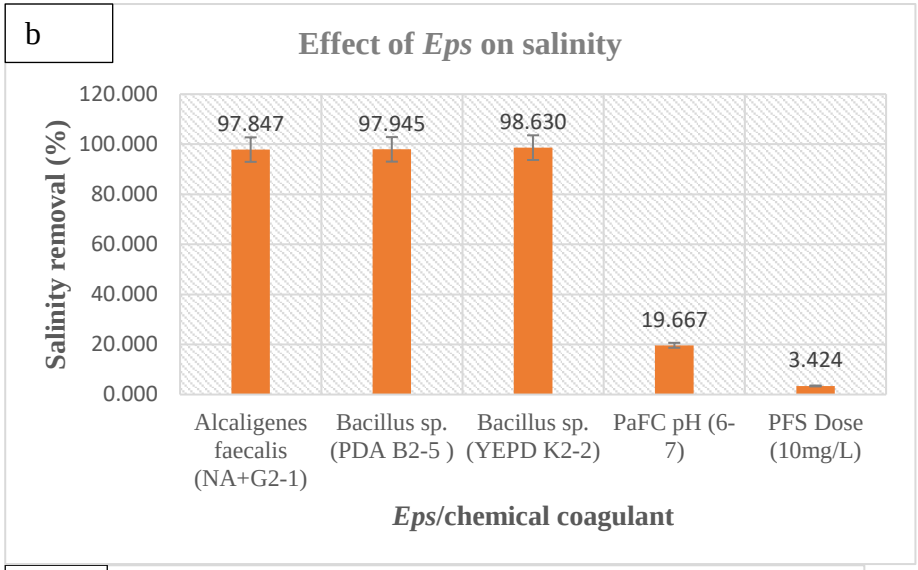
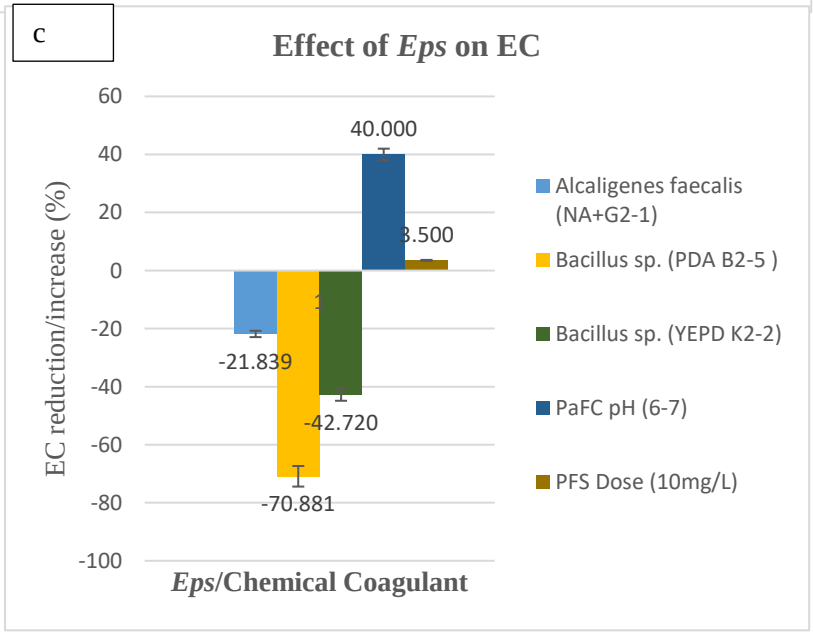
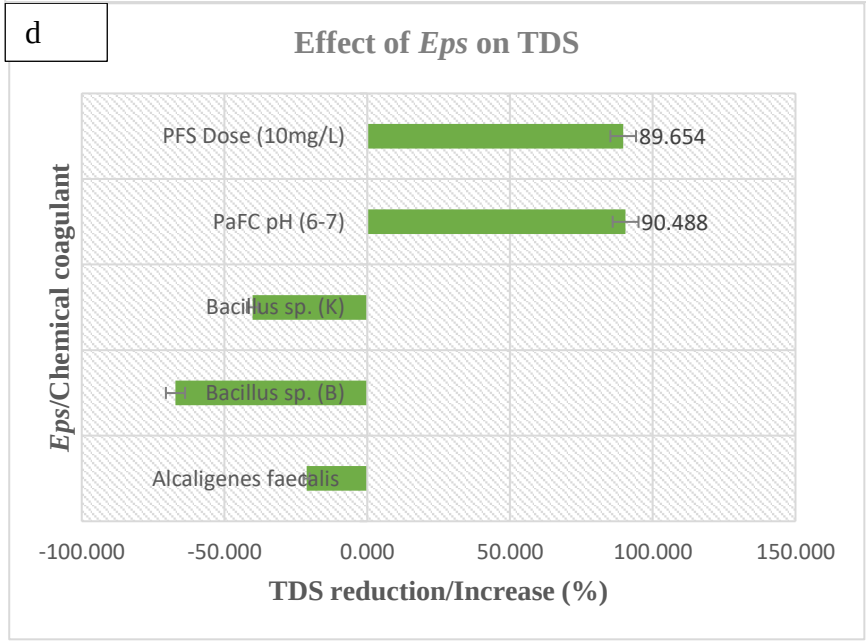

a
Colour Removal Efficiency Comparison
Colour Removal (%)
90
88
86
84
82
80
78
76
74
83.177
80.126
85.73
86.088
Alcaligenes faecalis (NA+G2-1)
Bacillus sp. (PDA B2-5)
Bacillus sp. (YEPD K2-2)
PFS
EPS /Chemical Coagulant


b
Effect of Eps on salinity
Salinity removal (%)
120.000
100.000
80.000
60.000
40.000
20.000
0.000
97.847
97.945
98.630
19.667
3.424
Alcaligenes faecalis (NA+G2-1)
Bacillus sp. (PDA B2-5)
Bacillus sp. (YEPD K2-2)
PaFC pH (6-7)
PFS Dose (10mg/L)
Eps/chemical coagulant


c
Effect of Eps on EC
EC reduction/increase (%)
60
40
20
0
-20
-40
-60
-80
-100
40.000
-21.839
-42.720
-70.881
Alcaligenes faecalis (NA+G2-1)
Bacillus sp. (PDA B2-5)
Bacillus sp. (YEPD K2-2)
PaFC pH (6-7)
PFS Dose (10mg/L)
Eps/Chemical Coagulant


d
Effect of Eps on TDS
Eps/Chemical coagulant
PFS Dose (10mg/L)
89.654
PaFC pH (6-7)
90.488
Bacillus sp. (K)
Bacillus sp. (B)
Alcaligenes faecalis
-100.000
-50.000
0.000
50.000
100.000
150.000
TDS reduction/Increase (%)

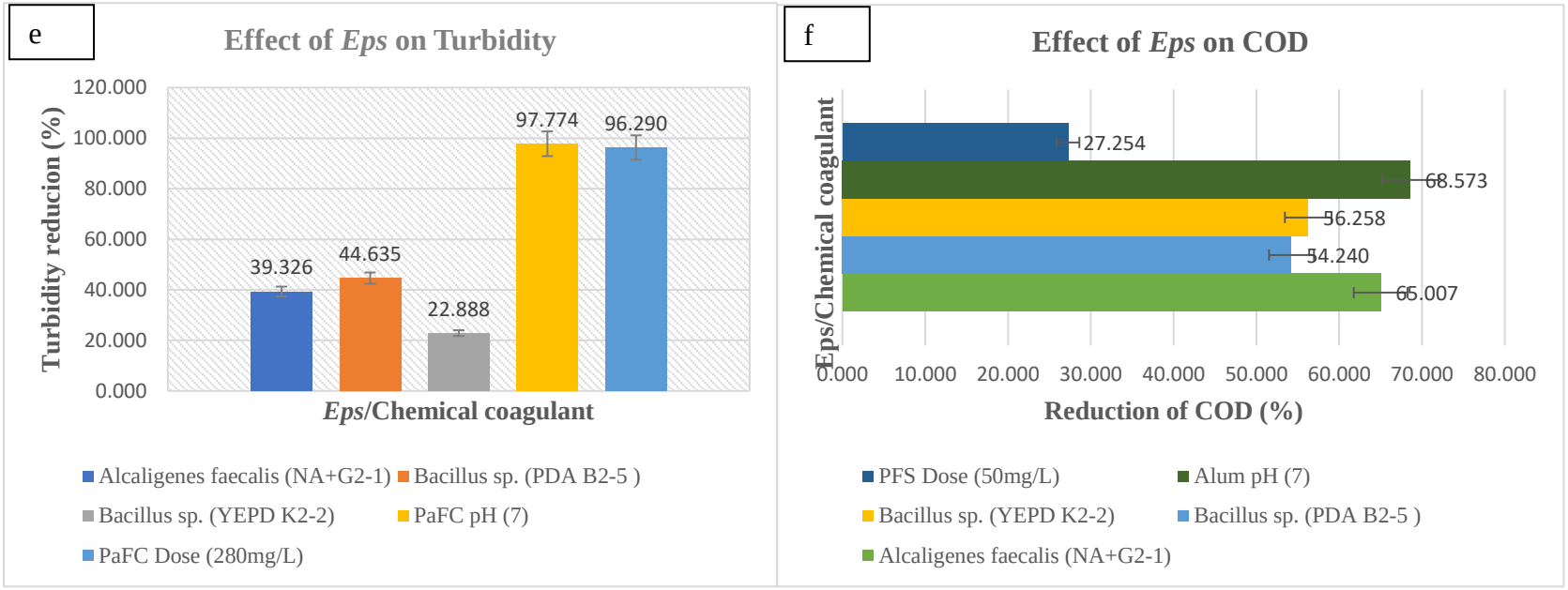


Fig. 4.7.2: Comparative results for the physicochemical properties of the chemical coagulants and *Eps*-treated dye wastewater.

Comparatively, Bisht and Lal (2019) reported a bioflocculant BF-VB2, produced by strain *Bacillus sp.* TERI VB2 that showed remarkable reduction of turbidity, dye colour, COD and TSS by 99.6%±1.0%, 82.78%±3.03%, 92.54%±0.24% and 73.59%±0.71% respectively from wastewater.

Table 4.6 gives a summary of physicochemical properties of the dye wastewater sample optimally treated at PH 6-8 by the respective *Eps*. The values for each of the parameters were compared with the water quality regulations set by National Environment Management Authority. All the *Eps*-treated samples met the pH, colour, TDS, BOD and COD limits.

Table 4.6 Physicochemical properties of the *Eps*-treated dye wastewater in comparison with NEMA limits

Parameter	**Untreated wastewater**	***Alcaligenes Faecalis* ($NA+G_2^{-1}$)**	***Bacillus sp.* (PDA B_2^{-5})**	***Bacillus sp.* (YEPD K_2^{-2})**	**NEMA Limits**
pH	10.23	6-8	6-8	6-8	6-9
Color (Hazen)	>250	80	85	70	≤40
TDS (mg/L)	132.8	161	222	150	2,000
BOD (mg/L)	350	186	84	107	500
COD (mg/L)	1486	520	680	650	1,000
Pb(mg/L)	nd				1.0
Cd(mg/L)	nd				0.2
Cu(mg/L)	0.0234	0.0171	0.0199	0.0181	1.0
Cr (Total)(mg/L)	0.097	0.0431	0.028	0.0611	2.0
[Nd] Not detected					

Bacillus sp. isolated from tannery activated sludge was reported to produce *Eps* able to reduce 50 mg/L of Cr (VI) within 24h under aerobic conditions (Zhu *et al*., 2019). *Bacillus sp.* has previously also been reported to remove 48% Cr from aqueous solution (Chug *et al*., 2016).

4.7.2 FTIR Analysis of the *Eps* -Treated Dye Wastewater

The untreated and *Eps*-treated wastewater displayed spectra as shown in Figure 4.7.3 (a-d) characteristic with the carbonyl and hydroxyl groups at 1500-1750 cm^{-1} and 3000-3500 cm^{-1}. Since there was no any new peak or shift of absorbance spectrum before and after the *Eps*- treatment, the decolorization was attributed to adsorption mechanism (Veena, Rao and Venkata, 2019; Mishra and Maiti, 2020; Maniyam, Ibrahim and Cass, 2020). This is because the dye structure remains unchanged during the dye removal process. The adsorption was associated to 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 adsorption of RB5 dye on carbon nanotubes occurred mostly on hydroxyl (-OH) and carboxyl (-COOH) groups which were predominant before and after the treatment. RB5 bioremediation could therefore be done in combination with membrane filtration in order to get rid of the flocs formed during the process to ensure safe disposal of the dye sediment.

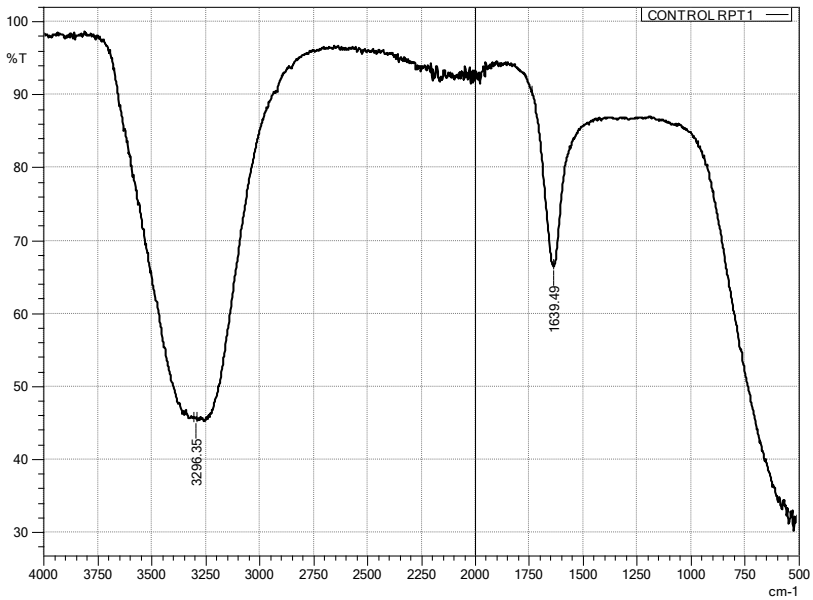


Fig. 4.7.3 (a): FTIR spectrum for untreated dye wastewater

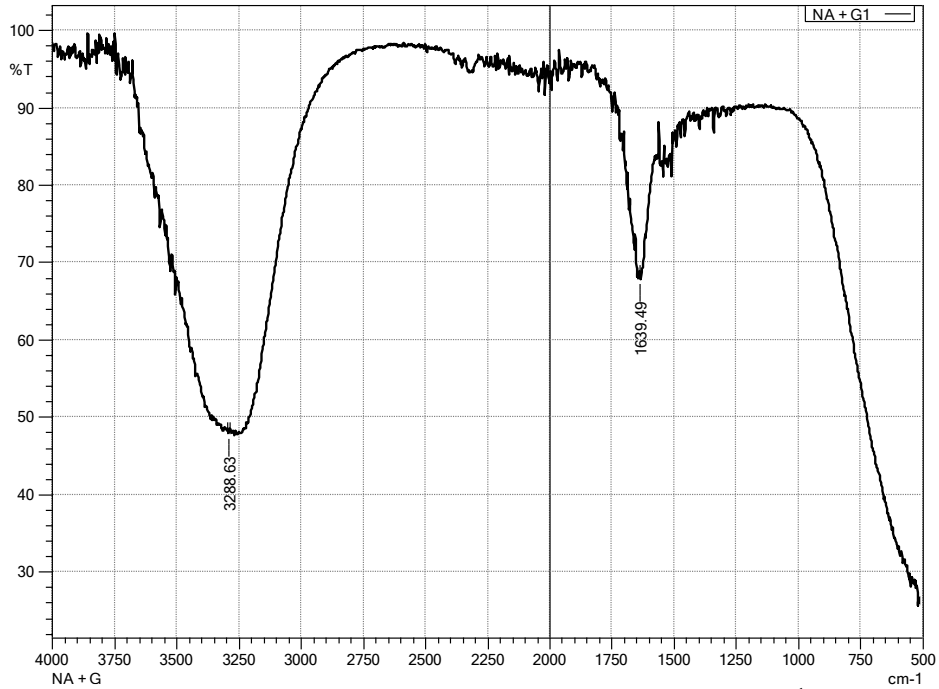


Fig. 4.7.3 (b): FTIR spectrum for *Alcaligenes Faecalis* (NA+G M_2 $^{-1}$) *Eps*-treated dye wastewater

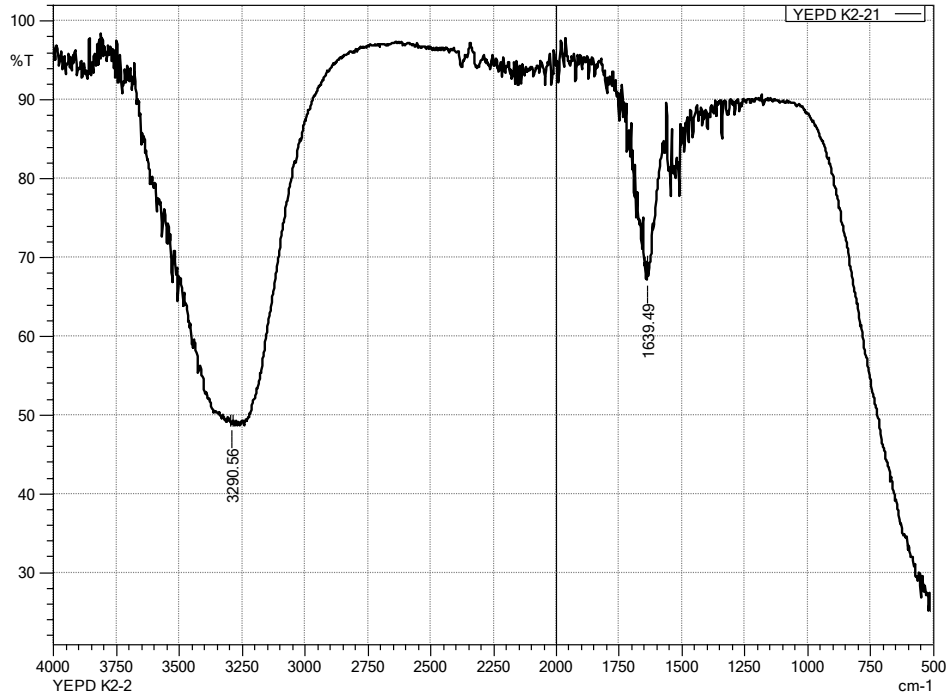


Fig. 4.7.3 (c): FTIR spectrum for *Bacillus sp.* (YEPD $K_2$$^{-2}$) *Eps*- treated dye wastewater

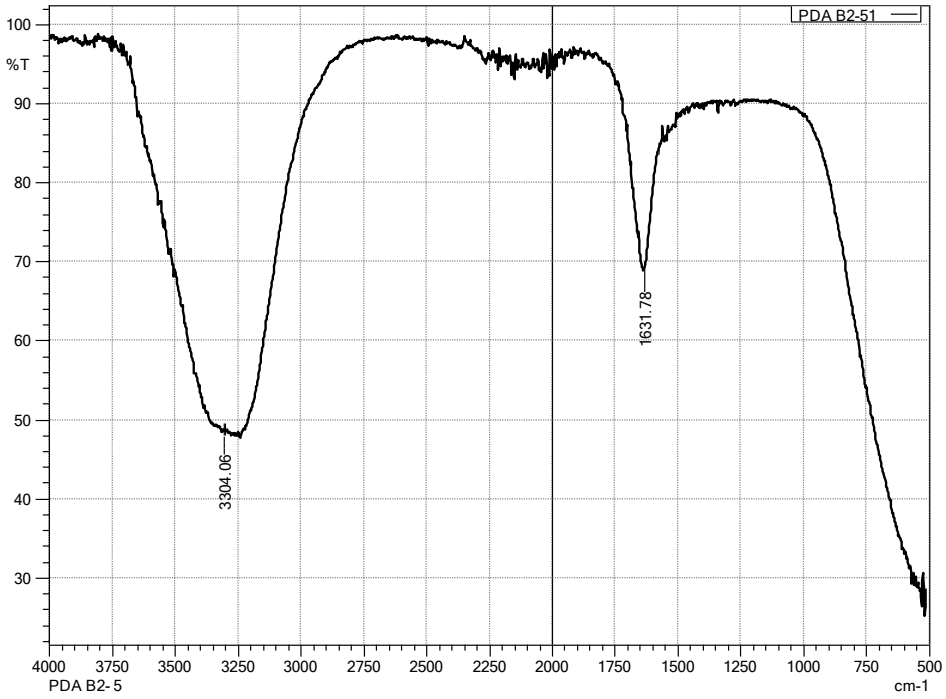


Fig. 4.7.3 (d): FTIR spectrum for *Bacillus sp.* (PDA B_2^{-5}) *Eps*-treated dye wastewater

4.7.3 UV-Vis Spectrophotometer Analysis of the *Eps* -Treated Dye Wastewater

Figure 4.7.4 (a) and (b) shows the spectra for the control (untreated) sample and the samples treated the three *Eps*. The peak for the Reactive black dye at 597nm in the untreated samples disappeared after the treatment with the *Eps* from all the three *Eps* confirming removal of the dye.

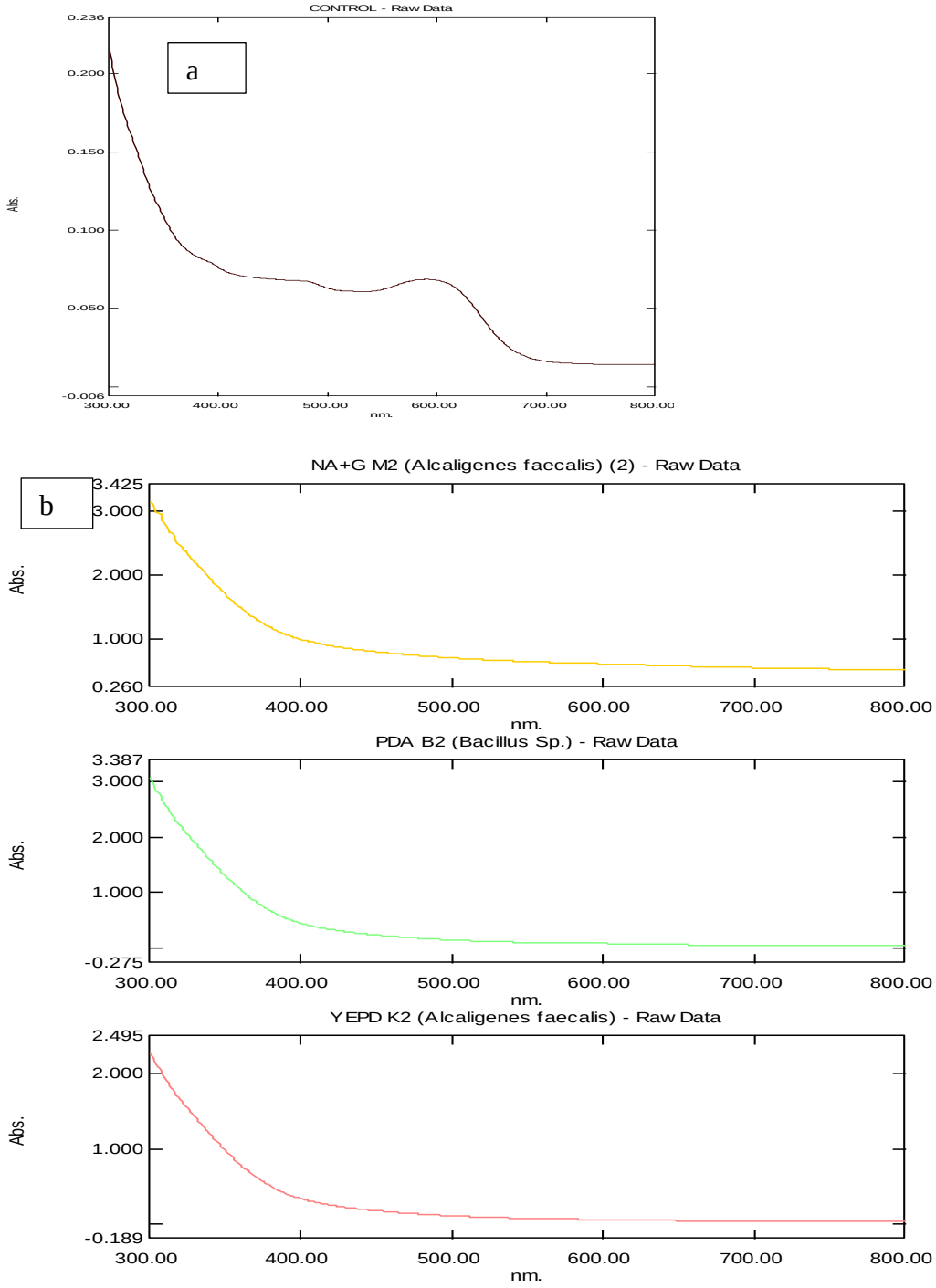


Fig. 4.7.4: Spectra for untreated (a) and *Eps*-treated wastewater (b)

4.7.4 Characterization of *Eps*-Producing Bacteria Isolates

4.7.4.1 Biochemical Characterization

Figure 4.7.5 (a, b & c) shows the gram staining results for the three bacteria isolates whose *Eps* were found to have optimal activity (at least 80%) on the dye wastewater.

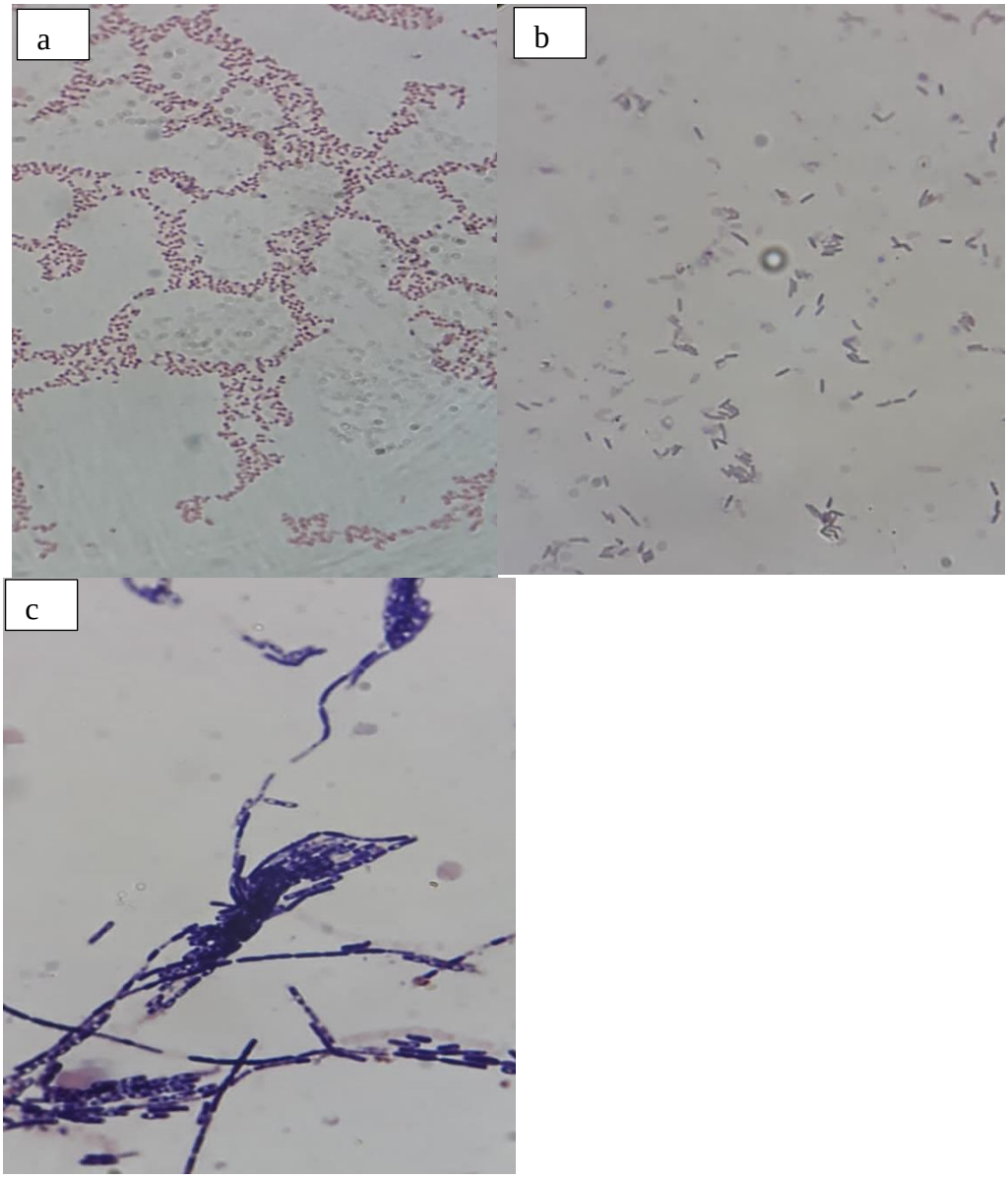


Fig. 4.7.5. Gram Stain results: (a) NA+G M_2^{-1} isolate; negative diplococci, (b) YEPD K_2^{-2} isolate; positive diplobacilli, (c) PDA B_2^{-5} isolate; positive streptobacilli

Table 4.7.1 and 4.7.2 show the respective biochemical and sugar fermentation results for the three isolates. All the three isolates were found to have the capacity to ferment simple sugars including sucrose in the sugarcane molasses.

Table 4.7.1. Biochemical test results

Isolate	**TSI**				**SIM**			**Methyl red**	**Voges Proskauer**	**Nitrate Reductase**	**Citrate Utilization**	**Urease Test**	**Starch Hydrolysis**
	Slant	**Butt**	**Gas**	**H_2S**	**H_2S**	**Indole**	**Motility**						
YEAPD K_2^{-2}	Alkaline	Acidic	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
NA+G M_2^{-1}	Alkaline	Acid	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve
PDA B_2^{-5}	Alkaline	No change	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve

Table 4.7.2. Sugars fermentation results

Sugar	YEPD K_2^{-2}		PDA B_2^{-5}		NA+G M_2^{-1}	
	Gas	Acid	Gas	Acid	Gas	Acid
Lactose	+ve	-ve	-ve	+ve	-ve	+ve
D.Glucose	+ve	+ve	-ve	+ve	+ve	+ve
Fructose	+ve	+ve	-ve	+ve	+ve	+ve
Sorbitol	+ve	+ve	-ve	+ve	+ve	+ve
Sucrose	+ve	+ve	-ve	+ve	+ve	+ve
Mannitol	+ve	+ve	-ve	+ve	+ve	+ve
Galactose	+ve	+ve	-ve	+ve	+ve	+ve
Rhaminose	+ve	+ve	-ve	+ve	+ve	+ve
Arabinose	+ve	+ve	-ve	+ve	+ve	+ve
Dulcitol	-ve	-ve	-ve	+ve	+ve	-ve
Mannose	+ve	+ve	-ve	+ve	+ve	+ve
Raffinose	+ve	+ve	-ve	+ve	+ve	+ve
Ribose	+e	+ve	-ve	+ve	+ve	+ve

Figure 4.7.6 illustrates the starch hydrolysis test results. NA+G M_2^{-1} (1), YEPD B_2^{-1} (4) and YEPD K_2^{-2} (6) isolates did not hydrolyze starch. YEA M_2^{-4} (2), PDA B_2^{-5} (3) and YEA K_2^{-5} (5) isolates had the capacity to hydrolyze starch as shown in Figure 4.7.6.

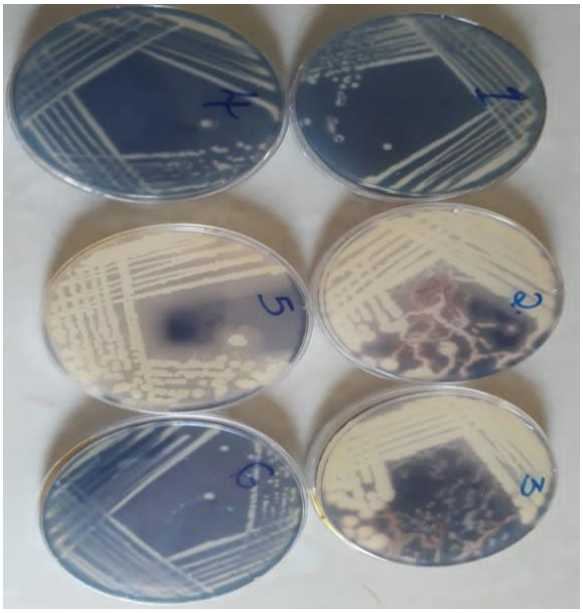


Fig. 4.7.6: Starch Hydrolysis Test Results

4.7.4.2 Molecular Characterization

Table 4.8 shows the results obtained from molecular characterization of the isolates

Table 4.8 Identification of the bacterial isolates

Isolate	NA+G M_2^{-1}	YEPD K_2^{-2}	PDA B_2^{-5}	YEA M_2^{-4}
Predicted Organism	*Alcaligenes faecalis*	*Bacillus sp.*	*Bacillus sp.*	*Alcaligenes faecalis*
% Identity	99.84%	99.93%	99.86%	98.15%

Two of the candidate bacteria isolates cultured from the gin trash soil collected from Meru ginnery were identified to be *Alcaligenes Faecalis* while the ones from Kitu and Baringo soils were both *Bacillus sp.*

4.7.5 Extraction and Characterization of *Eps*

4.7.5.1 Extraction of *Eps*

The *Eps* produced by the bacteria isolates in MSYMgSO$_4$ medium containing 20g/L molasses, and found to have optimal dye removal capacity were extracted and quantified. Table 4.9 gives the quantities of the *Eps* produced by the respective isolates. The same bacteria isolates, grown in the same medium but without adding molasses, SYMgSO$_4$, produced negligible amounts of *Eps*. This finding supports the report by Liu, Chu & Chou (2011) that sugarcane molasses contains sucrose, glucose and fructose as sugars, vitamins and mineral contents significant for *Eps* production.

Table 4.9. *Eps* production by isolates

***Eps* Producer Bacteria**	***Eps* Production (g/L)**
Alcaligenes Faecalis (YEA M_2^{-4})	1.48
Bacillus sp. (YEPD K_2^{-2})	3.74
Alcaligenes Faecalis (NA+G M_2^{-1})	4.84
Bacillus sp. (PDA B_2^{-5})	1.66

Alcaligenes Faecalis (NA+G M_2^{-1}) produced the highest amounts of *Eps* followed by *Bacillus sp.* (YEPD K_2^{-2} and *Bacillus sp.* (PDA B_2^{-5}) respectively. *Eps* yield varies with medium composition and the process parameters such as temperature, pH and time.

Figure 4.7.7 illustrates the production of *Eps* by the respective bacterial isolates.

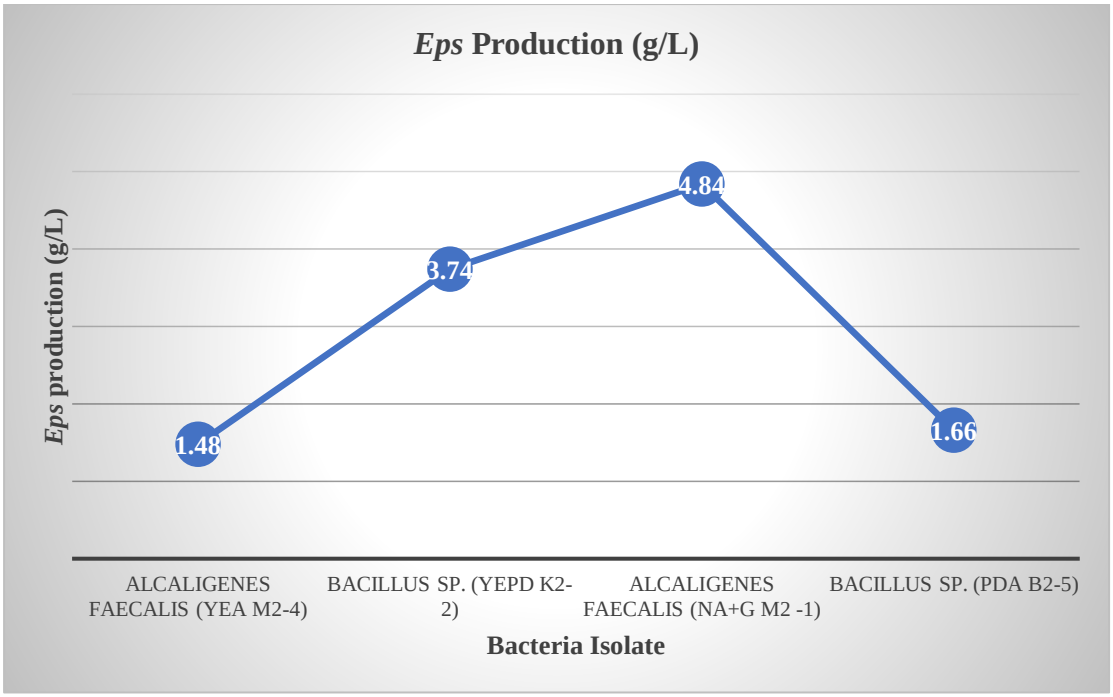


Fig. 4.7.7: *Eps* production by bacteria isolates

Compratively, Hassan and Ibrahim (2017) reported production of 18 gL^{-1} *Eps* by *Bacillus Subtilis* using YMG medium nd attained the highest yield of 44 gL^{-1} by the fouth day. Donot *et al*. (2012) also reported that *Bacillus sp.* produced was able to prouce 86.3 gL^{-1} *Eps* that was higher compared to that obtained from *agrobacterium sp*. (76 gL^{-1}) and *Alcaligenes faecalis*, *Xanthomonas campestris* and *Zymonas mobilis* (72, 53 and 50 g/L) respectively, among the *Eps*-producing bacteria.

Figure 4.7.8 ((a) and (b)) shows the comparative extraction of *Eps* between $MSYMgSO_4$ (precipitated) and $SYMgSO_4$ (non-precipitated) respectively. Figure 4.7.8 (c) shows the solid *Eps* extracted from the $MSYMgSO_4$ broth medium. $SYMgSO_4$ however did not support *Eps* production confirming that molasses played a critical role in promoting *Eps* production.

Fig. 4.7.8; Comparative extraction of *Eps* between MSYMgSO4 (precipitated) (a) and SYMgSO4 (non-precipitated) (b)

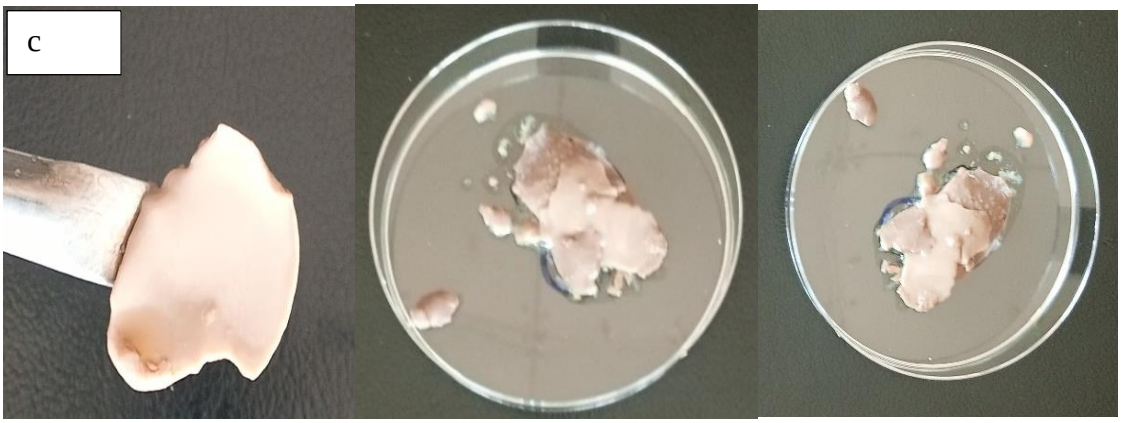


Fig. 4.7.8(c): *Eps* extracts from MSYMgSO4 broth medium

4.7.5.2 Characterization of *Eps*

4.7.5.2.1 Characterization of Eps by GC-MS

GC-MS results for *Alcaligenes Faecalis* (NA+G M_2 $^{-1}$) *Eps* indicated that it was composed of beta-D-Fructopyranose sugar derivative whose peak was produced at retention time, 17.476 min and constituted of 6.4 % area of the homopolysaccharide biopolymer. Figure 4.7.9 shows the GC chromatograph with peak number 5 representing the sugar and the corresponding MS spectrum (inset).

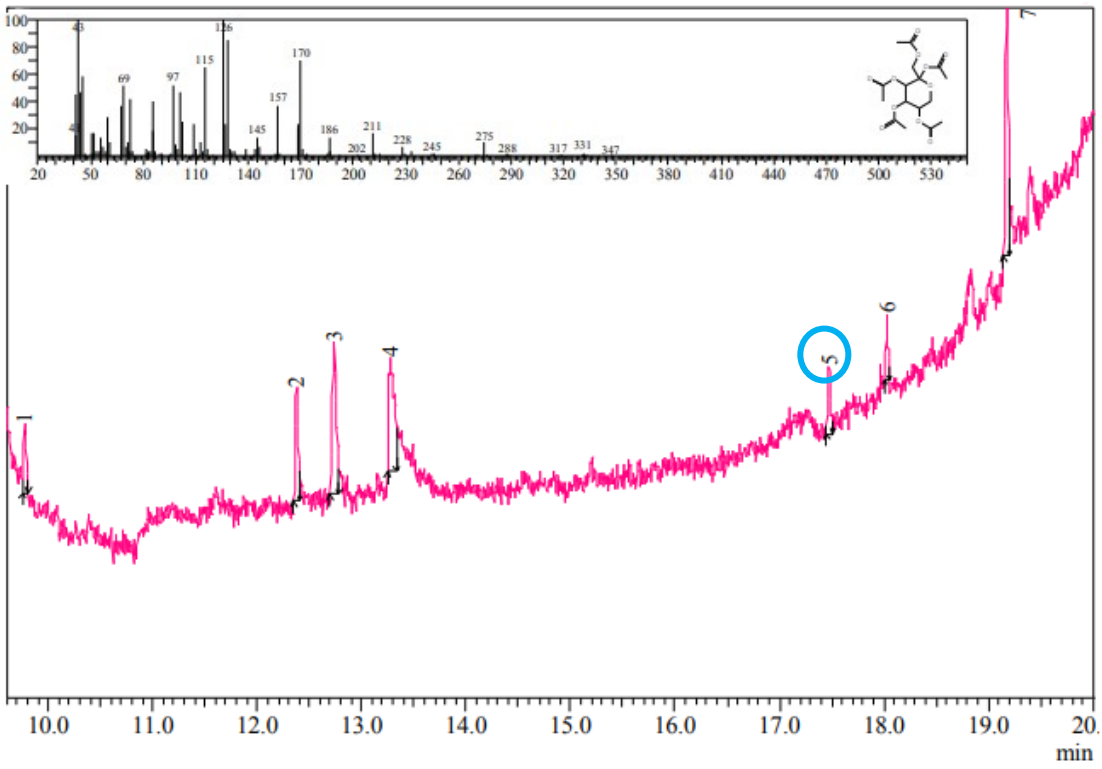


Fig. 4.7.9: GC-MS chromatograph and corresponding MS spectrum (inset) for *Alcaligenes Faecalis* (NA+G M_2 $^{-1}$) sugar derivative.

Bacillus sp., (YEPD $K_2$$^{-2}$) *Eps* was found to be composed of Tetra-O-acetyl-d-Manno pyranose and 1, 3, 4, 6-Tetra-O-acetyl-. beta. -d-glucopyranose sugar derivatives whose peaks were produced at retention time, 18.583 min and 18.512 min constituting 2.53 % and 2.24 % area of the heteropolysaccharide biopolymer respectively. Figure 4.7.10

shows the GC chromatograph and the respective sugars corresponding MS spectra (inset).

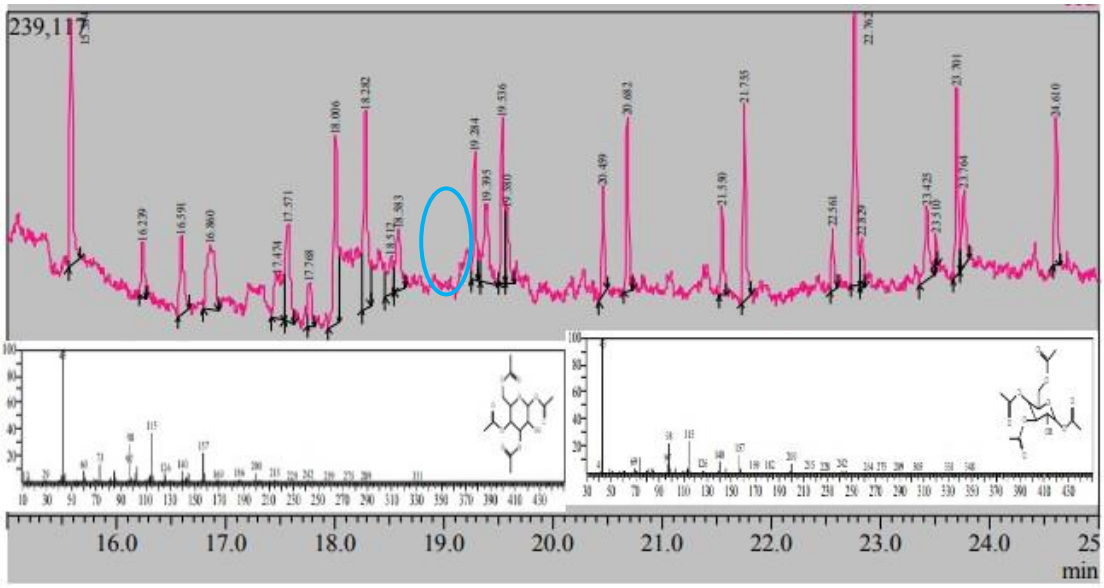


Fig. 4.7.10: GC-MS chromatograph and corresponding MS spectrum (inset) for *Bacillus sp.*, (YEPD K_2^{-2}) sugar derivatives.

Bacillus sp., (PDA B_2^{-5}) *Eps* was found to be composed of beta-d-Fructofuranose, Sorbose, alpha-D-Glucopyranose, D-Glucose and D-Mannitol sugar derivatives. The respective peaks were produced at retention time, 15.515, 17.469, 18.525, 18.595 and 19.180 min. The sugar derivatives constituted 4.26 %, 2.81%, 3.71%, 11.97% and 1.07 % area of the heteropolysaccharide biopolymer respectively. Figures 4.7.11 (a) and (b) show the GC chromatograph and the corresponding sugars MS spectra.

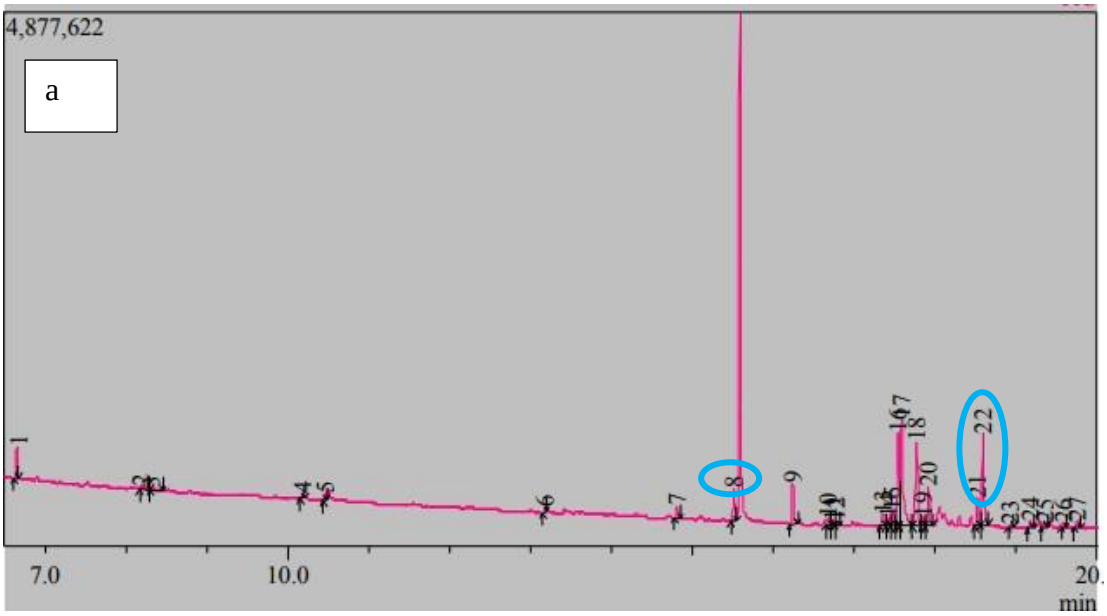
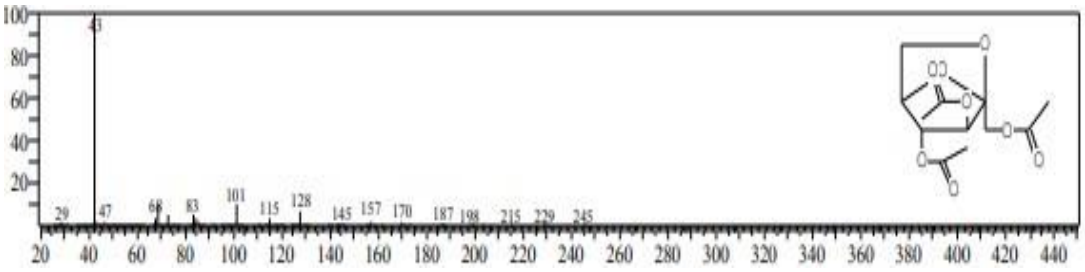
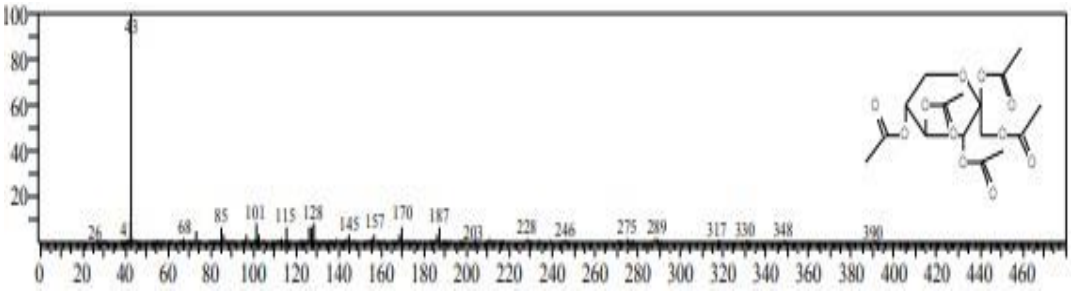


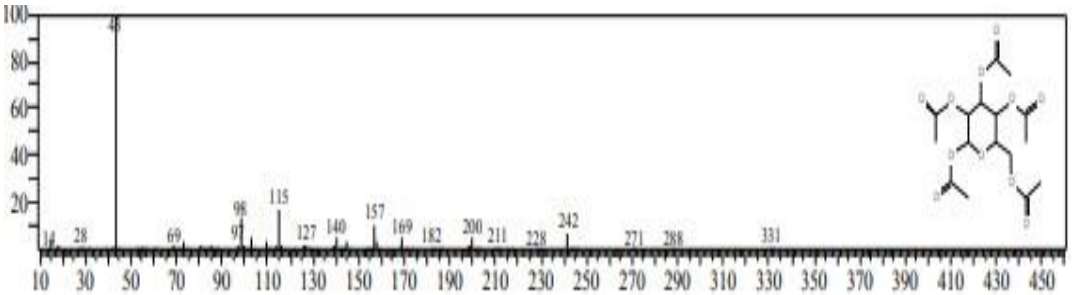
Fig. 4.7.11 (a): GC-Ms chromatograph for *Bacillus sp*., (PDA B_2-5) sugar derivatives.



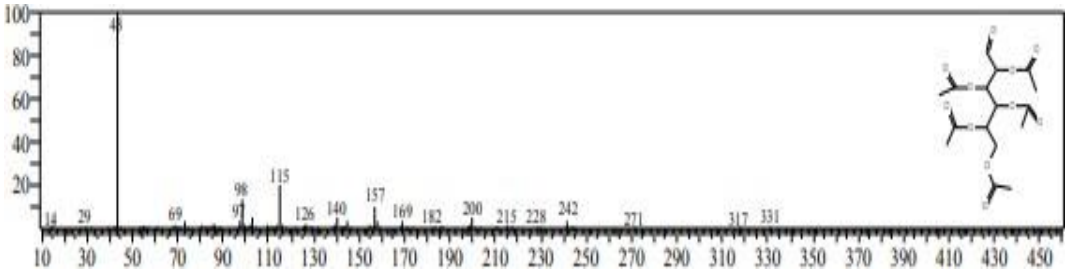
beta-d-Fructofuranose



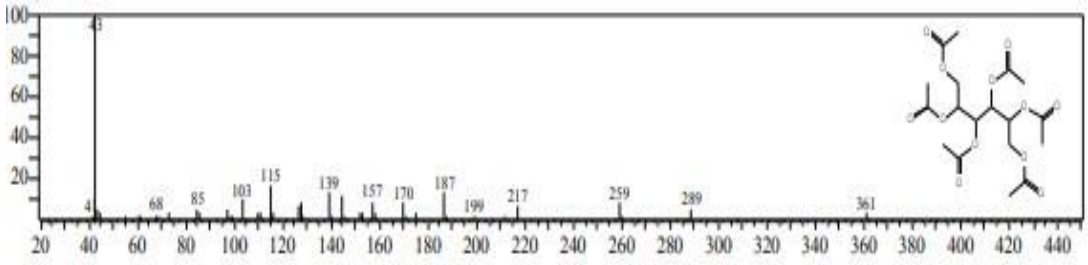
Sorbose



alpha-D-Glucopyranose



D-Glucose



D-Mannitol

Fig. 4.7.11 (b): MS spectra for the *Bacillus sp.*, (PDA B_2^{-5}) sugar derivatives

Monosaccharides contain five or six carbon atoms which can cyclize by condensation of an alcohol (OH) with the carbonyl carbon to form a new asymmetric carbon resulting into ring systems referred to as furanose (five-membered ring) or pyranose (six-membered ring). For example, the hydroxyl group on carbon 5 in D-glucose, attacks the carbonyl carbon forming α-D-Glucopyranose (Sohaib, 2015).

Monosaccharide composition of *Eps* by other Bacillus strains such as *Bacillus cereus* AR156 was found to be mannose (70.97%), galactose (17.59%) and glucose (11.45%) whereas that of *Bacillus licheniformis* was glucose (54.38%), mannose (25.24%) and galactose (11.32%) (Aadil *et al*., 2019).

4.7.5.2.2 Characterization of *Eps* by FTIR

All the samples gave comparable FTIR spectra as shown in Figure 4.7.12 (a-c). All the *Eps* displayed a broad band between 3000-3500 cm^{-1} that represents the stretching vibration of hydroxyl groups (-OH) characteristic with a carbohydrate ring (Hu *et al*.,

2019; Kumar, Anandapandian and Parthiban, 2011). A C–H stretching vibration was displayed at 2933 cm^{-1} for *Alcaligenes Faecalis* (NA+G M_2^{-1}), at 2879 cm^{-1} for *Bacillus sp.*, (YEPD K_2^{-2}) and at 2929 cm^{-1} for *Bacillus sp.* (PDA B_2^{-5}) *Eps*. This band is usually present in polysaccharides (Ismail and Nampoothiri, 2010). The stretching bands of carbonyl (-C=O), carboxyl (-COOH) and amide (+NH_2) groups were recorded between 1300-1750 cm^{-1} for all the exopolysaccharides (Zayed *et al*., 2018; Reda *et al*., 2022). In addition, a band within 1300-1150 cm^{-1} that was characteristic with the *Eps* from all the bacteria isolates is due to -C–O. C-O stretching band could be assigned to either, alcohol or sugar derivatives groups (Cao *et al*., 2020). Monic and Gurunathan (2022) reported similar results for *Eps* obtained from *Virgibacillus dokdonensis* isolated from water samples collected from a saltern region in Kumta, the Arabian Sea Coast of India. All the *Eps* displayed stretching bands between 900-1100 cm^{-1} that indicates the presence of a glycoside link (C-O-C) (Reda *et al*., 2022). Mohamed and Walaa (2016) reported that the -C=O and NH_2 groups of *Eps* are involved in the adsorption of dyes. Further, Saxena *et al*. (2020) observed that adsorbents containing hydroxyl (-OH), carboxyl (-COOH), and carboxylate (-COO-) functional groups can remove organic contaminants, especially dyes, through hydrogen bonding and electrostatic interaction.

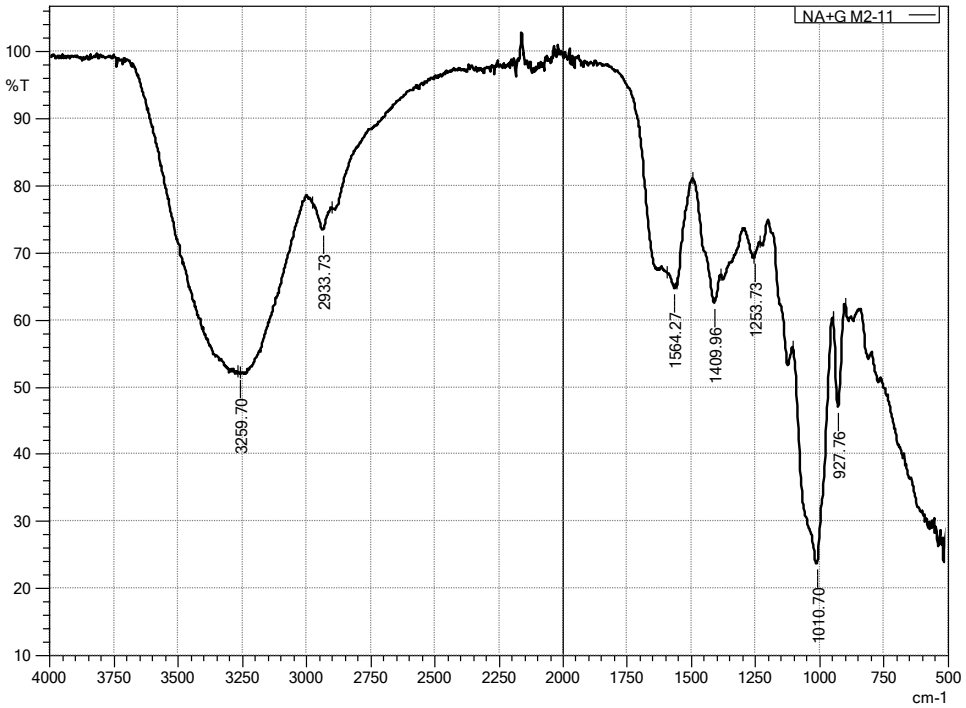


Fig. 4.7.12 (a): FTIR spectrum for *Eps* obtained from *Alcaligenes Faecalis* (NA+G M_2^{-1})

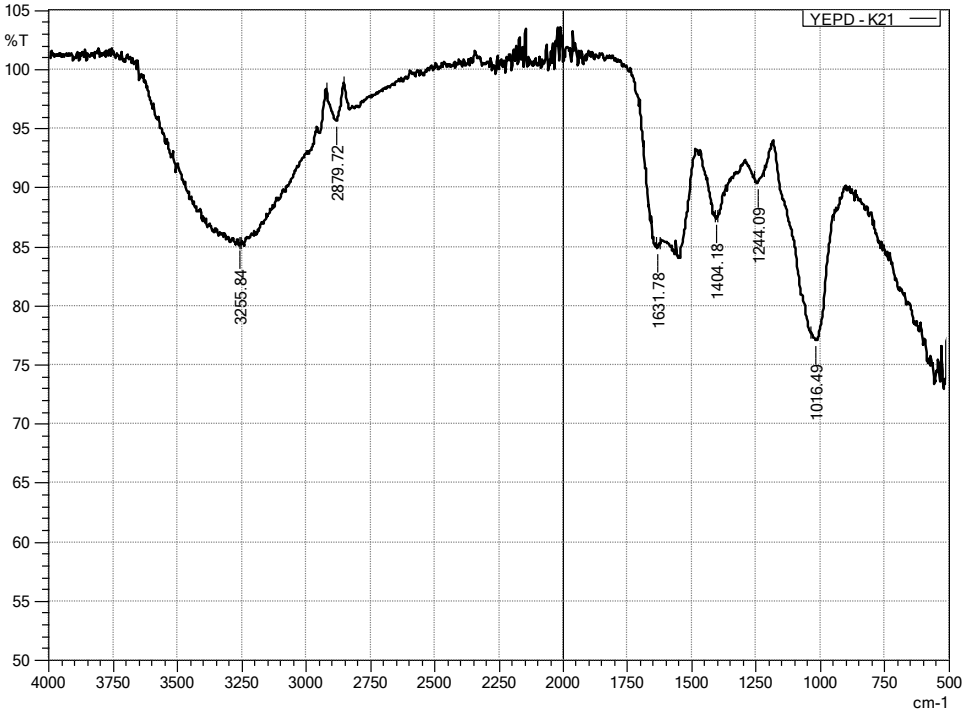


Fig. 4.7.12 (b): FTIR spectrum for *Eps* obtained from *Bacillus sp.* (YEPD K_2^{-2})

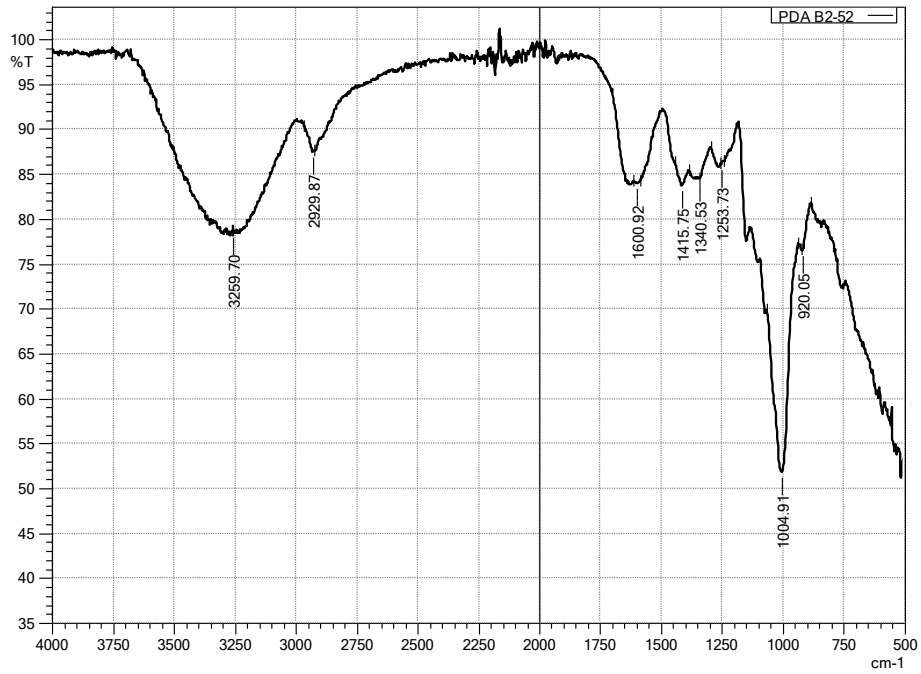


Fig. 4.7.12 (c): FTIR spectrum for *Eps* obtained from *Bacillus sp.* (PDA B_2^{-5})

4.7.5.2.3 Characterization of *Eps* by SEM Imaging

Eps produced by *Alcaligenes Faecalis* (NA+G M_2^{-1}) exhibited a dense porous microstructure asshown in Figure 4.7. 13 (a). *Bacillus sp.*, (YEPD K_2^{-2}) *Eps* on the other hand showed a dense and porous mcrostucture which was finer and denser than that of the *Alcaligenes Faecalis* (NA+G M_2^{-1}) as shown in Figure 4.7. 13 (b). The finer and denser structure of the *Bacillus sp.*, (YEPD K_2^{-2}) *Eps* justifies the slightly higher colour removal (85.73%) compared to that of *Alcaligenes Faecalis* (NA+G M_2^{-1}), 83.177%. *Bacillus sp.* (PDA B_2^{-5}) *Eps* microstructure was more compact and characterized with larger pores network as shown in Figure 4.7. 13 (c). This explains the lowest efficiency in dye removal due to lesser trapping and retention of the dye ions by the open structure.

Fig. 4.7. 13 (a): *Alcaligenes Faecalis* (NA+G M_2^{-1}) *Eps* microstructure

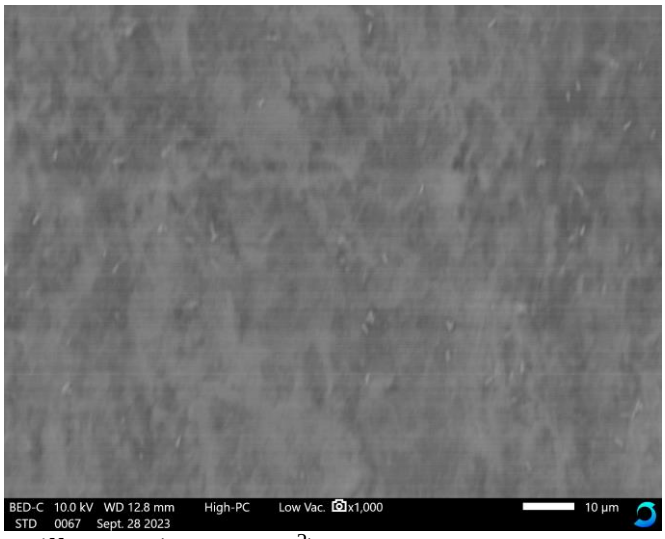


Fig. 4.7. 13 (b): *Bacillus sp.*, (YEPD K_2^{-2}) *Eps* microstuture

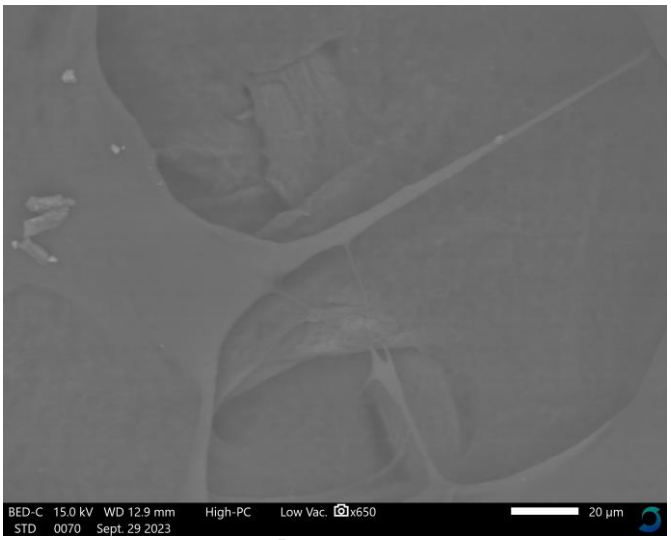


Fig. 4.7. 13 (c): *Bacillus sp.*, (PDA B_2^{-5}) *Eps* microstructure

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

RB5 textile dye wastewater collected from Rivatex East Africa Ltd was treated with commonly used chemical coagulants, alum, PFS and PaFC. The results were compared with those obtained after treatment of the same wastewater with *Eps* produced by bacteria that were cultured and isolated from gin trash soils collected from different cotton growing regions in Kenya; Meru, Baringo and Kitui ginneries.

5.1.1 Treatment of Textile Dye Wastewater Using Chemical Coagulants

5.1.1.1 Effect of pH and Dosage Variation

Treatment of the RB5 wastewater was carried by varying the pH and respective coagulant dosages in order to establish the appropriate conditions. The pH was varied between 2 to 10 while the respective coagulant dose was varied independently per coagulant. PaFC gave the lowest yet relatively constant values of TDS, EC and salinity across the entire pH range of 2-10 compared to alum and PFS whose results for the same parameters were relatively higher yet constant across the same pH range. Alum generated the highest sludge quantities of 20.12g/L at pH 6 while PFS generated relatively high amount of sludge that remained moderately constant (7.5-7.9g/L) across the pH range. PaFC gave negligible amounts.

Since each of the three coagulants gave relatively constant results of salinity, EC and TDS across the pH range, a neutral of pH 6-7 was chosen as the most appropriate and used to treat the wastewater at varied dosages of the coagulants. Alum, PFS and PaFC dosage variations were within the ranges of 60-140 g/L, 20-100 mg/L and 140-280 mg/L respectively. The pH was maintained between 6-7. PaFC gave the lowest yet

relatively constant values of TDS, EC and salinity across its entire dosage variation range while alum and PFS gave comparatively higher yet constant values of the same parameters across their dosage ranges. Alum generated the highest amount of sludge that increased with dosage, attaining 29.7g/L at the highest dose of 140g/L. PFS generated relatively lower amounts compared with those of alum, which were directly proportional with the dosage, attaining the maximum of 11.5g/L at 100mg/L. PaFC gave negligible sludge amounts across its entire dosage range. These results imply that PaFC is the most appropriate for treatment of the wastewater aimed at reduction of salinity, EC, TDS with little or no sludge generation at pH between 6 to 7. Alum and PFS were found to give relatively similar results on reduction of salinity, TDS and EC.

5.1.1.2 Assessment of Colour Removal of the Coagulant-Treated Wastewater

Extent of colour, COD and turbidity removal for the samples that gave the least values of the EC, TDS and salinity for each of the coagulants was assessed using UV-Vis spectrophotometer and Lovibond Day Light Equipment. The colour removal was found to be highest at pH 7 for PFS while alum gave lowest dye concentrations at pH 6, 7 and 10. PaFC gave highest concentrations, relatively constant results across the entire pH range (2-10). Colour removal was found to be proportional with coagulant dosages for alum and PFS. Alum clarified the dye at 140mg/L while PFS removed the highest percentage of the dye at 100mg/L. Gradual addition of PaFC dose from 140mg/L to 280mg/L however did not give significant dye removal results. Alum and PFS were therefore found to be the most appropriate for the RB5 dye removal from the wastewater although they generated high amounts of sludge.

5.1.2 Screening and isolation of bacteria with *Eps*-production capacity

Bacteria isolates characterized with mucoidal, ropy and slimy appearance, indicating the capacity to produce *Eps,* were cultured and isolated from three gin trash soils samples collected from Kitui, Meru and Baringo (Salawa) ginneries in Kenya. Nine different solid media were used, out of which YEA, YEPD, PDA and NA+G demonstrated better capacity for isolation of the *Eps*-producing bacteria. GPYMgSO_4 broth was found to support growth of at least 50 % of all the screened isolates whose *Eps* were able to remove at least 80% of the dye from the wastewater. About 16.7% and 33.3% of the screened isolates that supported production of *Eps* in the presence of the wastewater were supported by NB+S and NB+G respectively. *Eps* produced in MSYMgSO_4 broth containing 10g/L molasses, as substituted to GPYMgSO_4 was found to support removal of at least 60% of the RB5 dye from the wastewater. MSYMgSO_4 was therefore found to be a suitable substitute to the GPYMgSO_4 broth for production of bacterial *Eps* with capacity to bioremediate RB5 textile wastewater.

5.1.3 Optimization of RB5 dye wastewater bioremediation using bacterial *Eps*.

Optimization of the RB5 dye removal by the *Eps* found to have the capacity to remove at least 60% of the dye in MSYMgSO_4 was done by a full factorial design of three factors (temperature, incubation time and molasses concentration) each at three different levels as designed by MatLab 17 software. Isolate YEPD K_2^{-2} *Eps* exhibited the capacity to remove 81.67% of the dye at 30 ºC and 20g/L molasses concentration in MSYMgSO_4 broth for 24 h. Isolate NA+G M_2^{-1} *Eps* removed 82% of the dye at 30 ºC and 20g/L molasses concentration for 48 h. Isolates NA+G M_2^{-1}, YEPD K_2^{-2} and PDA B_2^{-5} *Eps* had the capacity to remove 83.18%, 85.73% and 80.13% of the dye at 30 ºC and 20g/L molasses concentration in MSYMgSO_4 broth for 72 h respectively. Comparatively, isolates NA+G M_2^{-1}, YEPD K_2^{-2} and PDA B_2^{-5} *Eps* removed 77.38%,

84.67% and 84.84% of the dye in the GPYMgSO$_4$ broth medium that was the substituted by the MSYMgSO$_4$ under the same conditions. Only isolate YEA M_2^{-4} *Eps* demonstrated the capacity to remove 83.8% of the dye at 34 ºC and 20g/L molasses concentration in MSYMg$_{SO4}$ broth for 72 h. Corresponding *Eps* from the same isolate grown in the GPYgMSO$_4$ within the same conditions removed 80.1% of the dye from the wastewater. None of the *Eps* from the six isolates had the capacity to remove at least 80% of the dye at 38 ºC for 72 h by using the different concentrations of the molasses in the MSYMgSO$_4$ broth medium. Isolate YEPD K_2^{-2} *Eps* exhibited strong linear relationship of R^2,0.99 and 0.98 between the percentage dye removal and incubation time for MSYMg$_{SO4}$ at 20g/L molasses concentration and GPYMgSO$_4$ broths respectively. R^2 for isolate NA+GM_2^{-1} *Eps* were 0.76 and 0.78 for MSYMgSO$_4$ at 20g/L molasses concentration and GPY MgSO$_4$ broths respectively. *Eps* efficiency in the dye removal was also found to be hampered by high temperatures, 34 ºC and 38 ºC. MSYMg$_{SO4}$ broth containing 20 g/L molasses as therefore found to support production of *Eps* found to have RB5 removal capacities that were comparable with those of the *Eps* produced in GPYMg$_{SO4}$ synthetic broth. These results imply that MSYMg$_{SO4}$ can substitute the GPYMgSO$_4$ synthetic broth which is chemical-based, expensive and environmentally unsustainable. MSYMgSO$_4$ was reconstituted using molasses and soya flour agromaterials, which would offer income to the farmers along their respective value chains in Kenya.

5.1.4 Characterization of *Eps*-treated wastewater, *Eps*-producing isolates and *Eps* extracts

Optimal *Eps*-treated RB5 dye textile wastewater was analyzed to study their physicochemical properties such as colour, salinity, turbidity, TDS, COD, EC and BOD. The properties of the *Eps*-treated wastewater samples were compared with

corresponding chemical coagulants-treated samples. Colour, salinity, turbidity, COD, BOD, Cu and Cr were found to reduce while EC and TDS increased after the *Eps*-treatment of the dye wastewater at the optimized conditions of 72 h, 30 ºC and 20g/L concentration of molasses in the MSYMgSO$_4$ broth by the NA+G M_2^{-1}, YEPD K_2^{-2} and PDA B_2^{-5} *Eps*. Colour removal efficiency by the *Eps* from the three isolates was found to be comparable with that of the best coagulant, PFS. While PFS had the highest colour removal capacity of 86.088%, isolate YEPD K_2^{-2} *Eps* gave 85.73%, the highest compared with that of the isolates NA+G_2^{-1} (83.17%) and PDA B_2^{-5}(80.13%) *Eps*. The three *Eps* were found to reduce the salinity of the wastewater by at least 90% compared with that of the optimized PaFC (19.667%) and PFS (3.424%) which can be associated to utilization of the salts in the wastewater and the broth medium for the *Eps* synthesis.

The untreated and *Eps*-treated wastewater FTIR spectra showed peaks characteristic with the carbonyl and hydroxyl groups at 1500-1750 cm^{-1} and 3000-3500 cm^{-1} respectively. There was no formation of new peaks or shift of absorbance spectrum before and after the *Eps*- treatment, therefore the decolorization was attributed to adsorption mechanism. UV-Vis analysis gave peak for the RB5 dye at 597nm in the untreated samples which disappeared after the treatment with *Eps* from all the three isolates confirming removal of the dye.

The three isolates were identified as follows through molecular characterization; YEPD K_2^{-2} as *Bacillus sp.,* NA+G_2-1 as *Alcaligenes Faecalis* and PDA B_2^{-5} as *Bacillus sp. Alcaligenes Faecalis* (NA+G M_2^{-1}) had capacity to produce 2.42g/L, *Bacillus sp.* (YEPD K_2^{-2}), 1.87g/L and *Bacillus sp.* (PDA B_2^{-5}), 0.83g/L of *Eps* at 30 ºC under a shaker at 150rpm for 72 h.

FTIR analysis of all the solid *Eps* samples from the three isolates gave comparable spectra that displayed a broad band between 3000-3500 cm^{-1} that represents the stretching vibration of hydroxyl groups (-OH). The stretching bands of carbonyl (-C=O), carboxyl (-COOH) and amide ($+NH_2$) groups were recorded between 1300-1750 cm^{-1} for all the *Eps*. A band within 1300-1150 cm^{-1} that was characteristic with the *Eps* from all the bacteria isolates is associated to -C–O that could be assigned to ether, alcohol or sugar derivatives groups. Carbonyl and amide groups of *Eps* are involved in the adsorption of dyes. Adsorbents containing hydroxyl (-OH), carboxyl (-COOH), and carboxylate (-COO-) functional groups can remove organic contaminants, especially dyes, through hydrogen bonding and electrostatic interaction.

GC-Ms analysis of the *Eps* showed that *Alcaligenes Faecalis* (NA+G M_2^{-1}) was a homopolysaccharide composed of beta-D-Fructopyranose sugar derivative. *Bacillus sp.*, (YEPD K_2^{-2}) *Eps* were found to be composed of Tetra-O-acetyl-d-Manno pyranose and 1, 3, 4, 6-Tetra-O-acetyl-beta-d-glucopyranose sugar derivatives. These findings agree with those previously reported that *Eps* possess the capacity to bind dyes and could be suitable substitutes to the chemical coagulants for dye wastewater treatment owing to their non-toxicity and eco-friendliness.

5.2 Recommendations

Treatment of RB5dye wastewater with chemical alum, PFS and PaFC coagulants and bacterial *Eps* was found to give comparable results. PaFC was found to be best at reduction of salinity, EC and TDS with minimal sludge generation but poorest in colour and COD removal while PFS had the best dye removal efficiency followed by alum although they generated high amounts of sludge. Therefore, none of the three coagulants had the comprehensive capability of dye removal and remediation of the

physicochemical parameters studied. A combination of PaFC and either PFS or alum is hence recommended for complementary effects during treatment of the dye wastewater. Gin trash soil demonstrated the capacity to host *Eps*-producing bacteria. Out of the nine different media used, YEA, YEPD, PDA and NA+G demonstrated suitability for isolation of *Eps*-producing bacteria from the gin trash soil hence recommended. GPYMgSO_4 broth medium is recommended for *Eps* production from the bacterial isolates since it supported at 50% of the isolates screened and identified to have the capacity to produce at least 80% *Eps*, compared with those obtained from NA+G and NA+S broths. *Eps* produced by *Bacillus sp.* (YEPD K_2^{-2}), *Bacillus sp.* (PDA B_2^{-5}) and *Alcaligenes Faecalis* (NA+G M_2^{-1}) are recommended for bioremediation of RB5 textile dye wastewater using MSYMgSO_4 broth at 20g/L molasses concentration at 30 ^oC under orbital shaker at 150rpm for 72 h since they were found to be effective in removing at least 80% of the colour alongside reduction of salinity, turbidity, COD and BOD pollutants. MSYMgSO_4 was therefore found to be an effective substitute to the GPYMgSO_4 broth. Since the *Eps* removed the dye through adsorption mechanism that resulted into formation of flocs it is therefore recommended that RB5 bioremediation should be done in combination with membrane filtration in order to get rid of the flocs and ensure safe disposal of the dye sediment.

5.2.1 Recommendations for Further Research

There is need to study the effectiveness of the three *Eps* produced by the bacteria isolates from the gin trash soils in remediation of effluents containing mixed dyes wastewater. Efficacy of the *Eps* in the removal of the RB5 dye from the wastewater can be studied and optimized further at varied concentrations of the dye, *Eps* and number of the orbital shaker rpms. The study can be conducted by using the individual *Eps* and/or their concoctions.

The isolates that demonstrated capacity to hydrolyze starch can further be studied for their possible use in desizing of cotton fabrics.

Synergistic effects of the bioflocculant *Eps* and the PFS and/or alum in bioremediation of the dye wastewater is also recommended for further evaluation for probable cost reduction and efficiency improvement. There is a need to study the suitability of re use of the *Eps*-treated wastewater within the textile factory processes such as scouring and/or for irrigation purposes.

Due to the low lack of equipment, it was not possible to fully characterize the *Eps* extracts. Further characterization of the *Eps* for determination of their molecular weight by Size-Exclusion Chromatography, antioxidant activity, thermogravimetric analysis (TGA) and morphological study by Scanning electron microscopy (SEM) is therefore recommended.

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APPENDICES

Table I *Eps* production using agro-industrial materials (Krina, Arpit and Meenu, 2021).

S/No	*EPS*	Producer	Carbon source	Carbohydrate concentration in carbon source (% total sugar)	Substrate concentration (g/L)	Maximum Yield obtained (g/L)	Time taken for *EPS* production (hours)	References
1	Succinoglycan	Agrobacterium radiobacter	Sugar beet molasses	51.2	10	22.7	103	Bakhtiyari, Moosavi-Nasab and Askari, 2015
2	Succinoglycan	*Agrobacterium radiobacter* NBRC 12665 (Immobilized cell	Sugarcane molasses	56.4	75	14.1	192	Ruiz *et al*., 2015
3	Levan	*Halomonas* sp.	Sugar beet molasses	55	30	12.4	210	Küçükaşik *et al*., 2011
4	Levan	*Bacillus polymyxa* (NRRL B-18475)	Sugar beet molasses	56.4	150	3.6	120	Han and Watson, 1992
5	Levan	*Bacillus polymyxa* (NRRL B-18475)	Sugarcane syrup	19.5	150	6.5	120	Han and Watson, 1992
6	Levan	*Zymomonas mobilis*	Sugarcane molasses	60-65	250	2.5	24	De Oliveira *et al*., 2007
7	Levan	*Zymomonas mobilis*	Sugarcane syrup	**	250	15.5	24	De Oliveira *et al*., 2007
8	Gellan	*Sphingomonas paucimobilis* ATCC-31461	Cane molasses	41	112.5	13.8	48	Banik, Santhiagu and Upadhyay, 2007

9	Xanthan	*Xanthomonas campestris* PTCC1473	Cheese whey	3.91	65.2	16.4	48	Niknezhad *et al*., 2015
10	Xanthan	*Xanthomonas pelargonii* PTCC1474	Cheese whey	3.91	80	12.8	48	Niknezhad *et al*., 2015
11	Xanthan	*Xanthomonas campestris* PTCC1473	Date extract	71.58	40	11.2	72	Khosravi-Darani *et al*., 2011
12	Xanthan	*Xanthomonas campestris* PTCC 1473	Broomcorn stem	65.34	40	8.9	0.75	Soleymanpour *et al*., 2018
13	Xanthan	*Xanthomonas campestris pv maniothis*	Apple juice	15.34 (pectin)	700	45	60	Druzian and Pagliarin,2007
14	Dextran	*Leuconostoc mesenteroides NRRL B512(f)*	Carob pod and cheese whey	45	20 (sucrose initial concentration in CPE) + 5 g/L (lactose from cheese whey)	7.23	12	Santos, Rodrigues and Teixeira, 2005
15	Dextran	*Leuconostoc mesenteroides* strain VKM V-2317D	Sugar beet molasses	50	175	49	96	Vedyashkina, Revin and Gogotov, 2005
16	Dextran	*Leuconostoc mesenteroides* NRRL B-512	Sugarcane molasses	47.2	200	9.44	17	Behravan, Fazly Bazzaz and Salimi, 2003
17	Dextran	*Leuconostoc mesenteroides* NRRL B512(f)	Carob pod extract	45	20 (sucrose initial concentration in CPE)	8.56	12	Santos, Rodrigues and Teixeira, 2005
18	Bacterial cellulose	*Gluconacetobacter xylinus* PTCC 1734	Date syrup	73.1	1,000 mL	43.5	336	Moosavi-Nasab and Yousefi, 2011
19	Bacterial cellulose	*Gluconacetobacter intermedius* SNT-1	Sugarcane molasses	8.4 (H_2SO_4-heat pretreated molasses)	45.8 (1:4 dilution)	12.6	168	Tyagi and Suresh, 2015

20	Bacterial cellulose	*Gluconacetobacter persimmonis* GH-2	Muskmelon	7	20	8.08	336	Hungund *et al*., 2013
21	Bacterial cellulose	*Gluconacetobacter persimmonis* GH-2	Orange juice	6.9	20	6.18	336	Hungund *et al*., 2013
22	Curdlan	*Rhizobium radiobacter* ATCC 6466	Date juice	86.65	120	22.83	51	Ben Salah, 2011
23	*EPS*	*Enterobacter* A47	Cheese whey	78.4	70	6.4	76.8	Antunes *et al*., 2015
24	*EPS*	*Bacillus* sp. ZBP4	Beet molasses	46.3		60	24	Ergene and Avci, 2018
25	*EPS*	*Pseudomonas fluorescens*	Sugarcane molasses	48.3		2.8	48	Sirajunnisa, Vijayagopal and Viruthagiri, 2012
26	*EPS*	*Bacillus* strain CMG1403	sugar beet	20	100	5.52	240	Muhammadi and Afzal, 2014
27	*EPS*	*Bacillus* strain CMG1403	cane molasses	50	100	4.85	240	Muhammadi and Afzal, 2014
28	*EPS*	*Lactobacillus confusus*	Coconut water	1.78	1,000	12.9	24	Seesuriyachan, 2010
29	*EPS*	*Lactobacillus confusus*	Sugarcane syrup	10	1,000	8.8	24	Seesuriyachan, 2010
30	*EPS*	*Bacillus sphaericus* 7055	Sugar beet molasses	48.6	25	0.8	48	Yilmaz *et al*., 2012

Table II. Monitoring guide for Industrial Discharge into Environment

FOURTH SCHEDULE

MONITORING GUIDE FOR DISCHARGE INTO THE ENVIRONMENT (r.12 (2))

DISCHARGING FACILITY	Gas and Oil	Dairy Products	Grain Mills	Canned Fruits & Vegetables	Canned & Preserved Sea Foods	Sugar Processing	Textiles	Cement	Feedlots	Electroplating	Organic Chemicals	Inorganic Chemicals	Plastics & Synthetics	Soap & Detergents	Fertiliser Manufacturing	Petroleum Refining	Iron & Steel Manufacturing	Non Ferrous	Phosphate Manufacturing	Steam Electric Power Generating
Water quality parameters																				
Biochemical Oxygen Demand, BOD	x	x	x	x	x	x	x		x		x	x	x	x	x	x				
Total Suspended Solids	x	x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x
pH	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Faecal Coliforms	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Oil & Grease	x				x		x				x			x		x	x	x	x	x
Temperature	x	x	x	x	x	x	x	x	x		x	x	x	x		x	x	x	x	x
Chemical Oxygen Demand, COD						x	x				x	x	x	x		x		x		
Colour/Dye/Pigment	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Elemental Phosphorus																			x	
Total Phosphorus						x				x					x				x	x
Ammonia (as N)												x			x	x	x	x		
Organic Nitrogen as N						x									x					
Nitrate						x									x		x			
Flow	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Phenols							x				x		x			x	x			
Sulphide							x									x	x			
Total Chromium							x			x		x				x				
Chromium VI										x		x				x				x
Chrome																				
Copper										x		x	x							x
Nickel										x		x								
Zinc										x			x				x			x
Zinc												x								
Cn total										x		x								
Cyanide A										x		x								
Fluorine										x		x	x					x	x	
Free Available Chlorine																				
Residual Chlorine	x																			x
Cadmium										x		x					x			
Lead										x		x					x	x		
Iron										x										
Tin										x		x								x
Silver										x										
Gold										x										
Iridium										x										
Palladium										x										
Rhodium										x										
Ruthenium										x										
Mercury (total)												x								
Total Organic Carbon												x					x			
Aluminium												x					x			
Arsenic												x					x		x	
Selenium												x								
Barium																				
Manganese																	x			
Tannin																				
Oil																				
Settleable Solids																				

Table III. Standards for Industrial Wastewater Discharge

(r. 13)

FIFTH SCHEDULE

STANDARDS FOR EFFLUENT DISCHARGE INTO PUBLIC SEWERS

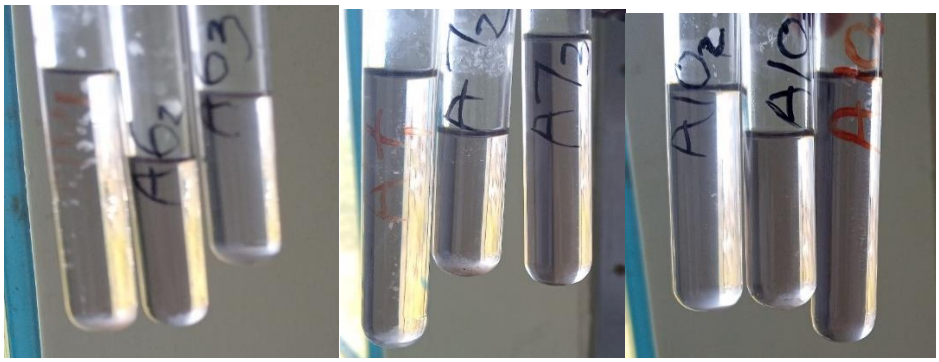
1 PARAMETER	Maximum levels permissible
Suspended solids (mg/L)	250
Total dissolved solids (mg/L)	2000
Temperature °C	20 - 35
pH	6-9
Oil and Grease (mg/L) -where conventional treatment shall be used	10
Oil and Grease (mg/L)- where ponds is a final treatment method	5
Ammonia Nitrogen (mg/L)	20
Substances with an obnoxious smell	Shall not be discharged into the sewers
Biological Oxygen Demand BOD_5 days at 20 °C (mg/L)	500
Chemical Oxygen Demand COD (mg/L)	1000
Arsenic (mg/L)	0.02
Mercury (mg/L)	0.05
Lead (mg/L)	1.0
Cadmium (mg/L)	0.5
Chromium VI (mg/L)	0.05
Chromium (Total) (mg/L)	2.0
Copper (mg/L)	1.0
Zinc (mg/L)	5.0
Selenium (mg/L)	0.2
Nickel (mg/L)	3.0
Nitrates (mg/L)	20
Phosphates (mg/L)	30
Cyanide Total (mg/L)	2
Sulphide (mg/L)	2
Phenols (mg/L)	10
Detergents (mg/L)	15
Colour	Less than 40 Hazen units
Alkyl Mercury	Not Detectable (nd)
Free and saline Ammonia as N (mg/L)	4.0
Calcium Carbide	Nil
Chloroform	Nil
Inflammable solvents	Nil
Radioactive residues	Nil
Degreasing solvents of mono-di-trichloroethylene type	Nil

A. MATERIALS

A.1.1: Untreated RB5 dye Wastewater

A.1.2: Gin Trash Soil Sample

B. CHEMICAL COAGULATION

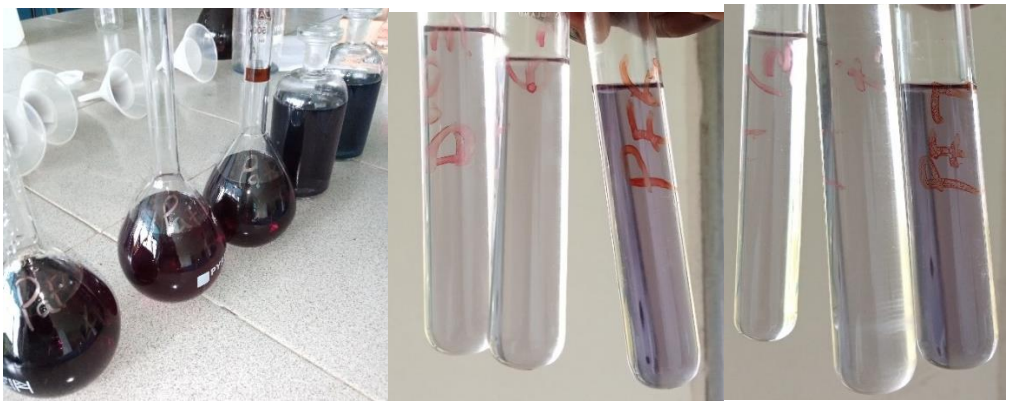


B.1.1: Alum Coagulation of Dye Wastewater-pH Optimization

B.1.2: Alum Coagulation of Dye Wastewater- Dosage Optimization

B.2.1: PFS Coagulation of Dye Wastewater-pH Optimization

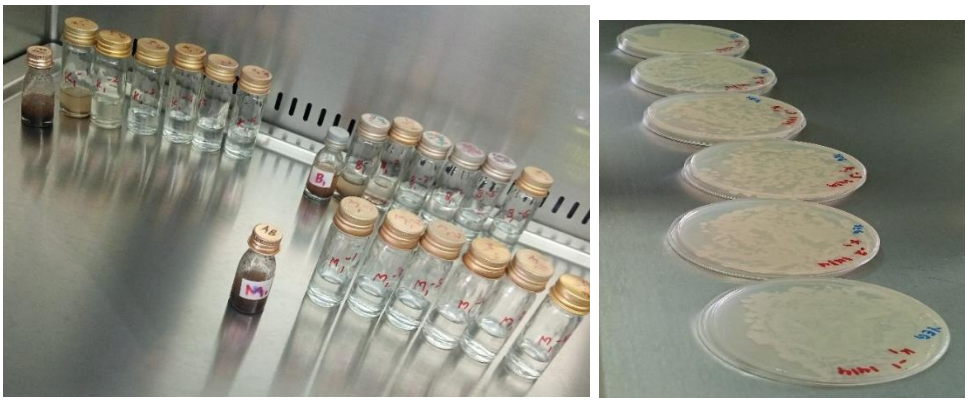
B.2.2: PFS Coagulation of Dye Wastewater-Dosage Optimization

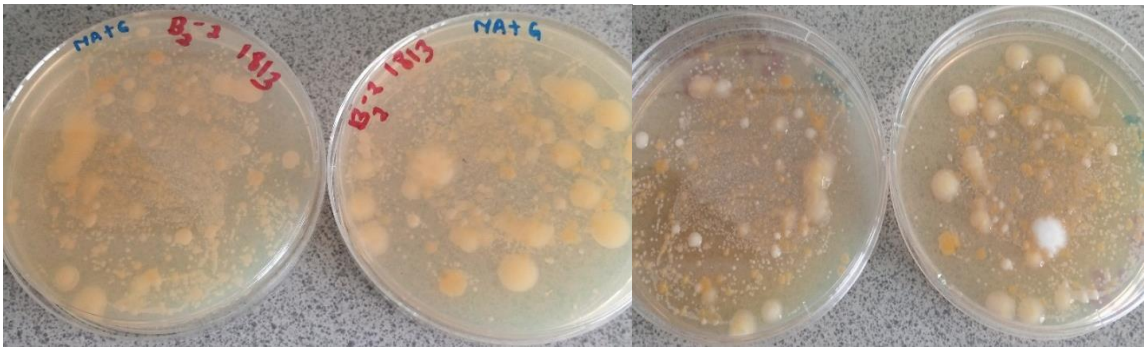


B.3.1: PaFC Coagulation of Dye Wastewater-pH Optimization

B.3.2: PaFC Coagulation of Dye Wastewater-Dosage Optimization

C. *EPS* PRODUCING BACTERIA ISOLATION FROM COTTON GIN TRASH SOIL





C.1: *Eps*-Producing Bacteria Culturing

C.2: *Eps*- Producers Bacteria Isolation

C.3: Characteristic mucoidal *Eps*-producing bacteria isolates

D. SCREENING FOR *EPS* PRODUCTION IN BROTH MEDIA (GPYMgSO$_4$, NB + G and NB + S)

D.1: Screening for *Eps* production

E. SCREENING FOR BACTERIA *EPS* AND ISOLATES CAPACITY FOR RB5 DYE REMOVAL IN NB+S GPYMgSO_4 AND NB+G BROTH MEDIA

E.1: Machine and synthetic dye wastewater samples inoculated and incubated with *Eps*

E.2: *Eps* and Isolates Screening for RB5 dye removal with NB+G, GPYMgSO_4 and NB+G broths

F. SCREENING FOR *EPS* PRODUCERS USING $MSYMgSO_4$

F.1.1: $MSYMgSO_4$ and $GPYMgSO_4$ broths inoculated with *Eps*-producer isolates and incubated

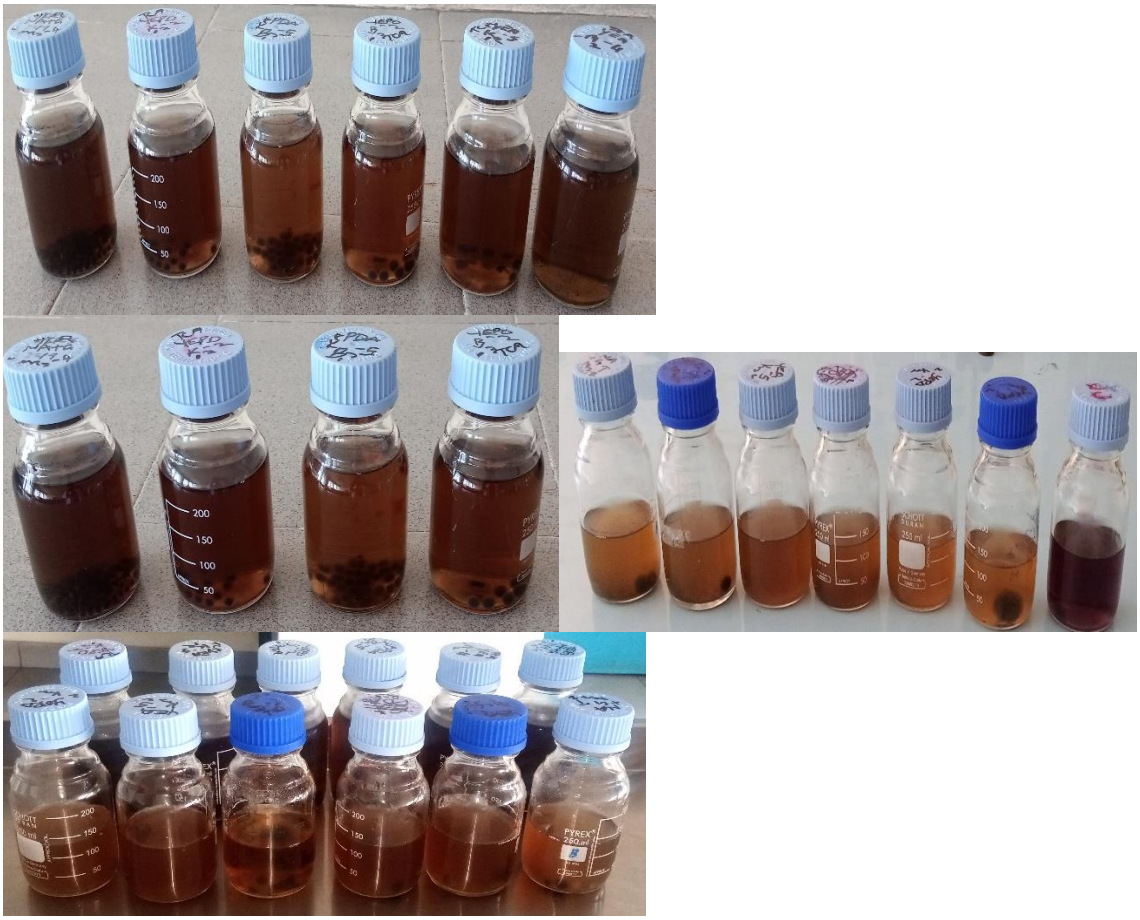
F.1.2 $MSYMgSO_4$ (10g/L molasses) decolorization at 30 ^oC for 24 hours incubation

F.1.3 $MSYMgSO_4$ (15g/L molasses) decolorization at 30 ^oC for 24 hours incubation

F.1.4 $MSYMgSO_4$ (20 g/L molasses) decolorization at 30 ^oC for 24 hours incubation

G. OPTIMIZATION OF DYE WASTEWATER BIOREMEDIATION

G1. Dye samples inoculated with *Eps*-before incubation



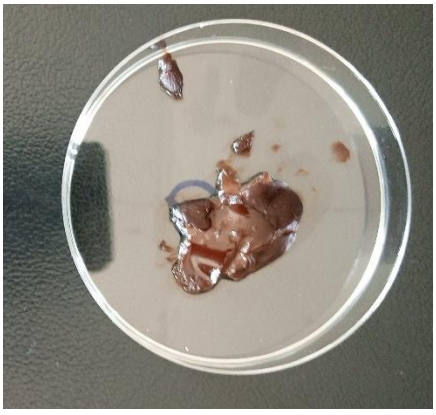
G.2: *Eps*- Bioremediated textile dye wastewater

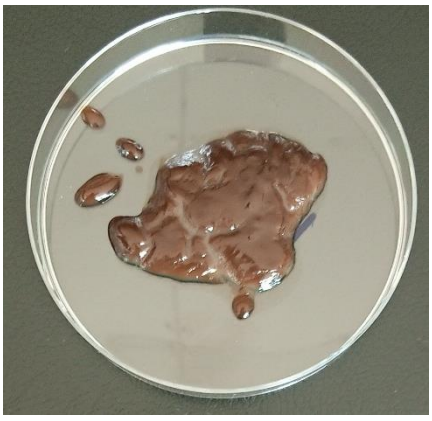
G.3: *Eps*-bioremediated (agglomerated) dye wastewater

H. *EPS* EXTRACTION USING MSYMgSO$_4$ BROTH

H.2: Centrifugation of broth media after incubation

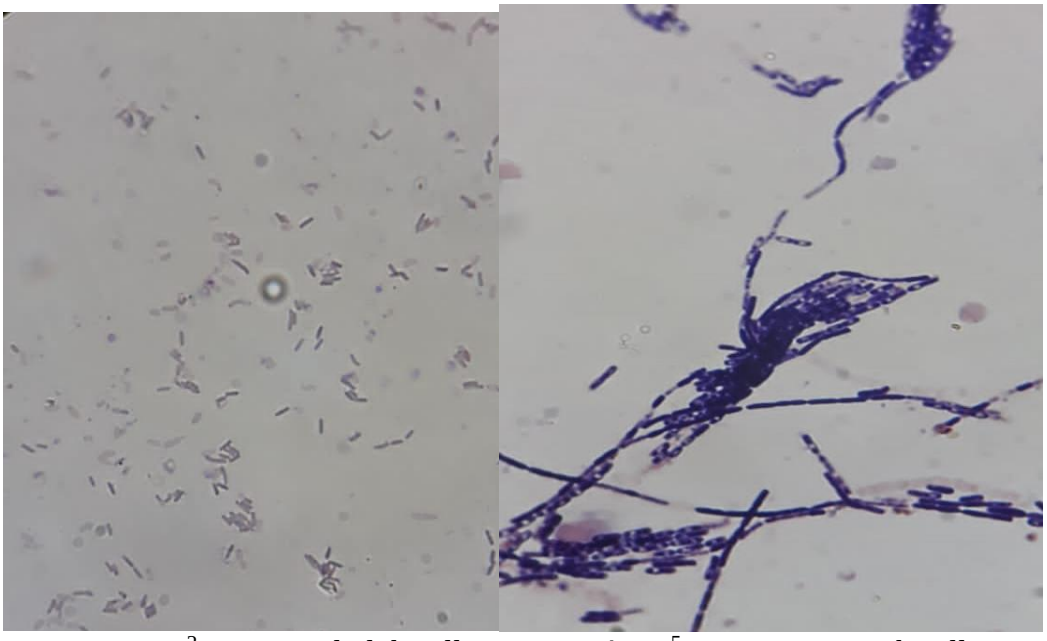
H.3: Ethanol precipitated *Eps*



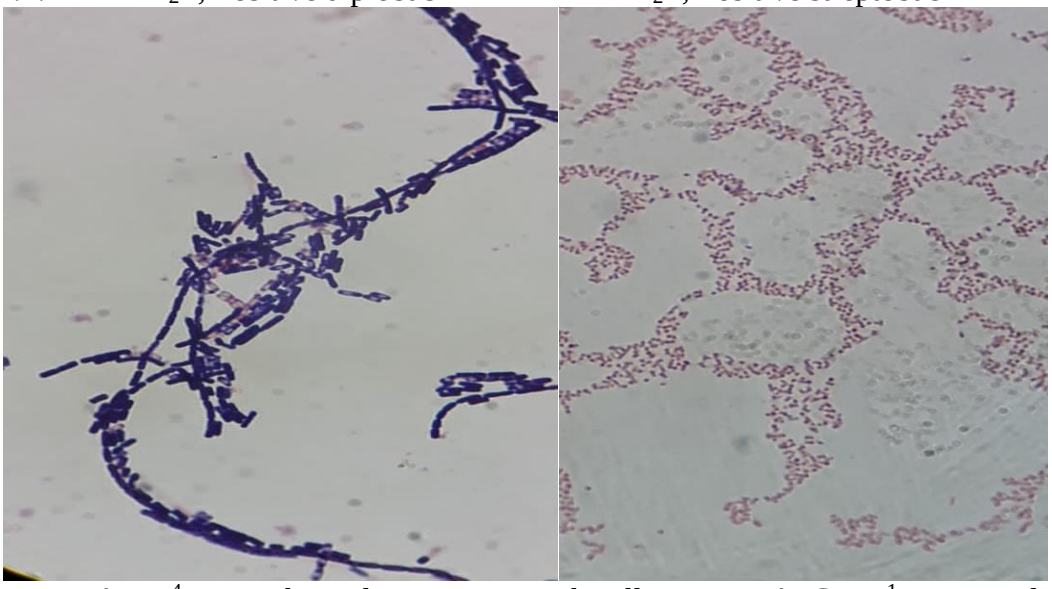


H.4: *Eps* extraction

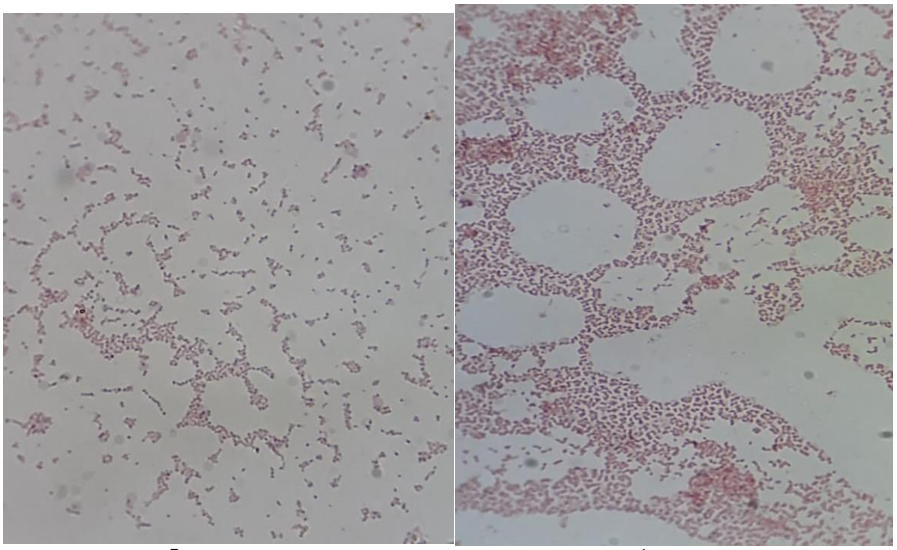
I.BIOCHEMICAL CHARACTERIZATION OF BACTERIA ISOLATES



I.1: YEPD K_2^{-2}; Positive diplobacilli PDA B_2^{-5}; Positive streptobacilli



I.2: YEA M_2^{-4}; Long chained positive streptobacilli NA+G M_2^{-1}; Positive diplococci



I.3; YEA K_2^{-5}; Positive streptococci YEPD B_2^{-1}; Negative bacilli

PUBLICATIONS

1. Mburu, A.W., Githinji, D. N., Musiemba, F., Nzila C.& Oyondi E. N. (2022). Comparative Study of Treatment of Reactive Super Black Textile Dye Effluent Using Different Chemical Coagulants. *Advances in Applied Science Research, 13* (1).

Papers Submitted (Under Review)

1. Bioremediation of Reactive Black 5 Textile Dye Wastewater Using Bacterial Exopolysaccharides- Submitted to *Research Journal of Textile and Apparel.*
2. Extraction and Characterization of Exopolysaccharides Produced by Bacteria Cultured from Cotton Gin Trash Soil-Submitted to *Express Polymer Letters*
3. Bioremediation of Reactive Blak 5 Textile Dye Wastewater Using Bacterial Exopolysaccharides-A Review – Drafting underway, to be submitted to Textile and Leather Reviews Journal

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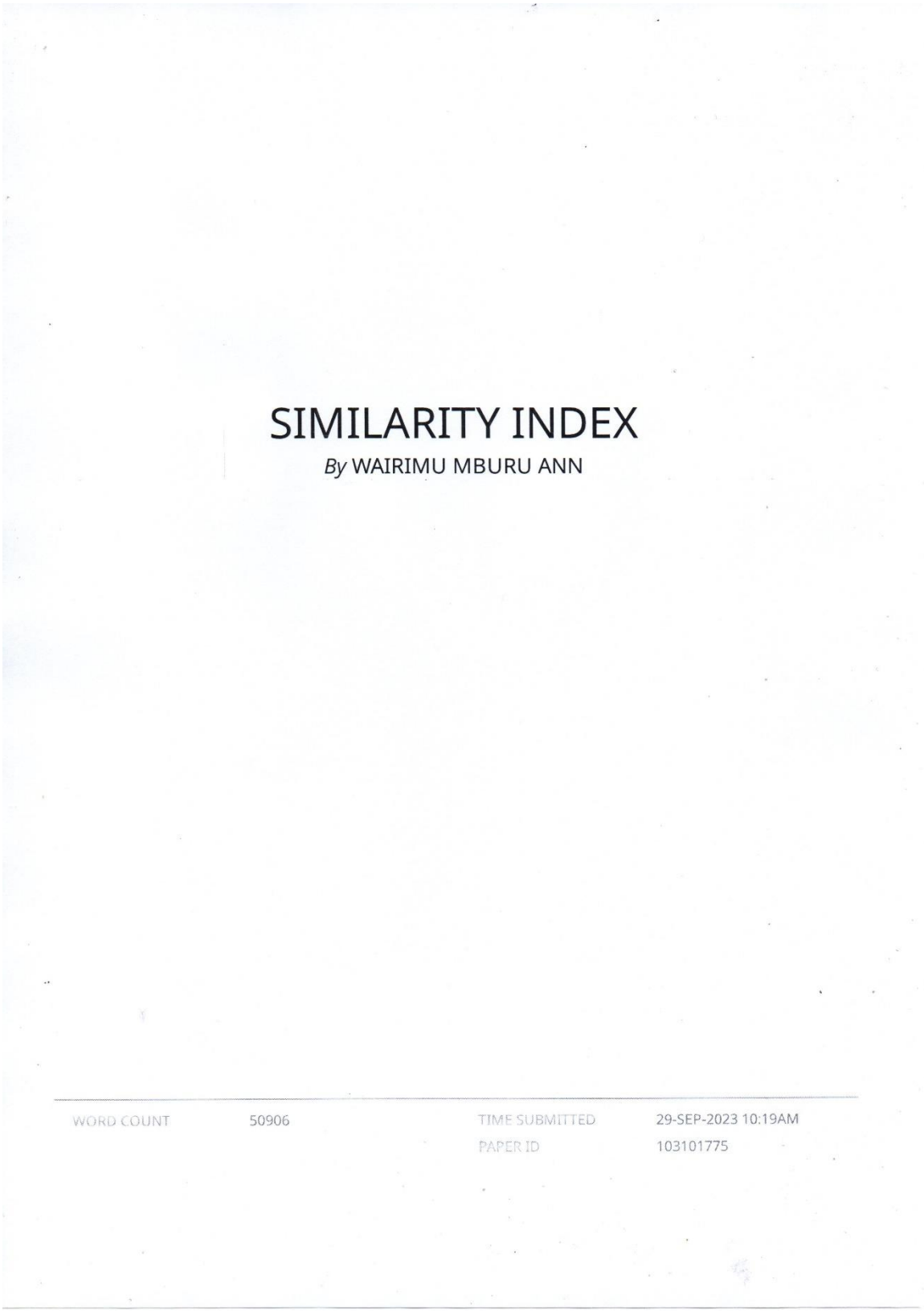

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